THE SOIL COLD TEST FOR MAIZE

by

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**TO MY FATHER
JAN VAN DER WOUDE**

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CHAPTER 1

INTRODUCTION

The fundamental objective of seed testing is to establish the quality level of seed. The most obvious component of seed quality is germination capacity and germination tests are used worldwide to determine the maximum germination potential of a seed lot under optimum conditions. The standard germination test is conducted under controlled laboratory conditions according to the specifications laid down in the rules of the International Seed Testing Association (ISTA, 1999).

Although the germination test can provide an estimate of the field emergence potential of a seed lot if seedbed conditions at planting are close to optimum, such conditions are seldom encountered in the field. Sub-optimal conditions may lead to field emergence values substantially lower than germination test results. In addition, seed lots with similar germination results may emerge from the soil quite differently when planted under identical, but stressful field conditions (Sherf, 1953; Delouche and Caldwell, 1960; Delouche, 1973; 1981). The major limitation of the germination test as an assessment of seed quality, is, therefore, its inability to detect differences in field emergence potential among high germination seed lots.

The component of seed quality that has a bearing on field emergence potential is seed vigour. Seed vigour is defined as "those properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions" (Association of Official Seed Analysts, 1983). Seed vigour testing has become an increasingly important component of seed testing and more accurately reflects the potential performance of a seed lot if stress is encountered in the field at planting. High vigour seed lots have the capacity for greater emergence and seedling survival than low vigour seed lots.

Seed vigour is a complex property which is influenced by factors such as the genetic constitution of seed and on events occurring during seed development, harvesting, conditioning and storage (Tatum, 1942; Burris, 1979). Seed ageing is the major cause of reduced viability and vigour. Physiological symptoms of ageing include reduced rate of germination, emergence and seedling growth; production of abnormal seedlings and decreased tolerance to suboptimal conditions (Burris, 1976; Johnson & Wax, 1978). Biochemical changes taking place during deterioration are membrane degradation resulting in solute leakage; enzyme, respiration and hormonal changes; impaired protein and RNA synthesis and accumulation of toxic metabolites (Woodstock & Grabe, 1967; Ching, 1973; Roos, 1980).

Vigour deteriorates faster than germination with age, *i.e.* the loss of vigour precedes the loss of the ability to germinate (Delouche & Caldwell, 1960). Although seed lots may have similar high germination percentages, they can differ in their physiological age, vigour, and thus their ability to emerge in the field.

Vigour is a complex phenomenon that cannot be quantified or measured directly. However, various tests that are able to rank seed lots according to vigour level have been developed for various crops. Vigour tests can be grouped into three general categories: a) seedling growth and evaluation tests b) stress tests (e.g. cold test and accelerated ageing test) c) biochemical tests (e.g. conductivity test and tetrazolium test) (Pollock & Roos, 1972; McDonald, 1975).

The soil cold test is one of the oldest and most acceptable seed vigour tests. It is the most widely used vigour test for maize in North America and Europe (Ferguson, 1990; Hampton, 1992; Te Krony, 1994) and is also widely accepted by the seed industry in many other parts of the world. In the soil cold test, seeds are placed in a moist medium containing soil at a temperature of 10°C for seven days. At this low temperature, solutes tend to leak from the seeds, encouraging growth of soil-borne pathogens. The soil cold test, therefore, entails two stress factors: low temperature and pathogens. Seedling emergence is assessed after subsequent incubation at 25°C . When counts obtained in the cold test are very close to those obtained in the germination test, the seed lots can be expected to emerge well over a wide range of moisture and temperature conditions.

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The moisture and temperature conditions and the pathogens provided in the cold test simulate the adverse conditions that seeds may encounter during early spring plantings.

Although most laboratories use the abovementioned temperatures in the soil cold test, methods differ in a variety of ways. Published methods include the deep box method (AOSA, 1983), and the tray and rolled towel methods (ISTA, 1999). The cold test can not be internationally standardized because of its use of field soil which is a variable material both physically and biologically. There is also a lack of uniformity among different laboratories and countries due mainly to variability in the germination mediums used (soil alone, soil mixed with sand, soil mixed with vermiculite, etc.), amount of soil used and different evaluation methods. The test is therefore limited primarily to an "in-house" vigour test within a laboratory or seed company.

Vigour tests are not yet routinely used by all seed companies in South-Africa, but the demand is increasing. Seed companies regularly receive complaints concerning poor field emergence from farmers who planted seed with a high germination percentage. Field emergence in the Eastern Free State and Highveld is often hampered by cold, wet conditions in the seedbed. This has prompted private seed companies to consider use of the soil cold test on maize with the primary goal to determine the carry-over potential of seed lots, to eliminate those lots that fall below company standards and to rank seed lots according to vigour level for trials, Gaucho treatment and export of seed.

The problem encountered by South-African seed companies in adoption of the soil cold test on a routine basis is the choice of method. Virtually no local research has been conducted on soil cold test procedures. The purpose of this study was, therefore, to compare different soil cold test methods and to determine the correlation of their results with field emergence under different field conditions. The objective was to determine the best method for adoption in South-Africa.

The seed lots used were obtained from Monsanto SA and consisted of twelve hybrid seed lots and ten parent line seed lots. The seed lots differed with respect to cultivar, production location and year of harvest.

Field emergence of the 22 seed lots was determined in four trials, two on a farm near Balfour (Highveld area) and two on the experimental farm of Monsanto SA at Petit. Different planting dates were selected for the four trials. Conditions were cool and wet, cold and wet and favourable.

The standard germination test and the soil cold tests were conducted in the laboratory. The different incubation mediums used in the cold test were sand, a sand-soil mixture, vermiculite, and a vermiculite-soil mixture. The deep box and rolled towel methods were used. Seedlings were evaluated by the criteria described in the AOSA Seed Vigor Testing Handbook,1983 (deep box method) and the ISTA Handbook of Vigour Test

Methods, 1995 (rolled towel method). The results of the laboratory tests were corre-

related with the emergence of the seed lots in the different field trials.

CHAPTER 2

LITERATURE REVIEW

2.1 History

Seed vigour is a complex phenomenon. The current understanding of the concept developed over many decades and many terms and definitions were formulated in the process.

2.1.1 Early workers

In the late 1800's observations that seed performance entailed more than simply germination percentage led seed analysts to coin the term "germination energy". Nobb (1876) is generally credited with the introduction of the term *Triebkraft* meaning "driving force". Early workers reported differences in the quality of seedlings associated with seed properties (Churchill, 1890; Hays, 1896; Clark, 1904; Cummings, 1914). Hiltner and Ihssen (1911) introduced the first "germination energy" test, the so-called brick grit test for evaluating differences in cereal seed emergence caused by *Fusarium* infection. This test was used for several years by testing stations in Europe (Eggebrecht, 1949).

During the early part of the twentieth century Stahl (1931, 1936) defined germination speed as the percentage of germination at first count in the standard germination test,

and for a number of years first counts were regarded as a useful criteria of emergence potential. Interest focused on the rate of germination as a supplemental measurement of the field value of seed and then shifted to seedling morphology and defects. During the following years many new ideas were developed and proposed.

2.1.2 Definition of seed vigour

The 1950 ISTA Congress was an important benchmark in the history of seed vigour. Franck (1950b) used and characterized the term "vigour" with subsequent widespread use. A Vigour Test Committee was established to define seedling vigour and standardize methods for vigour determination. It took the committee 27 years to agree upon an acceptable definition.

Some early individual attempts on defining seed vigour were made by Isely (1957) and Delouche and Caldwell (1960). Isely (1957) defined vigour as "the sum total of all seed attributes which favour stand establishment under unfavourable field conditions". Delouche and Caldwell (1960) proposed a modification of Isely's definition: "vigour is the sum total of all attributes which favour rapid and uniform stand establishment". There were many subsequently proposed definitions (Heydecker, 1960; Woodstock, 1965; 1969b; Perry, 1973b).

In 1977, the ISTA Vigour Test Committee proposed a definition of seed vigour which was adopted by the ISTA Congress (Perry 1978) : "Seed vigour is the sum of those

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properties which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence". The definition of seed vigour adopted by the Association of Seed Analysts (AOSA) in 1980 (McDonald 1980b) is much shorter and more direct, but quite similar: "Seed vigour comprises those seed properties which determine the potential for rapid, uniform emergence and development of normal seedlings under a wide range of field conditions". The adoption of these definitions represented a significant achievement.

2.1.3 Development of the soil cold test

The cold test was developed in North-America. During the period 1930-35, Reddy (1933, 1935), building on the earlier observations of Alberts (1927) and Dickson and Holbert (1926), devised a cold test for maize seed as a means of evaluating the effectiveness of seed fungicide treatments in protecting the seed in cold, wet soil. The idea of using environmental stresses to assay the "field" value of seed was introduced.

Reddy's procedures were subsequently developed by a hybrid seed company to evaluate conditioning methods (Isely 1950), by Tatum (1942) and Tatum and Zuber (1943) to evaluate the effect of genetic constitution and conditioning methods on the ability of maize seed to germinate in cold soil, and by Rice (1944, 1960), to routinely detect weaknesses and evaluate the stand producing ability of maize seed lots. By 1950, the cold test for maize seed was well developed and in routine use in seed testing laboratories.

Isely (1950) summarized the main specific applications of the cold test for maize seed:
(a) evaluation of the stand-producing and "carry-over" potential of maize seed lots, (b)
experimental evaluation of seed protectant chemicals and dosages, (c) monitoring and
control of conditioning operations which can influence cold test emergence, e.g. drying,
shelling, handling, and (d) identification and evaluation of sources of genetic resistance
to the stresses of the cold test. Subsequent work on the cold test has mainly concentra-
ted on identifying sources of variation and standardizing methodology (Pinnell,
1949; Clark, 1954; Hoppe, 1955; Svien & Isely, 1955).

Extensive research on the cold test for maize during the following years did much to
elucidate some of the factors influencing the performance of maize seed under cold test
conditions: pericarp injury (Crosier, 1957; Koehler,1957), deterioration due to seed age
and storage conditions (Goodsell *et al.*, 1955), seed maturity at time of harvest (Rush
& Neal, 1951), inherent resistance of seeds to seedling blight (Crane,1956), injury
during drying (Livingston 1951) and frost damage (Rossmen, 1949). The presence of
Pythium sp. in the soil used in the cold test was found to be particularly important in
the cold test results (Hoppe, 1949; Hooker & Dicksen, 1952; Crane, 1956). Seed and
environmental factors which influence cold test emergence were identified and extra-
polated to other kinds of seed.

Initially, emphasis was given to the physical characteristics of seed associated with low vigour (Perry, 1980) but more recent investigations have concentrated on the physiological and biochemical causes of vigour differences, especially the role of seed ageing and membrane integrity (Powell, 1988). Much work was done on the process of deterioration and degenerative changes (Delouche, 1969; Heydecker, 1972; Delouche & Baskin, 1973; Abdul-Baki, 1980; Roos, 1980).

2.2 Variations in technique

Different teqniques have been developed for the soil cold test and each of these methods has its advantages. A detailed description of the teqniques can be found in the ISTA Handbook of Vigour Test Methods (1999) and the AOSA Vigour Test Handbook (1983). Although the cold test is widely used, no consistent methodology has been developed and this has complicated standardization by ISTA and the AOSA.

2.2.1 Rolled towel method

The rolled towel procedure was first described by Hoppe (1956) and was modified by Fiala (1981). This method has gained widespread acceptance for its reliability, minimal space requirements and low volume of soil required.

The seeds are placed on a double layer of water-saturated paper towels and lightly covered with a soil/sand substrate. A top towel is placed over the two lower towels and the three towels are loosely rolled and placed upright in a cold room at 10°C for seven

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days. The towels are transferred to 25°C for five days and the seedlings are evaluated using the same criteria as for the standard germination test (ISTA 1999).

2.2.2 Tray method

The tray method was first reported by Burris and Navratil (1979). This test requires considerably more space than the rolled towel method but has several attractive features including: mechanization and standardization of substrate watering, vacuum-head planting, ease of interpretation and natural emergence of seed through a soil/sand substrate allowing faster evaluation and greater efficiency.

The seeds are planted on a soaked crepe cellulose substrate in a food service tray and covered with a soil/sand mixture. The resulting seed bed should approach 70% of water holding capacity. After planting, the trays are placed in a 10°C chamber for seven days and then moved to 25°C for four days. Seedlings are evaluated using the same criteria as for the standard germination test (ISTA 1999).

2.2.3 Deep box method

The deep box method was described by Clark (1953), Svien and Isely (1955) and Woodstock (1976) and has the same advantages as the tray method.

A layer of two centimeter of a soil/sand mixture is placed on the bottom of a plastic box (38cm x 12cm x 8cm) and leveled. The seeds are planted and pressed into the soil . The

same amount of soil is then placed over the seed and leveled. Enough water is added to bring the medium to 70% of its water holding capacity. The boxes are covered and put in a 10°C chamber for seven days, then transferred to 25°C for four days. The percentage germination is the percentage of seeds from which normal seedlings with plumules 2.5cm or more in length emerge from the germination medium (AOSA Seed Vigour Testing Handbook, 1983).

In 1983 the AOSA Seed Vigour Testing Handbook recommended the deep box method with the rolled towel and tray methods as alternatives. At about the same time, the ISTA Handbook of Vigour Test Methods recommended a rolled towel method. TeKrony (1987) reported wide variation among laboratories using the deep box method but more reliable and reproducible results with the rolled towel and tray methods.

In 1990, the AOSA revised the cold test procedure to recommend the rolled towel and tray method as primary methods and the deep box as an alternative method. Many referee tests have reported variation among laboratories using the recommended cold test methods (Ader & Fuchs, 1978; Tao, 1978; 1980; Fiala, 1987). This is due to variations in soil substrate (pH, pathogens, organic matter, oxygen supply), moisture and temperature differences, type and amount of fungicide used, duration of cold period and storage of soil substrate. Attempts were made to control the biological activity of the soil substrate by adding micro-organisms to a sterile substrate (Desai & Reddy, 1958; Garzonico & Larsen, 1981) but were unsuccessful.

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2.3 Correlations with field emergence

Vigour test results are not expected to predict an exact value for field emergence because soil and seedbed conditions vary from field to field. However, many studies have evaluated cold test methods and demonstrated a significant correlation between cold test results and field emergence in maize in the rolled towel method (Navratil & Burris, 1980; Loeffler *et al.*, 1985; Fiala, 1987), the deep box method (Garzonico & Larsen, 1981; Nijenstein, 1986; 1988; Bekendam *et al.*, 1987), and the tray method Burris & Navratil, 1979; TeKrony *et al.*, 1989). Numerous publications indicate the cold test's superiority over the standard germination test in predicting the relative field performance of maize seed lots (Schultz, 1992; Anfinrud ,1994). Many studies have demonstrated the cold tests ability to reflect relative field performance of other crops such as soybean (Ferris & Baker, 1990), sorghum (Ebrahim *et al.*, 1993), onion (Bekendam *et al.*, 1987), carrot (Hegarty, 1971) and pea (Clark & Baldauf, 1958).

Correlation between cold test results and field emergence seem to vary due to numerous factors, and may change over years for the same test (Martin & O'Neil, 1987). Correlations can be influenced by the inherent stress level of the laboratory test, quality levels of the seed lots, and environmental stress in the field. It is impossible to predict emergence percentage in the field correctly, because of the influence of weather conditions on the final percentages. Choosing cold test conditions which are not as severe as field conditions will not result in many statistically significant correlations. Factors in addition to those discussed above, may also be of importance. Storage conditions prior

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to sowing, quality of seed treatment, differences in sowing depth, sowing date, loss of

fungicide after heavy rainfall, salt stress and crust forming, may all lead to differences

in field performance of seed lots of the same quality.

2.4 Current use (popularity)

According to a 1990 survey conducted by the AOSA Seed Vigour Testing Committee,

the number of laboratories conducting vigour tests is increasing (Ferguson, 1994). The

survey showed that 85% of the laboratories in the U.S. and Canada participating in the

survey were performing vigour tests on a regular basis. The accelerated aging and cold

tests continue to be the most widely used vigour tests. TeKrony (1994) surveyed eight

USA seed componies and found that the cold test is the major vigour test for maize,

soybean and sorghum. All seed lots of maize and cotton are tested for vigour , while 80

and 85% of the soybean and sorghum seed is tested. The cold test is also the primary

vigour test used for sweet corn. Public and private laboratories participating in the

1993-1994 Illinois Seed Dealers Association testing program indicated that the rolled

towel and tray method are the most commonly used cold test methods.

A survey was conducted in South Africa by the author of this dissertation in 2001, with

12 seed companies participating. According to this survey, 50% of the laboratories

participating were using vigour tests as part of a quality program with only three labo-

ratories performing vigour tests on a day to day basis. Most of the laboratories started

using vigour tests only recently (1-3 years) while only two laboratories conducting

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vigour tests for a longer peri ![logo] :d were maize and soya beans, followed by cotton and sorghum. The cold test is the primary vigour test used for maize with the accelerated ageing test used for soya beans and the cool germination test used for cotton. Four companies used the tetrazolium test, but mainly for viability purposes.

CHAPTER 3

FIELD EMERGENCE TRIALS

3.1 Introduction

Seed vigour is a relative concept; there are no absolute values of vigour on a scale of, say, 0 – 100, as in the germination test. There is also no "standard" vigour test to which other tests can be compared to measure their efficiency. None of the vigour tests currently used has been standardized to the point where it has been recognized as an official test. There have been major problems, in some cases, with vigour test reproducibility, sensitivity, relevance, and a lack of understanding of the variables and assumptions involved. The interpretation of vigour test results is often difficult.

The only way in which the usefulness of a seed vigour test can be assessed is to determine whether there is a significant correlation between vigour test results and field emergence. Within a seed company, the level of the vigour test emergence can be related to field emergence and intelligent seed quality decisions made regarding potential seed lot performance. To ascertain the relationship between the different cold test methods conducted in this study (results presented in Chapter 4) and field emergence, a number of field trials were conducted on the seed lots used (results presented in this chapter). Correlations between the laboratory tests and field trial results are presented in Chapter 5.

Two groups of seed lots (*i.e* .a hybrid group and a parent line group) with different deterioration levels were planted in different field trials representing different sets of environmental conditions, including favourable and stress conditions. The conditions were subjectively designated as favourable (one trial with both seed lot groups), cool and wet (two trials with the hybrid group) and cold and wet (one trial with both seed lot groups). Emergence counts in each trial were performed daily. Variables determined were emergence rate and final emergence count.

3.2 Materials and methods

Two groups of seed lots were used in this study. The first group consisted of twelve seed lots selected from three commercially available cultivars and the second group consisted of ten seed lots selected from eight parent lines. The seed lots differed with respect to production locality and year of harvest. Details of these seed lots appear in Table 1 (hybrid seed lots) and Table 2 (parent line seed lots). Representative samples of the 22 seed lots were obtained from a private seed company, Monsanto SA. Because of confidentiality agreements, the seed lots were not identified but coded H1-H12 (hybrids) and P1-P10 (parent lines) with cultivars coded A - C and parent lines coded A - H. All seed lots had been treated by the supplier with Captan, Coopertox and sodium molybdate at the recommended dosages. All seeds were of the same grade (4F). Germination percentage of the hybrid seed lots, as determined in the standard germination test (ISTA 1999, procedure described in Chapter 4) ranged between 90% and 99% and the parent line seed lots between 91 and 95%. All seed lots, therefore,

Table 1 Details of the maize hybrid seed lots used in this study

Seed lots	Cultivar	Year of harvest	Production area	Germination %
H1	A	1993	Vryburg	91
H2	A	1994	Hoedspruit	99
H3	A	1995	Vryburg	97
H4	A	1996	Hoedspruit	97
H5	A	1997	Grootpan	99
H6	A	1998	Hoedspruit	99
H7	B	1994	Hoedspruit	93
H8	B	1996	Grootpan	92
H9	B	1997	Vryburg	98
H10	B	1998	Grootpan	98
H11	C	1993	Vryburg	90
H12	C	1993	Vryburg	90

Table 2 Details of the maize parent line seed lots used in this study

Seed lots	Line	Year of harvest	Production area	Germination %
P1	A	1996	Marble Hall	92
P2	A	1995	Groblersdal	92
P3	B	1998	Marble Hall	91
P4	C	1995	Groblersdal	95
P5	D	1995	Groblersdal	95
P6	D	1993	Groblersdal	92
P7	E	1994	Ohrigstad	94
P8	F	1994	Pietersburg	91
P9	G	1993	Ohrigstad	92
P10	H	1994	Pietersburg	91

showed germination percentages of an order which is commercially acceptable.

Field emergence of the 22 seed lots was determined in the different trials. Two of the trials were conducted on the experimental farm of Monsanto SA at Petit (26°10' S; 28°20' E) and two were conducted on a private farm near Balfour (26°32' S; 28°39' E). Both soils are a sandy loam. Experimental design in each case was a randomized block with four replicates of 100 kernels each. The kernels were hand-planted in 10m rows with a spacing of 0,5m , planting depth of 5cm and spacing within rows 10cm. Different planting dates were selected for the four trials to obtain different seedbed conditions.

Air temperature was recorded daily at 08:00 and 14:00 and the mean minimum and maximum temperatures were determined for each of the trials. A minimum- and maximum thermometer was used. The total irrigation for the period four days before planting and ten days after planting was 60mm for each trial. The days when irrigation took place were as follows: 20mm four days before planting, 20mm after planting was completed and 20mm ten days after planting. Sprayers were used and a rain-gauge was placed at each trial. No precipitation occurred during any of the four trials. Details of the planting dates and temperature conditions for each trial are supplied in Table 3.

Table 3   Details of the climatic conditions prevailing during the field

emergence trials with 22 maize seed lots

Trial	Field Condition	Location	Planting Date	Air Temperature °C Min	Max	Irrigation mm
1	Cold/wet	Balfour	April 1999	6	17	60
2	Cool/wet	Balfour	September 1999	11	19	60
3	Cool/wet	Petit	September 1999	10	21	60
4	Warm/wet	Petit	November 1999	18	25	60

Emergence counts in each trial were performed daily. Emergence rate was calculated for each seed lot according to a modified version of the index of Maguire (1962):

$$R = \Sigma (E_i / i)$$

Where R = emergence rate index

E = amount of seedlings newly emerged on day i

i = days subsequent to emergence of the first seedlings

Analyses of variance were conducted on the data of each trial and LSD values calculated according to the GLM procedure (SAS System, 1985). Analyses were conducted separately for the two groups of seed lots.

3.3 Results and discussion

The results of the emergence trials and ranking order of the hybrid seed lots are shown in Table 4. The germination percentages are included for comparison purposes. The range in field emergence from the highest to the lowest count was 17% against the variation of 9% in the germination test.

The emergence counts of some of the seed lots indicated a considerable difference between emergence under favourable conditions and germination percentage. Emergence counts of the hybrid seed lots in Trial 4 (favourable conditions) ranged between 80 and 97%. This was higher than the range of the germination percentages of the seed lots, with the lowest 90% and the highest 99%.

The lowest emergence counts in Trial 4 were obtained with seed lots H1 (84%), H8 (84%), H11(80%) and H12 (80%). Seed lots H11 and H12 were the lowest in the ranking order. The emergence counts of these seed lots indicated considerable differences between emergence values and germination percentages. Seed lots H5 and H6 were ranked first with an emergence count of 97% (germination was 99%). The emergence counts of the intermediate seed lots were 90% and higher. These seed lots showed only small differences between emergence and germination percentages.

Numerous publications indicate a good correlation between germination and field emergence in near ideal soil seedbed conditions (Delouche, 1973; Anfinrud, 1994).

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Table 4   Field performance of 12 hybrid seed lots of maize. Ranking

orders appear in parentheses

Seed lots	Emergence	%			Germination %
	Trial 1	**Trial 2**	**Trial 3**	**Trial 4**	
	Cold/wet	Cool/wet	Cool/wet	Warm/wet	
H1	75 (10)	82 (9)	83 (9)	84 (9)	91
H2	91 (2)	91 (5)	92 (5)	94 (5)	99
H3	87 (5)	91 (5)	96 (2)	95 (4)	97
H4	85 (6)	92 (3)	90 (6)	96 (3)	97
H5	91 (2)	95 (1)	93 (3)	97 (1)	99
H6	94 (1)	94 (2)	97 (1)	97 (1)	99
H7	84 (8)	86 (8)	88 (8)	90 (8)	93
H8	75 (10)	80 (10)	83 (9)	84 (9)	92
H9	85 (6)	92 (3)	93 (3)	93 (6)	98
H10	90 (4)	91 (5)	90 (6)	93 (6)	98
H11	77 (9)	79 (11)	80 (11)	80 (11)	90
H12	69 (12)	70 (12)	75 (12)	80 (11)	90
F value	11.36	16.07	9.35	11.37	
LSD	6.61	5.40	6.35	5.83	
CV %	5.53	4.34	5.02	4.52	

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The difference found in this study could be due to seed deterioration. Seed lots H1, H11 and H12 (lowest in ranking order) were produced in 1993, thus six years old and the oldest of the 12 seed lots. Seed lots H5 and H6 (highest in ranking order) were one and two years old, respectively.

Emergence differed considerably between different seed lots of the same cultivar. In cultivar A, emergence varied between 84% (H1, age 6 years) and 97% (H6, age 1 year). The lowest emergence counts were obtained from the oldest seed lots (most deteriorated). When the result of a germination test is less than a high standard (*i.e.* 90%) it indicates that the quality of the seed lot is suspect *i.e.* deterioration has occurred (Hampton & Coolbear, 1990) and field performance will be impaired. A small difference in percentage germination represents a large difference in the progress of deterioration (Ellis & Roberts, 1981). The differences found in emergence of the seed lots were the result of ageing and deterioration and thus differences in seed vigour. If the deterioration of a seed lot proceeds to below a critical point, emergence will be much lower than germination percentage (H11 & H12), even in ideal conditions.

Emergence under cool, wet conditions (Trials 2 and 3) differed from the emergence obtained in Trial 4, especially with the older seed lots. Emergence ranged between 70 and 97%, depending on the age of the seed lot and thus the vigour. The ranking order was generally the same as Trial 4 with seed lots H11 and H12 the lowest in ranking order and H5 and H6 ranked highest. The emergence of seed lots H5, H6 as well as

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the intermediate seed lots, did not differ much from Trial 4. The emergence of H12 (old seed lot) was the poorest and was 10% lower than in Trial 4 and 20% lower than in the germination test. The emergence of H6 (young seed lot) was the same in Trials 3 and 4. Emergence of different seed lots of the same cultivar, varied between 83% (H1, six years old) and 97% (H6, one year old), a difference of 14%. The germination percentages were 91 and 99%, respectively. The poorest emergence values were again obtained with the oldest seed lots.

Emergence count of the hybrid seed lots in Trial 1 (cold and wet conditions) ranged between 69 and 94%. Emergence of all seed lots, except H2,H5, H6 and H10, showed large differences from Trial 4. Performance of some of the seed lots was much poorer. The emergence of only four seed lots was 90% and higher (H2, five years old; H5, two years; H6, one year; H10, one year). The seed lots lowest in ranking order were H12 (six years old), H1 (six years), H8 (three years) and H11 (six years). The emergence of these seed lots was below 80% with H12 the poorest, namely 69%. Although the germination percentage of all hybrid seed lots was 90% and higher, emergence of some of the seed lots was poor under stress conditions, an indication of low vigour. Environmental stress results in varying field performance depending on the vigour status of the seed lot (Perry,1980; Powell,1988; Te Krony & Egli,1993). High vigour seed lots will perform better under environmentally stressed seedbed conditions than lower vigour seed lots, even though the laboratory germination of the lots may not differ.

Details of emergence rates of the hybrid seed lots are listed in Table 5. Emergence rate varied considerably between the trials (different stress conditions) and between the different seed lots (differences in vigour) in any particular trial. All of the seed lots emerged the most rapidly in Trial 4 (favourable conditions) and the slowest emergence rate was found in Trial 1 (cold and wet conditions). The performance of the different seed lots with regard to emergence rate corresponded with the emergence percentages of the seed lots in the different field trials. Seed lot H6 was ranked first with regard to emergence rate in all of the trials and H12 was the lowest in ranking order in three of the trials. The lowest emergence rates were obtained with the old seed lots H1, H11 and H12 *i.e.* seed lots low in vigour. High emergence rates corresponded with a high total stand in the field *ie* seed lots high in vigour (H5 and H6).

The results of the emergence trials and the ranking orders of the parent line seed lots are shown in Table 6. The emergence count of the parent line seed lots in Trial 4 (favourable conditions) ranged between 61 and 88%. The germination percentage of the seed lots was much higher, with the lowest 91% and the highest 95%. There was a considerable difference between emergence and germination percentage in all seed lots. The range in field emergence from the lowest to the highest count was 27% with a variation of only 4% in germination percentage. In Trial 4, seed lot P6 (six years old) was ranked tenth with an emergence count of 61% (germination 92%) and P5 (four years old) was ranked first with an emergence of 88% (germination 95%). These two seed lots were from the same parent line but differed with respect to production year.

Table 5 Emergence rates of 12 hybrid seed lots of maize in four trials.

Ranking orders appear in parentheses

Seed lots	Emergence	rate	(Index of Maguire, 1962)	
	Trial 1 Cold/wet	**Trial 2** Cool/wet	**Trial 3** Cool/wet	**Trial 4** Warm/wet
H1	45.2 (10)	56.3 (10)	65.3 (8)	84.0 (8)
H2	81.5 (2)	81.4 (4)	78.4 (6)	89.7 (5)
H3	63.4 (5)	72.3 (7)	82.6 (3)	89.7 (5)
H4	60.3 (6)	82.0 (3)	80.2 (5)	90.6 (3)
H5	78.7 (3)	83.6 (2)	82.6 (3)	91.6 (2)
H6	82.5 (1)	89.6 (1)	89.4 (1)	97.0 (1)
H7	55.6 (8)	65.7 (8)	63.8 (9)	79.9 (9)
H8	50.5 (9)	59.0 (9)	55.4 (10)	65.8 (10)
H9	59.3 (7)	81.4 (4)	85.6 (2)	90.6 (3)
H10	65.0 (4)	75.4 (6)	74.3 (7)	88.2 (7)
H11	44.0 (11)	44.0 (11)	43.3 (12)	54.0 (11)
H12	38.4 (12)	40.0 (12)	44.6 (11)	48.5 (12)
F value	39.46	81.46	97.62	102.84
LSD	6.88	5.18	4.62	4.49
CV%	7.94	5.22	4.57	3.86

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Table 6 Field performance of ten parent line seed lots of maize. Ranking orders appear in parentheses.

Seed lots	Emergence	(%)	Germination %
	Trial 1 Cold/wet	**Trial 4** Warm/wet	
P1	41 (8)	75 (7)	92
P2	57 (5)	79 (6)	92
P3	73 (1)	81 (4)	91
P4	65 (2)	80 (5)	95
P5	58 (4)	88 (1)	95
P6	40 (10)	61 (10)	92
P7	50 (7)	75 (7)	94
P8	65 (2)	86 (2)	91
P9	57 (5)	83 (3)	92
P10	41 (8)	70 (9)	91
F-value	9.55	10.45	
LSD	10.79	7.01	
CV %	13.64	6.26	

Emergence overall of the parent lines was much lower than the emergence of the hybrid seed lots. The performance of the parent lines was poor, even under favourable seedbed conditions, indicating low vigour of the seed lots. The parent lines are inbred lines or single cross hybrids (small plants) and the hybrids are double/multi cross hybrids, this could lead to differences in vigour.

Emergence counts of the parent line seed lots in Trial 1 (stress conditions) were very low and varied from 40 to 73%. Emergence counts differed considerably between trials 1 and 4. Seed lot P6 (six years old) was again ranked tenth with a poor emergence of 40% (germination percentage 92%) and P3 (one year old) was ranked first with an emergence of 73% (germination 91%). More variation was found in the ranking orders of the parent line seed lots between the different trials than with the hybrid seed lots. The overall performance of the parent lines was poor, both under favourable and stress conditions. These seed lots were obviously very low in vigour.

Details of emergence rates of the parent line seed lots are shown in Table 7. Considerable differences in emergence rate were found between Trial 4 (favourable conditions) and Trial 1 (stress conditions). Emergence rate ranged between 35.6 and 79.8 (Trial 4) and 23.4 and 58.3 (Trial 1). Emergence rate performance corresponded with emergence percentage. Seed lot P6 was ranked tenth with regard to both emergence rate and emergence percentage. First in ranking order was P5 (Trial 4) and P4 (Trial 1). The emergence rates of the parent line seed lots generally were considerable lower than the

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Table 7 Emergence rate of 10 parent line seed lots of maize. Ranking order of seed lots is indicated by the numbers in parentheses.

Seed lots	Emergence rate	(Index of Maguire, 1962)
	Trial 1 Cold/wet	**Trial 4** Warm/wet
P1	29.3 (7)	42.6 (9)
P2	32.4 (6)	65.3 (5)
P3	52.6 (2)	72.3 (2)
P4	58.3 (1)	72.3 (2)
P5	51.2 (3)	79.8 (1)
P6	23.4 (10)	35.6 (10)
P7	34.2 (5)	48.6 (7)
P8	38.2 (4)	62.2 (6)
P9	25.4 (9)	71.4 (4)
P10	25.5 (8)	45.3 (8)
F value	27.46	53.58
LSD	7.03	5.97
CV%	13.24	6.95

emergence rate of the hybrid seed lots.

In conclusion, the results presented in this chapter confirm that seed lots which show high percentages in the standard germination test, may show poor emergence in the field (Schultz, 1992). This demonstrates the need for vigour tests to supplement the results of the standard germination test.

The contrast between the germination and field emergence percentages in Trials 1, 2 and 3, probably explains the lack of correlation usually encountered between the standard germination test and emergence under stress conditions. In most areas of crop production, field conditions at planting are usually less than optimum. The general tendency of a decline in field performance with an increase in age of the seed lots, in the case of both hybrids and parent lines, is apparent in the results presented in this chapter. The differences found in emergence between the seed lots in the different trials appear to be the result of ageing and deterioration and thus differences in seed vigour.

CHAPTER 4

LABORATORY TRIALS

4.1 Introduction

Seed vigour tests are used to evaluate seed vigour and thus provide a more sensitive measurement of seed quality than the germination test. A vigour test more accurately reflects the potential emergence of a seed lot if stress is encountered in the field and can identify seed lots which may not perform well. The soil cold test has gained wide acceptance as a standard vigour test for maize as it simulates the most common stress conditions encountered in the seedbeds of the northern Hemisphere (cold, wet conditions which also enhance pathogen activity). Many publications have demonstrated the superiority of the cold test over the warm germination test regarding field emergence under stress conditions (Adegbuyi and Burris, 1989; Te Krony *et al.*, 1989; Bruggink *et al.*, 1991). The seeds are exposed to a cold temperature (10°C, seven days) in non-sterile field soil at 70% of water holding capacity prior to a four day grow out period in ideal conditions (25°C) (Hoppe, 1956; Fiala, 1981). Although widely used, no consistent methodology has been developed and this has complicated standardization by ISTA and AOSA.

As more and more seed companies use seed vigour tests in their quality control programmes, and consumer demands for vigour information become more pronounced, it

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is critical that all laboratories adhere to the same procedure. Standardization in testing procedures and interpretation and reporting of vigour test results between different laboratories is imperative. Even small deviations in a procedure can lead to large variation in test results.

Many seed companies utilize their own in-house cold test procedures and tests used at present vary in, for example, type of substrate, temperature, moisture content, soil type, duration of cold period and evaluation time. Because the cold test requires the use of soil it cannot be readily standardized between different laboratories. A specific cold test procedure adopted by a particular seed company has to suite its specific needs but, importantly, must be reproducible at least within the laboratory and correlated with field performance. The soil component is a very important aspect of the test and the selection of an appropriate soil source is critical to the reproducibility of the test. In this study various cold test methods were compared for their usefulness as a seed vigour test in South-Africa. Detailed descriptions of these and other vigour tests are contained in the ISTA Handbook of Vigour Test Methods (1999) and the AOSA Vigour Testing Handbook (1983).

The soil cold test was used to determine the vigour of the two groups of seed lots, H1-H12 and P1-P10. Details of the seed lots appear in Chapter 3, Tables 1 and 2. Six different cold test methods and the standard germination test were performed in the laboratory and the results of the different methods were correlated with emergence of the seed

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lots in the four different field trials. Correlations between the laboratory tests and field trial results are presented in Chapter 5. The deep-box and rolled towel methods were used and the substrates used were sand, a sand-soil mixture, vermiculite, a vermiculite-soil mixture and paper towels with and without soil. The soil used, temperature and duration of cold period were the same for the different cold tests.

4.2 Materials and methods

Samples of seeds from the seed lots described in Chapter 3 (Tables 1 and 2) were used in the laboratory trials. Seed moisture content varied between 10 and 12%.

The soil used was from a maize field on a private farm near Balfour. The soil was a sandy loam of the Avalon type with a clay percentage between 18 and 23. An adequate amount was stored in sealed containers to maintain soil moisture. Prior to use, it was screened through a 5mm sieve to remove large debris. The moisture content of the soil and waterholding capacity of the germination medium were determined and the amount of water that had to be added to the medium in order to achieve 70% of water holding capacity was calculated according to the procedure describe by the AOSA Seed Vigour Testing Handbook (1983).

The water used was chilled to 10°C.

To determine emergence percentage, the percentage of normal seedlings with plumules 2.5cm or more in length that had emerged from the germination medium were counted (AOSA Seed Vigour Testing Handbook, 1983).

Samples of all the seed lots were subjected to the laboratory tests described below. Four replicates of 50 kernels each were used in the different laboratory tests.

The tests were as follows:

a. Laboratory Test no.1

The deep-box method was used. The test was conducted in plastic boxes according to the procedure prescribed by AOSA (1983). The germination medium consisted of vermiculite. Four plastic boxes (38x12x14cm) were filled up to a depth of 2cm with vermiculite which was leveled. The seeds of each sample were evenly spaced on the vermiculite layer and covered with another 2cm layer of vermiculite, leveled and com-pacted. Enough water was added to bring the medium to 70% of its water holding capacity. The boxes were sealed and placed in an incubator maintained at 10°C for seven days. After seven days the boxes were transferred to another incubator at 25°C for four days. Percentage of normal seedlings emerged was determined.

b. Laboratory Test no.2

The procedure was the same as for laboratory test no.1 but soil was added to the germination medium. After the seeds were placed on the vermiculite layer, they were lightly covered with a soil layer (250 cm³). All seeds were in direct contact with the soil. The seeds and soil were covered with another 2cm layer of vermiculite, watered, sealed and placed in an incubator at 10°C for seven days and then transferred to 25°C for four days after which emergence counts were made.

c. Laboratory Test no.3

The deep-box method was again used but the germination medium consisted of sterilized quartz sand, particle size 0.05 – 0.8mm, so-called Germination Sand which was bought from Mixcrete. The procedure used was the same as for laboratory test no.1 with 2cm of sand below and 2cm of sand above the seeds.

d. Laboratory Test no.4

The procedure was the same as used for laboratory test no.3 but the germination medium consisted of a sand-soil mixture (50/50). The seeds were planted on a 2cm layer of the sand-soil mixture and covered with another layer of 2cm.

e. Laboratory Test no.5

The rolled towel method was used, without soil, according to the ISTA Handbook of Vigour Test Methods (1995). The paper towels (30 x 60 cm, Anchor Germination

Paper, obtained from Agricol) were soaked in chilled water and allowed to equilibrate at 10°C overnight. The seeds were placed on a double layer of cold saturated towels in two 25-seed rows, 6 and 12 cm respectively from the upper edge of the towel. A top towel was placed over the seeds and the three towels were loosely rolled and placed in a polyethylene bag to prevent loss of moisture. The towels were placed upright in a plastic container in an incubator at 10°C for seven days and then transferred to another incubator at 25°C for five days. Seedlings were evaluated using the same criteria as for the standard germination test.

f. Laboratory Test no.6

The procedure used was the same as for laboratory test no.5 but soil was added. After the seeds were placed on the paper towels they were lightly covered with soil (250 cm^3), ensuring that all seeds were in contact with the soil. A top towel was placed over the two lower towels containing the seeds and soil and the three towels were loosely rolled. The samples were incubated as in the previous test.

g. Laboratory Test no.7

The standard germination test was conducted in rolled paper towels at 25°C according to the rules of the International Seed Testing Association (1999). Four layers of germination paper were used (Anchor Germination Paper, 60 x 30 cm) with a layer of cellulose wadding placed between the second and third germination paper. The paper towels were moistened and the seeds placed on top of the third paper. The seeds were

covered with the fourth germination paper and the four layers were loosely rolled and placed in a polyethylene bag. The rolled papers were placed in a plastic container in an upright position in the incubator at 25°C and first and second counts of germination percentage were made after four and seven days, respectively.

Analyses of variance were conducted on the results of each test and LSD values calculated according to the GLM procedure (SAS System, 1985). The *t* Test (LSD) and *t* Grouping were used to determine any significant differences between the tests.

Each of the tests examined has its own features and advantages. The deep-box method has several attractive features including vacuum-head planting with uniform distribution of seeds onto the substrate allowing ease of counting. Natural emergence of seedlings through the substrate allows faster evaluation and greater efficiency. Primary limitations of this test are the relatively large amount of soil and floor space required to conduct the test which may reduce the number of samples tested. The major advantages of the rolled towel procedure is the minimal space requirement and low volume of soil needed.

4.3 Results and discussion

The results of the different laboratory tests conducted on the hybrid seed lots are shown in Table 8. The vigour percentage varied among the different laboratory tests as well as between the different seed lots within one test. The standard germination percentage

Table 8 Normal seedlings of 12 hybrid seed lots of maize in six different laboratory tests. Ranking order appears in parentheses

Seed lots	Normal seedlings %						
	Lab test 1	Lab test 2	Lab test 3	Lab test 4	Lab test 5	Lab test 6	Lab test 7
	Vermiculite	Vermiculite+soil	Sand	Sand + soil	Paper towel	Paper + soil	Standard germ
H1	88 (9)	76 (11)	84 (10)	71 (10)	84 (11)	79 (10)	91
H2	94 (4)	90 (4)	95 (2)	88 (4)	98 (1)	92 (4)	99
H3	95 (2)	90 (4)	95 (2)	88 (4)	96 (4)	92 (4)	97
H4	93 (5)	86 (6)	92 (5)	85 (6)	93 (7)	87 (7)	97
H5	95 (2)	91 (2)	99 (1)	90 (2)	98 (1)	93 (1)	99
H6	96 (1)	94 (1)	95 (2)	92 (1)	98 (1)	93 (1)	99
H7	90 (7)	85 (7)	89 (8)	82 (8)	91 (8)	85 (8)	93
H8	88 (9)	77 (9)	83 (11)	71 (10)	89 (9)	83 (9)	92
H9	91 (6)	85 (7)	92 (5)	85 (6)	95 (5)	90 (6)	98
H10	90 (7)	91 (2)	92 (5)	90 (2)	95 (5)	93 (1)	98
H11	82 (11)	77 (9)	85 (9)	70 (11)	88 (10)	75 (11)	90
H12	75 (12)	72 (12)	78 (12)	66 (12)	80 (12)	74 (12)	90
Mean	90	85	90	81	92	86	95
F value	11.19	14.31	13.45	21.57	18.61	10.80	11.81
LSD	5.33	5.52	4.82	5.78	3.90	6.32	3.17
CV %	4.15	4.57	3.75	4.97	2.96	5.13	2.33

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was high compared to the percentage normal seedlings in the vigour tests and ranged between 90 and 99%. The vigour percentage ranged between 66 and 99% within the different laboratory tests. The range in vigour percentage from the lowest to the highest value was 33% compared to the variation of 9% in the standard germination test. The largest differences were found with the older seed lots (H1, H8, H11 and H12) which showed the lowest vigour percentages. The difference between the standard germination test and lowest values in a cold test were 24% and 22% for seed lots H12 and H8, respectively. In some of the seed lots (H5, H6, and H10), the standard germination and cold test percentages differed only slightly. In seed lot H6 the results were 92 – 98% for the cold test and 99% for the warm germination test. When germination obtained in the cold test is close to that obtained in the standard germination test, the seed lot would be expected to emerge well over a wide range of seedbed conditions.

In the different laboratory tests the lowest vigour percentages were obtained from the older seed lots (H1, H8, H11 and H12). A decline in vigour percentage was found with an increase in age of the seed lot. Vigour percentages differed between the different laboratory tests but the ranking order of the seed lots did not show large differences between the different tests. Seed lots H1, H8, H11 and H12 were the lowest in ranking order with H12 consistently ranked twelfth in all the tests. The vigour percentages of these seed lots were below 88% with the lowest vigour score of 66% (H12, laboratory test 4). Seed lots H5 and H6 were ranked either first or second in all the laboratory tests. The vigour percentages of H5, H6 and H10 were above 90% in all the different

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tests. These results are indicative of high quality and field emergence could be expected to be the highest of all the lots studied under stress conditions. This was, indeed, found to be the case in the emergence trial results presented in Chapter 3.

Major differences were found in vigour percentage of a seed lot between the different tests, especially with the older seed lots. The variation between the tests can be attributed mainly to the use of soil in some of the tests (Tests 2, 4 and 6). Lower vigour percentages were obtained when soil was included in the medium and this effect was more pronounced with the older seed lots (H1, H8, H11 and H12). Large differences were found if the same substrate was used with and without soil. In seed lot H11 the differences found with the same substrate with and without soil, were 5% (vermiculite); 14% (sand) and 13% (paper). No major differences were found in vigour percentages between the different tests with the high vigour seed lots. The vigour percentage of seed lot H6 ranged between 92 and 98% in the different tests, a variation of 6%. The effect of soil was less obvious in the high vigour seed lots.

Results of the LSD means and t Grouping of the different laboratory tests for the hybrid seed lots are shown in Table 9. Means with the same letter in the t Grouping column are not significantly different. The standard germination test differed significantly from the cold tests. The three cold tests without soil (laboratory tests 1, 3 and 5) did not differ significantly from each other but differed significantly from the tests which included

Table 9 *t* Grouping and means of the different laboratory tests for the hybrid seed lots

t Grouping	Mean	Lab test	
A	95	7	Standard germination test
B	92	5	Paper towel
B	90	3	Sand
B	90	1	Vermiculite
C	86	6	Paper towel + soil
C	84	2	Vermiculite + soil
D	81	4	Sand + soil

F value	20.33	
CV%	2.8	
LSD	2.9	

soil. Significant differences were not found between laboratory test 6 (paper + soil) and laboratory test 2 (vermiculite + soil) but they differed significantly from laboratory test 4 (sand + soil). The lowest percentage emergence was obtained with laboratory test 4 (81%) and the highest percentage with laboratory test 5 (92%). Laboratory test 4 was the strictest of the cold tests followed by laboratory tests 2 (vermiculite + soil) and 6 (paper towel + soil).

Numerous publications have indicated the importance of including soil in the cold test. In 1966, Kietreiber found that when soil was not included in the cold test, germination percentages obtained in sand and paper were almost as high as in the standard or warm germination test. Loeffler , Meier and Burris (1985) found no differences between soil and paper for high quality seed lots, but for poor seed lots the use of soil resulted in much lower percentages. Bruggink *et al*. (1991) obtained similar results.

The results of the laboratory tests conducted on the parent lines are presented in Table 10. The vigour percentages obtained with the parent line seed lots were very low compared to the standard germination test. The germination percentages ranged between 91 and 95% and the vigour percentages between 20 and 88%, a variation of 4% and 68%, respectively. The largest difference was found with seed lot P6 (six years old) with a vigour percentage of 20% (test 4) and a germination percentage of 92%, a variation of 72%. The differences between germination and lowest cold test results were 56% and 52% for seed lots P10 (five years old) and P1 (three years old), respectively. The

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Table 10 Normal seedling percentages of 10 parent line seed lots of maize in six different laboratory tests. Ranking order appears in parentheses

Seed lots	Normal seedlings %						
	Lab test 1	Lab test 2	Lab test 3	Lab test 4	Lab test 5	Lab test 6	Lab test 7
	Vermiculite	Vermiculite+soil	Sand	Sand + soil	Paper towel	Paper + soil	Standard germ
P1	70 (8)	49 (8)	60 (9)	40 (8)	68 (10)	45 (8)	92
P2	80 (5)	61 (3)	75 (4)	50 (6)	84 (4)	57 (7)	92
P3	86 (1)	60 (5)	80 (1)	62 (2)	88 (1)	72 (2)	91
P4	85 (2)	73 (1)	79 (3)	70 (1)	87 (3)	75 (1)	95
P5	85 (2)	68 (2)	80 (1)	60 (3)	88 (1)	70 (3)	95
P6	70 (8)	30 (10)	65 (8)	20 (10)	75 (7)	43 (10)	92
P7	80 (5)	57 (7)	75 (4)	45 (7)	78 (6)	60 (5)	94
P8	83 (4)	60 (5)	75 (4)	58 (4)	80 (5)	68 (4)	91
P9	72 (7)	61 (3)	69 (7)	55 (5)	75 (7)	58 (6)	92
P10	68 (10)	49 (8)	60 (9)	35 (9)	70 (9)	45 (8)	91
Mean	78	57	72	50	79	59	93
F value	10.77	13.83	12.64	18.64	15.31	20.60	2.66
LSD	6.16	9.39	6.40	9.83	5.39	7.53	2.84
CV %	5.47	11.44	6.19	13.75	4.70	8.78	2.13

smallest difference (25%) was found with seed lot P4 (four years old).

The vigour percentage varied between the different seed lots and between the different laboratory tests, but the ranking orders of the seed lots did not show large differences between the tests. Seed lots P1, P6 and P10 were the lowest in ranking order in all of the laboratory tests with the highest vigour percentages obtained being 70 and 75% and lowest values being 40%, 20% and 35%. Seed lots P3, P4 and P5 were ranked first, second and third with highest vigour percentages of 88% and 87% and lowest values of 60 and 70%.

Major differences were found in the vigour percentages between the different laboratory tests. Large differences were found when soil was used (pathogenic activity). In seed lot P6 the differences found with the same substrate with and with-out soil, were 40% (vermiculite), 45% (sand) and 32% (paper). The effect of soil was the most obvious with the lowest vigour seed lots but differences were found with all the seed lots. With poor quality seed lots the use of soil results in a much lower vigour percentage (Bruggink *et al.*, 1991). Soil-borne fungi , particularly *Pythium* spp., are known to be an important cause of maize seed mortality under stress conditions (Nijenstein, 1986).

Results of the LSD means and t Grouping of the different laboratory tests for the parent line seed lots are shown in Table 11. Means with the same letter in the t Grouping column are not significantly different. Results were similar to those obtained with the

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Table 11   *t* Grouping and means of the different laboratory tests for the parent line seed lots

t Grouping	Mean	Lab test	
A	92	7	Standard germination test
B	79	5	Paper towel
B	78	1	Vermiculite
C	72	3	Sand
D	59	6	Paper towel + soil
D	57	2	Vermiculite + soil
E	49	4	Sand + soil

F value	84.39
CV%	14.87
LSD	4.55

hybrid seed lots. The standard germination test differed significantly from the cold tests. Laboratory test 5 (paper towel) and laboratory test 1 (vermiculite) were not significantly different from each other but differed significantly from laboratory test 3 (sand). Laboratory test 6 (paper + soil) and laboratory test 2 (vermiculite + soil) did not differ significantly. The lowest percentage emergence was obtained with laboratory test 4 (sand + soil) and the highest percentage with laboratory test 5 (paper towel). This was similar to the results with the hybrid seed lots.

The parent line seed lots showed an average of 93% in the standard germination test. This compares favourably with the average germination of 95% of the hybrid seed lots and is a value which is acceptable for commercial seed lots. The values obtained for the parent line seed lots in the vigour tests were, however, generally lower than the values for the hybrid lots, in some cases much lower. This is indicative of lower seed quality (emergence potential) of the parent line seed lots and emphasizes the need to use vigour tests in conjunction with the standard germination test. The emergence of the parent line seed lots was, indeed, lower than the emergence of the hybrid seed lots (Chapter 3).

CHAPTER 5

CORRELATIONS BETWEEN LABORATORY AND FIELD TRIALS

5.2 Introduction

A vigour test can only be regarded as useful if it has been demonstrated that significant correlations exist between its results and field emergence. Thus vigour scores should be related to potential field performance, but they cannot be expected to be highly correlated with emergence under *all* conditions that occur in the field. The levels of stress in the field differ from year to year, and from field to field. Correlations may differ over years for the same test and vary due to numerous factors.

Correlations can be influenced by the inherent stress levels of the laboratory test, number of seed lots tested, quality levels of seed lots, and type and level of environmental stress in the field. Factors such as storage conditions prior to sowing, quality of seed treatment, differences in sowing depth, sowing date, loss of fungicide after heavy rainfall and even salt stress following an application of manure, may lead to differences in field performance of seed lots of the same quality (Ader & Fuchs, 1978; Van der Werf *et al.*, 1985; Bruggink *et al.*, 1991). Standardization of all procedures in the laboratory as well as storage conditions, seed treatment and plant specifications are very important to obtain valuable results.

In this study, various cold tests were compared for their usefulness as a seed vigour test. Correlations were determined between the results of the six different cold tests as well as the standard germination test (Chapter 4) and emergence in the different field trials of the 12 hybrid seed lots and the 10 parent line seed lots (Chapter 3).

5.2 Results and discussion

The correlation coefficients between the laboratory tests and the emergence trials of the hybrid seed lots are shown in Table 12. All correlation coefficients were found to be significant at the 1% level. With the hybrid seed lots the standard germination test was highly significantly correlated with emergence in all four trials but the correlation coefficients obtained were generally of a lower order than those obtained with the cold tests. This was especially the case with emergence under cold, wet conditions (Trial 1). A correlation coefficient of 0.59 was found between emergence and the standard germination test results while the correlation coefficients between emergence and the different cold tests ranged between 0.72 and 0.82.

Although differences in the correlations between the different cold test results and emergence in the different field trials were not of a large magnitude, values for laboratory test 4 (sand + soil) was the highest in three cases (Trials 1, 2 and 4). Laboratory test 1 (vermiculite) tended to produce the lowest values. In general, inclusion of soil in the laboratory test medium resulted in a small increase in correlation coefficients.

Table 12 Correlation coefficients between laboratory tests and emergence of 12

hybrid seed lots in four trials. (Significant at P = 0.01)

Lab test	Trial 1 Cold/wet	Trial 2 Cool/wet	Trial 3 Cool/wet	Trial 4 Warm/wet
Lab test 1 Vermiculite	0.75	0.73	0.71	0.67
Lab test 2 Vermiculite+soil	0.81	0.77	0.68	0.79
Lab test 3 Sand	0.80	0.80	0.77	0.77
Lab test 4 Sand + soil	0.82	0.88	0.76	0.82
Lab test 5 Paper towel	0.72	0.80	0.74	0.76
Lab test 6 Paper + soil	0.79	0.72	0.75	0.74
Lab test 7 Germination	0.59	0.73	0.65	0.72

Correlation coefficients between emergence and laboratory test results were lower with Trial 3 than Trials 1&2. Stress conditions were less severe in Trial 3. The correlation coefficients between the different cold test results and emergence under favourable conditions (Trial 4) were surprisingly high. This could be due to the poor emergence of the low vigour seed lots in all of the trials, even under favourable conditions.

Correlation coefficients between the laboratory tests and emergence trials of the parent line seed lots are presented in Table 13. With the parent line seed lots no significant correlations were found between the results of the standard germination test and emergence under favourable as well as stress conditions. The germination test, therefore, had no predictive value for field emergence.

Highest correlations with emergence in Trial 1 were found in the case of laboratory tests 3 (sand) and 4 (sand + soil). Correlations with the paper towel cold test (5 and 6) were slightly lower, while those with the vermiculite cold tests (1 and 2) were the lowest. In contrast to the results with the hybrid seed lots, inclusion of soil in the different cold tests did not have much effect.

Correlations with emergence in field Trial 4, however, were higher when soil was included in the cold test medium. Correlation coefficients between cold test results and emergence in field Trial 4 were of a lower order than in the case of field Trial 1. There were only small differences in the correlations between emergence in Trial 4 and the results

Table 13 Correlation coefficients between laboratory tests and emergence of 10 parent line seed lots in two trials. Correlation coefficients between emergence and all cold tests were highly significant (P = 0.01). The correlation coefficients between emergence and the standard germination test were not statistically significant.

Lab test	Trial 1 Cold/wet	Trial 4 Warm/wet
Lab test 1 Vermiculite	0.70	0.51
Lab test 2 Vermiculite+soil	0.64	0.69
Lab test 3 Sand	0.80	0.57
Lab test 4 Sand + soil	0.80	0.73
Lab test 5 Paper towel	0.74	0.53
Lab test 6 Paper + soil	0.78	0.68
Lab test 7 Germination	-0.45	0.22

of laboratory tests 1 (vermiculite), 3 (sand) and 5 (paper towel). Similarly, there were only small differences in the correlations between emergence in Trial 4 and tests 2 (vermiculite + soil), 4 (sand + soil) and 6 (paper + soil).

CHAPTER 6

GENERAL DISCUSSION

The major limitation of the standard germination test as an assessment of potential field performance of a seed lot is its inability to detect quality differences among high germination seed lots. The object of this test is to determine the maximum germination potential of a seed lot under optimum conditions. These conditions are seldom encountered in the field which may lead to field emergence values substantially lower than that indicated by the germination test results. The vigour status of a seed lot more accurately reflects the potential performance of a seed lot if stress is encountered in the field. High vigour seed lots will perform better under environmentally stressed seedbed conditions than lower vigour seed lots, even though the warm germination percentages of the lots may not differ. Seed companies in South-Africa annually receive complaints about field emergence from farmers who have bought seed with a high germination percentage, especially from the areas which experience cold and wet conditions during spring.

The cold test is one of the oldest and most acceptable vigour tests as it simulates stress conditions commonly occurring in the field. Various cold test procedures have been adopted by different laboratories, and the test has, therefore, not been standardized. In this study various cold test procedures were compared for their usefulness as a seed vigour test. A number of field trials were conducted to ascertain the relationship be-

tween the different cold tests methods used in this study with field emergence. Correlation coefficients were determined between the different cold test procedures and the emergence trials.

Two groups of seed lots, each group representing seed lots with different levels of deterioration, were planted in field trials representing different sets of environmental conditions and field emergence was determined. Emergence between the different trials and between the different seed lots differed significantly. Although the germination percentages of all seed lots were above 90%, emergence of some of the seed lots were poor even under favourable as well as stress conditions. These differences found in emergence of the seed lots were the result of ageing and deterioration and thus differences in seed vigour. Significant differences were found between emergence pertage under favourable conditions and the warm germination percentage of some of the seed lots. The poorest emergence counts were obtained with the older seed lots in all of the trials. The overall field performance of all parent line seed lots was poor under both favourable and stress conditions due to the very low vigour of these seed lots. The standard germination test was found to be a poor indicator of field emergence of poor quality seed lots. The performance of the different seed lots with regards to emergence rate corresponded with the final emergence percentage of the seed lots in the different field trials. High emergence rates corresponded with a high total stand in the field *i.e.* seed lots high in vigour. The lowest emergence rates were obtained with the older seed

lots (low in vigour). The general tendency of a decline in field performance with an increase in period of ageing was apparent from this study.

Large differences were found between the results of the different laboratory tests as well as between the different seed lots in the same laboratory test. The lowest vigour percentages were obtained from the old seed lots as well as all of the parent line seed lots. Percentage emergence obtained in the different cold tests with the poor quality, older, seed lots were much lower than their warm germination percentages. Smaller differences were found in the case of the high quality seed lots. A decline in vigour percentage was found with an increase in age of the seed lot. This corresponded with the results of the emergence trials.

With the hybrid seed lots the standard germination test was significantly correlated with emergence in all four trials but the correlations obtained were lower than those obtained with the cold tests. No significant correlations were found between the standard germination test results and field emergence of the parent lines. Thus the standard germination test was poorly related to field emergence under both favourable and stress conditions, demonstrating the need to supplement the standard germination test with vigour tests to assess seed quality. The different cold tests had better predictive value for emergence than the warm germination test.

In certain cases, inclusion of soil in the cold test medium gave higher correlations and the sand + soil cold test gave the best correlations in most of the cases. However, the differences between correlations obtained between field emergence and the different cold test procedures were not of such an order that a clear case can be made for the adoption of one particular cold test above any other.

Although the correlations between laboratory test results and field emergence do not provide grounds for a clear recommendation of adoption of one cold test procedure over another, other factors could influence adoption. The results of the different cold tests (Chapter 4) clearly showed that lower values were obtained when soil was included in the medium and that the sand + soil cold test proved to be the strictest, *i.e.* gave the lowest vigour percentage values followed by the vermiculite + soil and paper + soil procedures.

Taking both the correlations with field emergence and strictness of the test into account, the sand + soil cold test can be recommended for adoption by South African seed companies. From the results of this study, however, the sand + soil cold test is judged to be only marginally more suitable than the vermiculite + soil and paper + soil tests. In fact the vermiculite + soil test uses less soil, is therefore, less expensive, and is more likely to be favoured by seed analysts. The paper + soil test is more time consuming and cumbersome than the sand + soil or vermiculite + soil tests and is more difficult to evaluate. Its adoption is, therefore, unlikely.

CHAPTER 7

SUMMARY

1. A seed vigour test provides a more sensitive measurement of seed quality than the standard germination test. The cold test is one of the most widely used vigour tests for maize but procedures had not been standardized. In this study different cold test methods were compared for their usefulness as a seed vigour test.

2. Field emergence of 12 hybrid seed lots and 10 parent line seed lots were determined in four different field trials, representing different environmental conditions. Conditions were cold and wet, cool and wet, and favourable. Percentage emergence and emergence rates were determined for the different trials.

3. Considerable differences were found between percentage emergence in the different trials and the standard germination test results. The lowest emergence counts as well as emergence rates were obtained with the oldest seed lots. The differences found in emergence counts between the different seed lots appear to be the result of ageing and deterioration and thus differences in vigour. The ranking order of the different seed lots according to percentage emergence were similar for the different trials.

4. Six different cold test methods and the standard germination test were conducted in the laboratory and the results of the different tests were correlated with field emergence. The deep-box and rolled towel methods were used and the germination substrates used were sand, a sand-soil mixture, vermiculite, a vermiculite-soil mixture and paper towels with and without soil.

5. Considerable differences were found in percentage emergence between the different laboratory tests. The lowest vigour percentages were obtained with the older seed lots, thus a decline in percentage emergence with an increase in age of the seed lot. The same tendency was found with the field emergence trials. The ranking order of the seed lots did not show large differences between the different tests.

6. Lower vigour percentages were obtained when soil was included in the germination medium and this effect was more pronounced with the older seed lots. The sand + soil cold test was found to be the strictest of the cold tests with the lowest percentage emergence.

7. Correlations were determined between the results of the different cold tests and emergence in the different field trials. All correlation coefficients of the hybrid seed lots were found to be significant at the 1% level. However, the correlation coefficients obtained between the standard germination test and field emergence were generally of a lower order than those obtained with the cold tests. No significant correlations were

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found between emergence and the germination percentages of the parent line seed lots.

8. Although the correlations between the different laboratory test results and field emergence do not provide ground for a clear recommendation of adoption of one cold test procedure over another, inclusion of soil in the germination medium resulted in a small increase in correlation coefficients. Correlation coefficients between emergence and the sand + soil cold test were the highest in most of the trials .

9. Although any of the cold tests which include soil in the medium appear to be suitable for adoption by South African seed laboratories, the vermiculite + soil test has advantages over the sand + soil and paper + soil tests.

CHAPTER 8

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