A STUDY OF THE RELATIONSHIP BETWEEN SEED VIGOUR AND SEED PERFORMANCE IN TRIFOLIUM PRATENSE L. CV. GRASSLANDS PAWERA

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ABSTRACT

Significant differences in seed vigour within lots of red clover cv. Pawera, white clover cv. Huia and lucerne cv. Wairau were recorded in a preliminary experiment. Subsequently, 7 high viability Pawera seed lots were used to explore the relationship between seed vigour, as measured in the laboratory, and seed performance, both in the field and in storage. Four vigour testing techniques i.e. conductivity, accelerated aging (AA), controlled deterioration (CD) and speed of germination were employed. Results for standard germination (SG) and each of the vigour tests were related to seed performance by correlation analysis. Field performance was monitored for 6 seed lots, sown at 8 dates through spring and autumn. Seed storability was determined by measuring the viability of 4 seed lots under 5 storage conditions (including ambient open storage and simulated temperate controlled storage :20 ^{o}C, 45% to 90% RH) over a total of 11 months. The effects of mechanical damage, thousand seed weight, imbibition rate and storage fungi on seed viability and vigour were also investigated and seed quality changes during storage were monitored.

The vigour rankings found in the laboratory were consistent with those for field emergence, emergence rate over the 8 sowings and performance during storage. Low vigour lots also showed a significant reduction in seedling dry weight for the autumn sowings when soil temperatures were very low.

Each of the four vigour techniques were able to provide more accurate parameters for predicting seed performances than the SG test. For predicting seed field emergence over all the sowings, the best result was provided by the CD test at either 16% or 18% seed moisture content ($r = 0.933$ and 0.911 resp.), followed by AA (2-day AA of surface sterilized seed) ($r = 0.840$) and conductivity ($r = -0.602$). For predicting seed storability, the best result was obtained from the CD test (at either 18% or 20% seed moisture content) for ambient storage conditions, and from both CD (either 18% or 20% seed moisture content) and AA (3-day aging) under controlled storage conditions. Correlation coefficients for vigour tests and storage performance tended to vary between storage periods.

Seeds which imbibed water rapidly (within 4h) were low in viability and vigour, but this was generally related to the extent of mechanical damage to the seed coat. Seed weight was not related to seed vigour.

Seed deterioration during storage was associated with increasing conductivity, abnormal seedlings and dead seed content, and decreasing germination rate, normal seedlings, and field emergence. Vigour was lost before viability. The deterioration rate was quicker at high RH (75 and 90% RH), since the seeds were quickly invaded by storage fungi.

The present experimental results strongly suggest that standard germination was a poor predictor of seed performance, both in the field and during storage. Both accelerated aging and controlled deterioration seem promising techniques for determining vigour of red clover seed lots. The further development of these vigour tests is discussed.

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CHAPTER 1

INTRODUCTION

Red clover is a valuable legume plant, widely distributed through the temperate zones of both hemispheres, for pasture, hay, and silage, for soil improvement, and for use in cropping rotations (Fergus and Hollowell, 1960). Seed viability in this species is usually high and once hard seed has been broken through scarification, germination values of around 90% are common (Hampton, unpub. data). However, seed field emergence and establishment may be poor or variable (e.g. Whitcomb, 1924), particularly under adverse field conditions such as has been reported (Hampton et al., 1987) in New Zealand unploughable hill country. There have been some reports of vigour differences between seed lots of red clover (e.g., Delouche and Baskin, 1973; Hampton , 1987; and Helmer as cited in Helmer et al., 1962). However, whether such vigour differences affect seed performance, and whether the vigour test can efficiently predict seed field performance has not been reported.

Seed lot germination results provide information about seed planting value under optimum field conditions, but in fact field conditions are often far from optimum. For this reason the concept of vigour has been accepted, and subsequently many vigour tests have been developed and proposed. However, test methods are based on different principles, and have different merits and limitations for practical application under certain environments and for a given species. Therefore, the critical evaluation of vigour methods has been given increasing attention. A number of agricultural and horticultural species have been used in these studies, and the work of standardizing some vigour test methods is being conducted by ISTA and AOSA (e.g., Heydecker, 1968; Perry, 1978; 1981; Tao, 1980a; b). However, seed vigour research on herbage crops has received little attention.

Among the many different vigour testing methods, accelerated aging, controlled deterioration, conductivity and speed of germination tests have the advantages of being relatively quick, easy to conduct in a seed laboratory, and most importantly show

good reproducibility in some crops and often high correlations with field emergence (AOSA, 1983; ISTA, 1987).

Perry (in ISTA, 1987) stated that : 'A vigour test should provide a reproducible result which is more closely correlated with seed performance in the field under some conditions than the germination test. It is clearly important that any vigour test can be only evaluated by comparing it's result with the results obtained from standard germination and field performance. It is also very important that the test should be conducted using a number of seed lots which are similar in laboratory germination, and under a range of field conditions.

Seed field performance and seed storability are two important end results of seed quality. Seed vigour can encompass potential seed performance both in the field and in storage (Hampton and Coolbear, 1989). Seed vigour properly evaluated, therefore, is not only a measure of the capacity of seed to survive and emerge under adverse field conditions, but also a measure of the storability of seed (Helmer and Delouche, 1962). Studies of vigour tests which relate to seed storability are not as extensive as those which relate to field emergence, but several kinds of vigour test methods have been experimentally used in studies of seed lot storability and seed deterioration. For instance, the seedling growth and evaluation test (Delouche and Baskin, 1973; Egli et al., 1979), controlled deterioration test (Powell and Matthews, 1984 a; b), accelerated aging test (Byrd and Delouche, 1971; Baskin et al., 1980), cold test (Grabe, 1965; Delouche and Baskin, 1973) and conductivity test (Helmer et al., 1962; Ching, 1972). Among the tests used, the accelerated aging test has usually been more applicable and effective than the others (Delouche and Baskin, 1973; Egli et al., 1979), but others have also been valuable in some cases (Byrd and Delouche, 1971). The controlled deterioration test has been used to successfully predict seed lot storability in a number of small seeded legumes (Powell and Matthews, 1984a; b), but as yet the test has not been reported for small seeded herbage legumes.

Many factors may cause variation in seed vigour. The seed weight, physiological age, mechanical integrity and microorganism contamination are some of the commonly known factors listed by ISTA (1987). Other characteristics of seed e.g., imbibition rate (Nabeesa, et al., 1988) have also been considered important factors to indicate initial seed quality in small seeded legumes.

The general objectives of this study were:

(1) to confirm the existence of vigour differences in several small seeded herbage legumes, and particularly red clover ;

(2) to assess the suitability of several vigour testing methods for predicting field performance under a range of field conditions;

(3) to evaluate these vigour test methods for predicting the relative storability of seed lots under simulated temperate storage conditions;

(4) to study some aspects of the sequence of changes in seed deterioration during seed storage;

(5) to quantify some of the physical characters of seed differences between seed lots that might account for differences in quality, with particular attention being paid to seed weight, mechanical damage, seed coat permeability and storage fungi.

CHAPTER 2

REVIEW OF LITERATURE

2.1 Seed vigour and vigour testing

2.1.1 Current concepts of seed vigour

Currently, there are two concepts of seed vigour which are adopted internationally. One was proposed by The Vigour Test Committee of the International Seed Testing Association (ISTA) in 1977 (Perry, 1978), and another by The Vigour Testing Committee of the Association of Official Seed Analysts (AOSA) in 1980 (McDonald, 1980).

2.1.1.1 ISTA concept of vigour (Perry, 1978)

" Seed vigour is the sum total of those properties of seed which determine the level of activity and performance of the seed or seed lot during germination and seedling emergence. Seeds which perform well are termed high vigour seeds and those which perform poorly are called low vigour seeds ".

The aspects of performance which may show variation associated with differences in seed vigour include:

" (1) biochemical processes and reactions during germination such as enzyme reaction and respiratory activity,

(2) rate and uniformity of seed germination and seedling growth,

(3) rate and uniformity of seedling emergence and growth,

(4) emergence ability of seeds under unfavorable environmental conditions.

The effect of vigour level may persist to influence mature plant growth, crop uniformity and yield. .....".

2.1.1.2 AOSA concept of vigour (McDonald, 1980)

" 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 ".

2.1.1.3 Discussion of the two concepts

Both concepts place emphasis on rapid and uniform seedling emergence under a range of field conditions. Undoubtedly, this quality aspect is very important in agricultural practice. In addition, they indicate that the only reliable way to assess vigour test methods is to make a comparision with field emergence under a range of field conditions. This comparison is very important, particularly as most of the techniques of vigour testing are still at the developmental stage.

There are dissimilarities between the two concepts. The ISTA concept is more comprehensive and much longer, and perhaps more academic than operational. In contrast, the AOSA concept is more direct, short and operational. However, as mentioned in the AOSA Vigour Test Handbook (1983) "there is no case for selecting one definition over the other; all that can be said at this time is that AOSA definition may be less ambitious than the ISTA definition".

2.1.2 Factors influencing vigour potential

Many factors are considered to influence the level of seed vigour (ISTA, 1987). They can be grouped as genetic effects, conditions during seed development, techniques of seed harvesting and processing, and deterioration during subsequent seed storage (aging).

2.1.2.1 Genetic effects

The vigour potential inherent in seeds such as the ability to germinate and rate of germination or emergence, is partly controlled by the genotype. For example, Kooistra (1971) demonstrated that there were differences in germination ability between cultivars of bean (Phaseolus vulgaris L.) especially at soil temperatures below 15 °C; Ching and Kronstad (1972) in a comparative study on two cultivars of wheat (Triticum aestivum L.) reported that seed weight, embryo weight and rate of radicle emergence did not differ, but shoots of one cultivar were at least 30% longer than another after 10 days growth.

2.1.2.2 Conditions during seed development

Conditions during seed development such as the environment and the nutrition of the mother plant are key factors causing variation in vigour. Heydecker and Walter (1965) stated that environmental conditions may influence the degree to which the seed's potential can be reached. There are two approaches to the study of this aspect of seed vigour:

1) Manipulating the seed crop environment and studying the effects on the seed produced

In growth chamber experiments, Bulisani and Warner (1980) found that the addition of nitrogen significantly increased both the seed nitrogen content and subsequent seedling vigour in wheat. In the same crop, similar results were also obtained by other authors (e.g. Holzman, 1972 as cited in Perry, 1976;), Holzman (as cited in Perry ,1972) further demonstrated that seedling growth differences were related mainly to embryo weight.

In glasshouse studies of the effect of nutrition of the mother plant on seed yield and seed vigour in pea (Pisum sativum L.), Hadavizadeh and George (1988) showed that increasing the nitrogen supply increased seed dry weight. However, an increase in seed vigour as determined by the seed leachate conductivity was only achieved by the interaction of high nitrogen (i.e. 1000 mg per plant) with medium phosphorus supply (i.e. 250 to 500 mg per plant). Potassium nutrition alone was found to have very little effect on yield and vigour. However, Browning and George (1981) demonstrated reduced pea seed vigour following a high nitrogen regime (i.e.800 and 1050 mg N per plant), due to bleaching of the cotyledons, and the low vigour of seed produced under low nitrogen and high phosphorus (i.e. 450 mg N, 255 mg P and 450 mg N, 422 mg P per plant) conditions as a result of a high incidence of hollow heart.

Seed vigour may also be affected by soil and climatic conditions. For example, air temperature and soil moisture regimes during seed production not only affected seed yield, size and germination but also influenced the vigour of subsequent seedlings in alfalfa (Walter and Jensen, 1970); high air temperatures and hot sunny weather during seed ripening and maturation can induce low vigour in peas (Browning and George,

1981). Rainfall has been reported to influence the vigour of seed, not only by fungal infection, (Flentje, 1964), but also by physical stresses set up by cycles of swelling and contracting under changing moisture content (Moore, 1965). In an experiment which examined the effects of weeds on seed vigour, Liptay and Friesen, (1982) showed that tomato plants grown in weedy conditions produced a significantly greater percentage of seeds with high vigour than those grown in weed free conditions.

2) Analyzing seeds and relating differences in seed characteristics to differences in seed performance

a) Chemical composition

Lowe et al. (1972) found that seedling dry weight of wheat grown in the laboratory showed a high positive correlation with seed protein content, and analysis of amino acids in seed hydrolyates indicated that glutamic acid was positively related to seedling growth. The yield in the field was found to be positively correlated with both seed protein and glutamic acid. However, Welch (1977) reported that high protein seeds in the greenhouse produced larger plants than low protein seeds initially, but later the differences were lost.

b) Seed size (or weight)

Seed size or weight is the most obvious of the several characteristics which are influenced by the environment during seed development; the large seeds presumably have larger quantities of stored reserves available for seedling growth. Differences in size commonly exist within any lot or between lots of the same cultivar, and such differences can be detected readily and easily; therefore, much attention has been paid to the study of seed size as it relates to seed vigour. Some of this work is summarized in Table 2.1. The majority of the studies showed that a positive relationship exists between seed size and seed field performance. For instance, larger seed produced greater plant yield (Smith and Camper, 1975; Smittle 1979; Smittle and Williamson, 1977), larger seed size (Smith and Camper, 1975) and higher emergence percentage (Stickler and Wassom, 1963; Taylor, 1972). However, some reports showed little or even negative relationships to seed performance (Table 2.1). It seems that no universal benefit can be obtained from sowing large seeds. This may be due to the fact that most of this research was conducted by separation and comparison of the size among different cultivars or seedlots, so that the effects of other factors, for example pathogens, aging, mechanical damage, may be different between individual lots and so might obscure the effects of seed size on vigour.

Furthermore, separation of seed size within seed lots where the individual seeds have a common background, may provide a greater possibility to predict seed vigour than comparisons between seedlots or cultivars. McKersie et al. (1981) reported that the large seed from within bird's-foot trefoil (Lotus corniculatus L.) lots tended to have better field establishment than the original lots. Evans (1973) presents clear evidence that the heaviest fraction of seeds from within seed lots each of perennial ryegrass (Lolium perenne) and red clover produced seedlings of greater root weight and length and shoot weight than plants from lighter seeds. Similar results were also found in Italian ryegrass (Lolium multiflorum L.) (Hampton, 1986).

Table 2-1 Effects of seed size on field performance in large and small seeded legumes

Kind of seed	Effect	Reference
	Positive effect on	
Glycine max	Seed size, plant height and yield.	Smith & Camper, 1975
Glycine max	Grain yield per plot and plant.	El-Zahab et al as cited in McDonald, 1975
Lotus corniculatus	Emergence.	Stickler & Wassom, 1963
Lotus corniculatus	Plant height and dry matter.	Henson et al. 1961
Lotus corniculatus	Establishment within lot.	McKersie et al. 1981
Melilotus officinalis	Plant height and weight.	Haskins & Gorz, 1975
Phaseolus lunatus	Plant yield.	Smittle, 1979
Phaseolus vulgaris	Plant yield.	Smittle & Williamson, 1977
Trifolium subterrannrum	Emergence.	Taylor, 1972
Vicia faba	Plant yield.	Zeyada et al. as cited in Perry,1980
	No effect on	
Astragalus falatus	Emergence.	Townsend, 1972
Glycine max	Emergence.	Smith & Camper, 1975
Glycine max	Emergence, yield.	Johnson & Luedders, 1974
Lotus corniculatus	Establishment between lots,	McKersie et al., 1981
Melilotus officinalis	Emergence.	Haskins & Gorz, 1975
	Negative effect on	
Trifolium subterranneum	Plant size, growth rate,	Taylor, 1972

c) Seed colour

Several authors have shown that colour sorting is a very effective procedure for determining seed germination potential in small seeded legumes, Much of this work was concerned with natural colour of seeds rather than with colour changes during storage. Eastman (1912) attributed differences in red clover seed coat colour to difference in maturity, while Williams (1930) (cited by Dalianis, 1980), stated that colour was influenced by both genetic and environmental factors.

Dymond (1921) found that the weight of purple seeds of red clover exceeded that of yellow seeds. Intermediate colour seeds averaged about the same weight as purple seed. Smith (1940) and Liberal at al. (1975) also reported that dark coloured seeds were heaviest in alsike (T. hybridum), red and white clovers.

Dymond (1921) demonstrated that yellow seeds gave a higher germination percentage than other seeds. Vaugham (1962) as cited in Dalianis, 1980) separated dark brown seeds from crimson (T.incarnatum L.), white and red clovers and found that germination percentages of dark brown seeds were as much as 50% less than those of the lighter coloured seeds. West and Harris (1963) reported essentially the same results on colour separations of alfalfa, crimson and white clover seeds. They also found that water extracts from dark alfalfa (Medicago sativa L.) seeds inhibited germination of yellow seeds and reduced root growth of the seedlings.

2.1.2.3 Techniques of seed harvesting and processing

Improper techniques during harvesting,threshing, transporting, drying and other processing can cause lower quality seed; for example seed lots with high percentages of immature, over-mature, and mechanically damaged seeds.

1) Immature and over-mature seed

It is very important for the seed producer to determine the correct method and time of harvesting in order to produce seeds of maximum quality. Premature harvesting of immature seed, sometimes followed by artificial or natural drying at high temperature may cause low vigour in peas (Harrison, 1971; Myers, 1948; Perry and Howell, 1965).

Over-maturity and /or deterioration may occur on the plants before harvest. For instance, low vigour in lima bean (Phaseolus lunatus) was caused by bleaching when the seeds were exposed to strong sunlight before harvest (Pollock and Toole, 1964), and a reduction in vigour due to a delay in harvest was also found in carrot (Daucus carota L.) seed (Austin and Longden, 1968).

2) Mechanically damaged seed

Powell et al. (1984b) state that there are three processes during which the handling of grain legumes has been shown to result in mechanical damage to the seed: the harvesting and processing stages of seed production, and sowing. The damage can be internal and/or external damage.

Mechanically damaged seeds are usually associated with a high incidence of abnormal seedlings leading to a low percentage of germinated seeds. For example, Link et al. (1984) reported that even slight damage significantly reduced germination percentage and increased abnormal seedlings under laboratory and field conditions. Mason et al. (1982) also found that the percentage of broken seed coats of soybean seed was reciprocal with germination and seedling emergence.

Damaged seed may influence seed vigour. For instance, damaged seeds produced a higher level of solute leakage (Oliveira et al., 1984; Powell and Matthews, 1979), reduced seedling growth in the laboratory (Barriga, 1961), lowered final field emergence (Mason et al., 1982) and affected mature plant performance (Abdalla and Roberts, 1969).

Damaged seed is also a frequence cause of poor quality during storage (Section 2.2.2.1).

Damaged seed is prone to injury during the early stages of imbibition. Powell et al. (1984b) demonstrated that low vigour seed lots included a large proportion of seeds with damage to the testa. These seeds imbibe rapidly, leading to dead tissue on the cotyledons, and leakage. In contrast, there is little damage in high vigour lots which imbibe relatively slowly.

2 1.2.4 Deterioration during seed storage (or aging)

Maximum seed quality occurs at physiological maturity after which vigour and viability decline, both during aging on the plant (Delouche, 1980; Powell et al., 1984b) and during storage (Powell et al., 1984b; Roos, 1980). Seed aging has come to be recognized as the major cause of reduced vigour and storability. Aging involves the process of deterioration, that is the accumulation of irreversible degeneration changes until eventually the ability to germinate is lost (Powell, 1988). The rate of deterioration is dependent on many factors, e.g. storage environment and initial seed quality (see section 2.2.2).

Many changes in metabolism which occur during seed aging are frequently referred to as biochemical changes. These involve changes in solute leakage, enzyme activity, respiration and TAP content, protein and DNA synthesis, the chemical content of seeds and genetic changes (Powell, 1988). Along with these biochemical changes, there are also a number of physiological symptoms and alterations of seed which include reduced rates of germination and emergence, decreased tolerance to suboptimal conditions and poorer seedling growth (Powell et al., 1984b), resulting in the induction and loss of dormancy, changes in seed germination requirements, reduced seedling growth, and the production of abnormal seedlings (Roos, 1980).

2.1.3 Methods of vigour testing

2.1.3.1 Requirements of a testing method

A vigour test is a reproducible laboratory method which distinguishes seeds of different level of vigour and which produces results which should be more closely correlated with seed performance in the field under some conditions than the germination test (Perry, in ISTA 1987). Because seed vigour is not a single property like germination, the events which precede loss of germination and could be associated with seed vigour have been widely measured and developed into different tests. To be practicable and acceptable, a vigour test must meet certain requirements (AOSA, 1983 and ISTA 1987). Hampton and Coolbear (1989) reviewed the previous literature and offered the following requirements of vigour testing:

1) to provide a more sensitive index of seed quality than the germination test
2) to provide a consistent ranking of seed lots in terms of potential performance
3) to be objective, rapid, simple and economically practical

4) to be reproducible and interpretable

2.1.3.2 Characterestics of each type of method

The various vigour test methods can generally be separated into the following groups, based on their main characteristics; either the parameters of seed measured, component of seed vigour monitored or the experimental conditions used:

(1) seedling growth and evaluation tests
(2) stress tests
(3) biochemical tests
(4) physical tests
(5) multiple vigour index

Most of the tests in category (1) (2) and (3) are as recommended in the Vigour Handbooks of AOSA (1983) and ISTA (1987).

1) Seedling growth and evaluation tests

The tests belonging to this group are seedling growth, seedling evaluation and speed of germination tests. These tests are conducted under the same conditions as the standard germination test but seedling growth and seedling evaluation are measured in different ways (AOSA, 1983). The basis for these tests is that the ability for rapid and uniform germination of seeds, and seedling growth in the laboratory, are the main aspects of recording of seed vigour.

Seedling growth and evaluation tests have important advantages for seed testing organizations, in that they are inexpensive, relatively rapid, require no specialized equipment, and do not necessitate additional technical training. However, they also exihibit specific limitations e.g variables such as moisture and temperature are difficult to standardize among laboratories.

2) Stress tests

Usually field conditions are less than optimum when seeds are sown. Under suboptimum or stress conditions, high vigour seeds have a greater potential for emergence and establishment. Therefore, the stress tests are those which simulate certain stress conditions which seeds may encounter in the field (AOSA,1983). These stress conditions include high temperature and relative humidity (e.g. accelerated aging

and controlled deterioration tests), low temperature (e.g. cold and cool germination tests), osmotic stress (Osmotic stress), brick grit stress (Hiltner test) and complex field stress which involves oxygen deficiency, imbibition damage and low temperature chilling (CSVT). In these tests, seeds are stressed either prior to imbibition or during germination. However, seed germination remains the criterion for evaluation.

The stress tests are easily within the competence of seed technologists since germination is assessed. The main limitation is that the stresses in the laboratory are to some extent different from those in the field.

3) Biochemical tests

Radicle protrusion and seedling growth during seed germination are the end results of biochemical changes. The level of metabolic activity determines seedling growth rate, and therefore, biochemical tests measure certain metabolic events in seeds associated with germination. The events measured are mainly membrane degradation (conductivity test), enzyme activities (tetrazolium and glutamic acid decarboxylase activity tests), respiration, energy and synthesis mechanisms impaired (i.e. ATP content test).

Biochemical tests offer the advantages of requiring less testing time , but they generally necessitate more specialized equipment and training than other methods.

4) Physical tests

Some seed physical characteristics may be considered as part of the causes of variation in seed vigour. These measurements such as seed size, weight, colour, hollow heart, and mechanical integrity (x-ray test), are generally quick and inexpensive, can be conducted on a large scale, and also have value for agricultural practices e.g in crop breeding and management. However, for seed vigour testing, each of these methods can only measure part of the causes of differences in vigour potential. Therefore physical tests were not accepted by ISTA and AOSA as main vigour tests methods (AOSA, 1983; ISTA 1987).

5) Multiple vigour index

Since vigour comprises many properties of seed, any single test is unlikely to be measuring all the potential of vigour. Mainly for that reason, multiple vigour indeces which may combine two or more test parameters have been proposed (e.g. Barla-Szabo and Dolinka, 1988; Scott and Close, 1976; TeKrony and Egli, 1977). The limited published information has shown that multiple vigour indeces for predicting field emergence are often better than the standard germination test and single vigour tests (Barla-Szabo and Dolinka, 1988; Scott and Close, 1976; TeKrony and Egli, 1977). However, these tests need further evaluation with different cultivars under different planting conditions (Hampton and Coolbear, 1989)..

Overall, each type of vigour test and even each individual test has its own principle or basis for measuring vigour, and has different merits and limitations for practical use. Different methods may also have different suitabilities for testing certain species and assessing performance under specific environments. Therefore, the evaluation of vigour test methods has received much attention. For example, in attempts to standardize vigour tests, ISTA and AOSA have organized global and regional vigour referee (collaborative) tests. Results have been published (e.g. Fiala, 1987; Heydecker, 1969; Perry, 1978; 1981; Tao, 1980a; b; TeKrony, 1987a; b)) and partly summarized in the Handbooks (AOSA, 1983; ISTA, 1987).

2.1.3.3 Some individual vigour testing methods

Among the various testing methods, some have been extensively studied, have shown high ability for predicting potential field emergence and are reasonably consistently reproducible. For instance, the conductivity test for some large seeded legumes and accelerated aging test for soybean have been proposed as recommended standard methods in the new AOSA Handbook (TeKrony, 1987a, b). It was also suggested by Hampton and Coolbear (1989) that conductivity testing, controlled deterioration and possibly the combined stress tests seem to be the most promising areas for continued study. In addition, germination speed tests have been widely used for vigour testing, and some physical tests may indicate the cause of variation in vigour between seed lots. Therefore, these tests will be individually reviewed in the next section. The review of each test generally includes: the principle of the methodology, how test results are related to field performance, advantages and limitations, and some key precautions which should be taken during the test.

1) Electrical conductivity test

The basis of conductivity as a vigour test is that poor membrane structure and leaky cells are usually associated with deteriorating, low vigour seed (AOSA, 1983). This results in a greater loss of electrolytes such as amino acids and organic acids from imbibing seeds which increase the conductivity of the soak water. A higher soak water conductivity, therefore, may indicate a low vigour seed lot (AOSA, 1983).

The conductivity test has been shown to correlate well with vigour in seeds of peas (cited in ISTA, 1987), rice (Agrawal, 1977), corn (Gill and Delouche, 1973; Tao, 1980a, 1980b), bean (Matthews and Bradnock, 1967), soybean, barley , crimson clover and ryegrass (cited in AOSA, 1983). However, a few results have also shown that the conductivity test for predicting field emergence was not as good as the standard germination test , e.g. in Chickpea (Cicer arietinum L.) (Ram. et al., 1989). It was also reported that the test result expressed as a function of seed weight in bird's-foot trefoil was significantly correlated with seedling length and field establishment, whereas when it was related to seed number it was not (McKersie et al., 1981). A 4 h soaking time gave results consistent with the 24 h soak in soybean (Brouwer and Mulder, 1982).

The conductivity test has the great advantage for seed testing laboratory work of being simple, quick, and reproducible. The conductivity of the water in which a certain quantity of seeds has been soaked for a specific time depends on the leaching of electrolytes from the seed, which itself indicates the physiological stability of the seed cell membrane. The equipment, a conductivity bridge, is relatively cheap and its handling should present no difficulty to the analysts.

The limitation of the present conductivity test is that it expresses results as an average conductivity evaluation for a certain number of seeds. However, each individual seed has its own unique potential to perform in the field. Conductivity tests, therefore, may better reflect the vigour capability of a seed lot if they were presented on an individual seed basis. A commercial instrument is now available which monitors the electrolyte leakage of individual seeds. Some studies suggest that this instrument provides a more accurate appraisal of seed vigour (AOSA, 1983) but it is not yet widely available. There is still debate about the supposed advantages, because conductivity results are best expressed on a per unit weight basis, and therefore in theory, each seed should be individually weighed (Hampton, personal communication).

Several precautions should be taken in this test:

a) Initial seed moisture content may affect the result. It was reported that seeds with a high initial moisture content e.g 30% or more for pea, produced low conductivity readings (Simon and Wiebe, 1975), while seeds with a low moisture content, such as 7.2% for soybean, produced significantly higher readings (Pollock, et al. 1969, Tao, 1978b). Therefore, it was suggested that seed lot seed moisture prior to conductivity measurement should be between 10-14% (AOSA, 1983).

b) The use of mechanically injured seeds may result in high conductivity results (Tao, 1980c). With the removal of injured seeds, the reproducibility of test results was improved in the 1979 and 1980 AOSA referee's tests (Tao, 1980a, and 1980b). However, this tends to be less promising in a practical sense, since firstly, the damaged seeds are usually not removed when seeds are sown or stored; secondly, if damaged and undamaged seed has to be measured separately, the test procedure becomes very complicated unless the individual seed measuring instrument is used.

c) Seeds with a high percentage of microorganisms, could significantly lower the conductivity since microorganisms use the electrolytes as a nutrients source (Tao et al., 1980). In this case fungicide treatment e.g Captan prior soaking is required. However, chemical and fungicide seed treatments can also affect test results (Tao, 1980a). This effect can be eliminated by an acetone wash which has no effect on other test results (Tao, 1980a).

d) The temperature during soaking and recording should be as constant as possible as small variations in temperature may result in big variations in results (AOSA,1983).

2) Accelerated aging (AA) test

The basis for the AA test is that high vigour seeds tolerate the high temperature and high humidity of the treatment and thus retain their capability to produce normal seedlings in the germination test.

In the AA test, seeds are stressed prior to the germination test by being placed at a high temperature (40-45 oC) and high humidity (nearly 100%)was recommended, for varying length of time, depending on the kind of seeds (ISTA, 1987). The methods of

'plastic box' (tray) and 'sealed jar' are commonly suggested to be used in the AA test (AOSA, 1983; ISTA, 1987).

At present, the AA test is used primarily for soybeans. It has been extensively researched and put through many referee tests (Fiala, 1987; Perry,1981; Clark, 1980; Tao, 1980a; TeKrony, 1985). The results have been highly correlated to field emergence and are very repeatable in most cases. The use of the AA test for crops other than soybeans is also under investigation. For example, predicting storability in some small seeded legumes (Delouche and Baskin, 1973) ,and the referee test for corn (McDonald, 1980).

The AA test offers the advantages of being inexpensive, relatively easy, and meaningful for predicting seed potential performance both in the field and in storage. However, the test has some limitations, particularly because under high temperature and humidity conditions, the seeds may absorb water at a different rate during the initial period of aging, which will lead to differences in the extent of aging among tested samples.

The following precautions should be taken to reduce variability in the tests:
a) The range of percentage of initial moisture content among seed samples should not be very big. It was reported that soybean seed with lower initial moisture content (7%) failed to reach the same moisture content at the end of the AA period as compared to seeds with a higher initial moisture content (13%) (McDonald, 1977). However, it was found that no significant difference in germination occurred between soybean samples with moisture contents of 13% and 8.8% after 3 days of aging (Tao, 1979). These moisture content ranges may occur routinely in a seed laboratory.

b) The results between different methods, e.g. 'plastic box' and sealed jar' can not be compared with each other. For instance, significant differences in final seed moisture content and germination percentage occured between the different methods (Tao, 1979).

c) With both the sealed jar and plastic box method, the distance between the seeds and water surface, and the size of the container (boxes or jars) should not differ. It was found (Tao,1979; 1980a) that the higher the seed sample is located from the water surface, the higher the vigour of the seedlings obtained, and placing multi- samples in one container could result in variation among the samples.

d) Accurate temperature and time control is also very necessary for test repeatability. Delouche and Baskin (1973) suggested that the temperature should be controlled with a tolerance no greater than $\pm$ 0.5 ^{o}C, and that the test duration must be vigorously adhered to.

e) All samples should be either treated or untreated with fungicides and results should not be interspersed because the treatment may affect the test result (ISTA, 1987).

3) Controlled deterioration (CD) test

The basis of the CD test is an aging technique similar to the accelerated aging test. The main difference is that in the CD test the initial seed moisture content is raised to the same level for all lots prior to the period of deterioration at high temperature. A temperature of 40-45 ^{o}C (ISTA, 1987) and seed moisture contents from 16 to 30% (ISTA, 1987; Marshall and Naylor, 1985) have been used in previous studies.

The CD test now is mainly used in UK as a vigour test. With a number of small seeded vegetables, the test result has been found to correlate well with field emergence (Powell and Matthews,1981; 1985); to be highly repeatable both within one laboratory and between laboratories (Powell et al, 1984a). and also to be a good indicator for predicting seed storage potential both under good and poor storage conditions (Powell and Matthews, 1984a, b). However it was reported that emergence of Italian ryegrass (Lolium multiflorum Lam.) from soil at moderate temperature could be predicted by both standard germination and CD tests, but not in a soil under a cool or a hot temperature regime (Naylor and Syversen, 1988).

The test method has important advantages:
a) good control of seed moisture content during the aging period.

b) it is reasonably simple and inexpensive, no additional analyst training is required, complicated equipment is not needed, and the test requires less aging space in the chamber.

Precautions which should be taken with this test are:
a) accurate control of the test temperature.

b) even mixing of the seed sample with water to ensure that each individual seed within the sample has the same opportunity to absorb water.

c) Radicle appearance as the criterion of germination in the test was proposed in the Vigour Test Handbook (ISTA, 1987). However, it is possible that seeds having good radicle growth may further develop into abnormal seedlings, and as a result, the test may possibly over estimate seed vigour for some lots. Therefore, normal seedlings should be used as the criterion in this test.

4) Speed of germination

The speed of germination test is one of the oldest seed vigour tests, and the seedling growth and evaluation tests could be considered as modifications of this test. The test is based on the concept that more vigorous seeds germinate faster than less vigorous seeds. On a lot basis, individual seeds within a vigorous lot normally germinate in a shorter time span than do those in a non-vigorous lot.

Various specific parameters have been used to measure speed of germination, and some common ones are listed below:

a) First count

The first count of the standard germination test has been used as an index of vigour. The method has been used to provide an estimate of seedling vigour (e.g. Burries et al., 1969; Larsen, 1964). According to McDonald (1975), the method was recommended as an unofficial vigour test by ISTA, but the procedure was later eliminated due to inconsistencies in results between laboratories .

b) Germination rate

$$\text{Germination rate } (x) = \frac{\text{number of normal seedlings}}{\text{days of first count}} + \ldots\ldots + \frac{\text{number of normal seedlings}}{\text{days of final count}}$$

This is generally an adaptation of the daily counting method. Using this method, it has been found the higher the rate, the higher the seed vigour (Maguire, 1962).

The advantages and limitations of the test are the same as mentioned in tests of seedling growth and evaluation.

5) Physical tests

Some seed physical characteristics such as seed weight and mechanical damage can be used to indicate seed quality and vigour (see Section 2.1.2.2).

a) Seed weight

The weight is calculated by weighing seeds and expressing the values as weight per seed number, e.g. thousand seed weight.

b) Mechanical damage

Internal damage can be detected by x-ray radiography (Kamra, 1964) or by tetrazolium staining (Moore, 1972). External damage can be measured by using either visual observation or chemical (e.g. fast green) staining (e.g Peiffer, et al., 1972a; b; Powell and Matthews, 1979).

c) Imbibition rate

Legume seed lots often contain a varying proportion of 'hard' seeds; that is, seeds with impermeable coats. The resulting non-uniformity in water uptake will necessitate special care in sampling of seeds for biochemical analysis during germination, and in interpretation of results (Nabeesa, et al. 1988).

There are two common methods which have been used in determining seed imbibition rate:

First, seed weight basis: Seeds are soaked in water. At predetermined intervals the seeds are removed, blotted dry, and weighed. Changes in weight due to imbibition are expressed in terms of an increase in the percentage moisture content (as a percentage of seed wet weight) (e.g. Powell and Matthews, 1979).

Second, seed number basis: Seeds are placed between moistened, germination blotters. At predetermined intervals seeds that have become swollen are removed ,and the percentage of swollen seeds at each time calculated (e.g. Vaughan et al., 1960). This

method has mainly been used in the small seeded legumes (Fayemi, 1957; Vaughan, 1960).

Fayemi (1957) demonstrated that there was considerable variation in the rate of swelling of individual seeds in small seeded legumes. He found that seeds which were visibly swollen on moist blotters after 6 hours did not germinate. Non-germinability of these seeds was attributed to mechanical damage and disease. Vaughan (1960) also reported that the rate of seed swelling in red clover and crimson clover was closely associated with viability, particularly during the first four hours of the incubation period. Powell and Matthews (1979) further found that seedlots of peas with high rates of water uptake contained a large proportion of seeds with cracked testae which were low in vigour, as indicated by electrolyte leakage and poor field emergence. They also showed that the cracks in the testa could cause cell death by increasing the rate of water uptake.

The rate of seed imbibition is influenced by temperature, the extent of contact with the water film, the seed kind (Brown, 1912; Dungan, 1924; Shull, 1920; cited in Vaughan, 1960), the incidence of cracks in the testa (Fayemi, 1957, Peiffer et al., 1972b) and even the colour of the seed coat. It was reported that dark coloured seed coats are more impermeable to water (Tully et al., 1981).

2.1.4 Vigour and field emergence

2.1.4.1 Relationship between standard germination, vigour test, and field performance

The standard germination test as prescribed in the Rules for Seed Testing should predict the planting value of a seed lot under favorable conditions. However, conditions at sowing and immediately after are frequently sub-optimum and variable, which usually means that, first, the result of the standard germination test often overestimates field emergence. As early as 1924, Whitcomb analyzed the data from ten tests in red clover, and reported that the laboratory standard germination test gave results 38% higher than the field emergence. He considered however that the germination test, in conjunction with the size and nature of the seed, was a good criterion to predict field emergence. Second, the result is largely affected by the field environment. For example, MacKay (1972) illustrated the interaction between establishment and environment in trials with several lots of onion and carrot seed. No

correlation between germination and emergence was found when 12 seed lots were sown deeply in dry soil, but when the seeds were sown less deeply and conditions were more favorable, significant correlations were obtained in two trials with several lots of onion seeds. Third, results are affected by the seed lots tested. Sometimes a positive correlation can be obtained between standard germination and field emergence; however, these relationship are usually attributable to the large difference in the laboratory germination of the seed lots tested. For example, Hegarty (1977) demonstrated a high correlation between laboratory germination and field emergence in field bean with germinations ranging from 73 to 99%. However, when seed lots with poor germinations were excluded, the correlation between standard germination and field emergence was no longer significant. Field environments are often unfavorable, and seed lots to be sown in most cases have a high percentage germination. Therefore, the standard germination test as a predictor for field performance may often not be reliable.

Vigour tests are designed and used to provide information about the expected level of field performance of seed lots under a wide range of field conditions. To be of any use vigour tests must be better indicators of field emergence than the standard germination test (see section 2.1.3). Some experiments have also demonstrated that the vigour of seed may further influence the plant stands (Hampton and Scott, 1982) and seed yield (Roberts, 1986). However, seed vigour is not the only factor determining successful establishment, and many factors in the field may affect seed vigour potential expression (see next section). Vigour tests do not replace the standard germination test, but supplement it by providing more information, especially as to the field planting value (Powell, et al., 1984b).

2.1.4.2 Factors affecting the expression of vigour potential

The factors affecting the formulation of vigour potential have been discussed in section 2.1.2 of this Chapter. The field environment limits the expression of this potential and, moreover, interactions with seed vigour. Many factors associated with the soil may impose limitations on seed establishment:

1) Soil temperature

The physical soil environment is dominated by the controlling factors of temperature and water availability, both of which have optima above and below which the

emergence declines. Temperature controls the rate of germination and the response to it is predictable, if it is assumed that no other factors are limiting. Blacklow (1973) demonstrated that the rate of imbibition in corn was temperature dependent and the subsequent rate of seedling elongation showed a linear relationship with temperature between 10-32 ^{o}C. The observed values for growth in a glasshouse were similar to the field. The rate of emergence of cowpea (Vigna unguiculata), melon (Colocynthis citrullus), tomato (Lycopersicon esculentum) and Amaranthus (Amaranthus caudatus) was delayed by a heat stress of 45 ^{o}C (Onwueme and Adegoroye, 1975) and the effects were most pronounced when seeds were sown deeply. Field emergence in wheat was closely correlated with 5 cm soil temperature (Hampton, 1981).

2) Soil moisture content

Lack of available soil water is a major limiting factor to crop establishment in some parts of the world. Sharma (1973) used solutions of mannitol and polyethylene glycol to demonstrate differential abilities of several pasture grasses and white clover to germinate in drought conditions. The results showed that the rate and total germination of all the species declined with decreasing levels of water potential. The extent of such reduction varied considerably among species and with the type of osmotic medium.

Excessive water levels can be deleterious to emergence in soil; for example, high soil moisture inhibited barley seed lots which were shown to be differentially sensitive (Matthews and Collins, 1973). The sensitivity could be reproduced in laboratory tests and the affected seed lots had a lower oxygen uptake. The effects of excessive soil water were taken to the extreme by Anderson (1974) who studied the tolerance of seed of six tropical crops to submersion during flooding. Some seeds were sensitive and rapidly lost viability, while others were more tolerant.

3) Soil structure

The changes in soil structure leading to the development of soil crusts may affect seed establishment have been recently reviewed (Powell, 1988). Increased crust strength can reduce the emergence of soybean and cowpea (Ranganatha and Satyanarayana, 1979). In cotton the increased impedance presented by the soil crust delayed emergence and reduced both the emergence rate and final emergence (Bilbro and Wanjura, 1982). Futhermore, as the emergence delay increased from 33 to 60 and

Wanjura, 1982). Futhermore, as the emergence delay increased from 33 to 60 and 100%, the final population decreased even further (Nuttall, 1982). The consequences of crusting were also reflected in plant vigour and lint yield of cotton (Wanjura, 1982, cited in Powell, 1988).

4) Sowing depth

Depth of sowing is often critical to successful seedling emergence (Perry, 1976). Deep sowing reduced soybean seed emergence by 40% and cultivar differences in tolerance to deep sowing were associated with the rate of hypocotyl growth in the laboratory germination test (Fehr, 1973). Similarly, carrot seed lots were also differentially sensitive to deep sowing and their ability to survive was closely related to seed weight (Bedford and MacKay. 1973).

5) Plant density or sowing rate

Plant density may affect seed vigour performance after seed emergence. For instance, reduced yields were obtained from both low vigour peas (Hampton and Scott, 1982) and cotton (Bishnoi, 1981 as cited in Powell, 1988), when poor emergence resulted in reduced plant stands. No differences were however, found in the yield from low and high vigour peas (Hampton and Scott,1982) and soybean (Egli et al., 1979) at equal plant densities, but in cotton, high vigour seed gave 21% higher yields than did low vigour seed when grown on an equal stand basis (Bishnoi, 1981, as cited in Powell, 1988). Roberts (1986) showed that in spring wheat the reduction in yield from low vigour seeds is less when plants are grown at high densities than at low densities. He attributed this to the greater competition between plants from more vigorous lots in high population densities compared with the less vigorous lots.

2.2 Seed longevity and its prediction

2.2.1 Seed longevity in red clover

According to Harrington (in Kozlowski, 1972), red clover is a species with long-lived seeds (10 years or more). However, the life span for any given species is largely dependent on the storage conditions. Longevity in red clover seeds will be reviewed below in order to understand: (1) the longevity under specific conditions; (2) the factors influencing the longevity, and (3) the storage studies which have been done in this species. The literature includes that either published or as cited in other publications in English since the 1950' s, and excluding that in which the investigation was conducted under accelerated aging or soil burial conditions.

Under open storage in laboratory conditions, the viability (% germination) of red clover seed started to decline after 1 year (Evans, 1957) and 3 years (Davies, 1956), decreasing to less than 50% in 5 years (Davies, 1956, Evans, 1957), to less than 10% in 7 years (Davies, 1956), and the seeds were all dead within 15 years (Davies, 1978).

In a humid climate such as is found in Britain, it is unsafe to hold red clover seeds for more than 1 year, e.g., an initial viability of 98% dropped to 27% after 1 year storage in an open farm store (Evans, 1957).

Seeds stored in vacuumn sealed tubes and in nitrogen were all dead in 5 years under laboratory temperatures (Davies, 1956); the same sealed seeds, however, maintained near 70% viability for 27 years under low temperature (0 ^{o}C) (Davies, 1978).

Seeds with an initial viability of 93% were dried to different seed moisture contents (SMC) and stored in sealed polyethylene containers in a basement by MacKay and Flood (1969). They demonstrated that seeds with a SMC of 19% were nearly all dead after 8 months. The time for seed viability to decline to less than 50% in the other treatments was: 12 months at 16% SMC, 27 months at 12% SMC, 36 months at 11% SMC and 66 months at 9% SMC. However, seeds at 6% SMC maintained a viability of more than 80% for 5 years. In the same experiment they also demonstrated that the longevity of seeds with the same initial moisture was also affected by the different initial seed viabilities and packaging materials.

A combination of low moisture content (8.1%), cold storage (0-5 ^{o}C) and replacement of air by CO_2 enabled red clover seed to germinate 71% after 23 years' storage and produce vigorous seedlings (Evans,1957).

Seeds stored at low temperature (-15 ^{o}C and 60% RH) maintained their original germination (96%) over a storage period of 16 years (Rincker, 1980).

Seed samples stored under ambient conditions in two museums have had a germination of 3% after 81 years (Turner, 1933, as cited in Justice and Bass, 1978), and 100 years (Youngman, 1951, as cited in Evans, 1957) respectively. However, viability information before final testing seems not to have been recorded.

Overall a number of factors which may affect red clover seed longevity have been investigated, i.e.,seed moisture content, storage temperature, relative humidity, atmospheric gases and packaging materials. Each of them play an important role in determining seed longevity (see section 2.2.2). The experiments conducted were not only on the same seed under different storage conditions, but also on seed with different initial quality (e.g. viability and moisture content) under the same conditions. However, the effect of seeds with similar initial viability and moisture content but different vigour on seed longevity has not been reported.

2.2.2 Factors affecting longevity

Seed longevity is determined by the initial seed quality before storage, the environment, pests and management during storage. These factors have been discussed by Bass et al. (in Hanson, 1988), Brett (1952), Evans (1952), Justice and Bass (1978), Kozlowski (1972), Owen (1956) and Roberts (1973). The effects of the initial seed quality, storage environment and storage fungi on seed longevity, particularly on red clover and some small seeded legumes will be reviewed:

2.2.2.1 Seed initial quality

The initial quality of seeds determines their potential longevity under any storage conditions (Roberts and Black, 1989). Quality factors include:

1) Genetic effects

The longevity of seed of different plant species varies widely under identical storage conditions. The life span of some aquatic and woody plants is only a few weeks, whereas seeds of some legume species retain viability for over a hundred years. The principles prevailing at the species level are also effective at the cultivar level (Justice and Bass 1978). Differences in longevity between red clover and some closely related species have been reported. For example, seeds of red clover stored under laboratory temperatures were all dead within 15 years while those of white clover took 27 years. Red clover seeds were also shorter-lived than white clover under lower temperature and sealed storage conditions (Davies, 1978). The longevity of subterranean, crimson and berseem clover is less than red clover (Justice and Bass, 1978). Information on the longevity between different cultivars of red clover has not been reported.

2) Hard seed

Red clover is one of several species which can be expected to contain hard seed. Martin (1945) found that most red clover seed obtained from 13 growers in Iowa USA contained a considerable number of hard seeds, varying from 0-35%. Scott and Hampton (1985) reported that 84.7% of certified red clover seed lots in New Zealand contained a hard seed content of 10% or less, 14.7% of lots had a hard seed content of 10-19% and 0.6 % of lots had more than 20% hard seed.

Hard seed can develop under certain environmental conditions during seed production. Rolston reported that seed from lower altitude, or from sites with lower RH and higher temperature, tend to produce high levels of hard seed (Rolston, 1978). However, Cavazza (1950 cited in Evans,1957) maintained that hard seed in red clover was for the most part due to premature ripening under conditions of drought. The percentage of impermeable seeds usually increases as the stage of maturity increases (Harrington, 1915,; Lute, 1928; cited in Justice and Bass, 1978; and Rolston, 1978), and drier storage conditions tend to produce a higher hard seed content (Evans, 1957, Rolston, 1978). However when red clover seed was dried to a moisture content of 4% there was no significant effect on percentage germination or the proportion of hard seed (Vlk, 1986). Varying percentages of hard seed of most species become permeable during over winter storage in the temperate zone and softening depends largely on temperature and relative humidity (Justice and Bass, 1978). Harrington (1916) reported nearly all hard seeds of alsike clover, white clover, and sweetclover in dry storage

remained impermeable for at least 2 or 3 years. Impermeable red clover seeds gradually become permeable, but 30 to 65% of the hard seeds remained impermeable for 4 years or longer.

Although Justice and Bass (1978) stated that in many species, hard impermeable seeds have a longer lifespan than permeable seeds, information on how hard seeds are directly related to seed longevity is limited.

3) Mechanical damage

Mechanical damage of seeds might not only reduce seed viability and vigour (see section 2.1.2.3) but also affect storability. Damaged seeds are easily invaded by micro-organisms and are less able to tolerate stress storage conditions, so that deterioration in these seeds will be quicker than in undamaged seeds (Hill and Johnston, 1985). Powell and Matthews, (1979) found that scarification of the testa reduced vital staining and increased leakage of solutes from the cotyledons as the aging period increased. Broken seeds are also more readily attacked by certain types of insect pests (Hill, 1988). Seed coat cracking induced by too rapid drying has a marked effect on seed storability (Escasinas (1986, as cited in Coolbear, 1988b) and the higher the percentage damage, the greater the number of abnormal or diseased seedlings produced (Effmamn, 1963, as cited in Hill 1988). In discussing the storage of clover and medic seeds, Brett (1952) demonstrated that scarified seed loses its ability to germinate at a significantly greater rate than does unscarified seed when stored under the same conditions. Moore (1972) pointed out that injuries to vital organs, i.e., various parts of the embryo, become more serious with age than injuries to nonembryonic tissues.

4) Seed moisture content

The moisture content of seeds during storage is the most influential factor affecting their longevity. Harrington's (1960) so-called Rules of Thumb for seed storage state that (1) for seed between 5 and 14% moisture content, the length of time in storage before seed viability significantly declines is doubled for every 1% reduction in seed moisture content, (2) For every 5 ^{o}C reduction in storage temperature, the length of time before seed viability declines significantly is doubled. Justice and Bass (1978) stated that it is very important to harvest mature, relative dry seeds or to reduce the

moisture content soon after harvest. The ways in which moisture affects seed storability may be:

a) Moisture content is the key factor controlling sensitivity to mechanical damage; the higher the moisture content, the more damage may occur, resulting in lower longevity of seeds (Justice and Bass, 1978).

b) Moisture content is usually closely associated with maturity. Immature seeds with high moisture content which are exposed to the beating action of the threshing process may be bruised instead of broken (Kozlowski, 1972). Mature, plump seeds generally store better than immature seeds (Justice and Bass, 1978).

c) High seed moisture content during storage can induce several problems, which were generalized by Harrington (in Kozlowski, 1972) as follows:

Seed moisture above 40-60% -- germination occurs
Seed moisture above 18-20% -- heating may occur
Seed moisture above 12-14% -- fungi grow on and in seed
Seed moisture above 8-9% -- insects becomes active and reproduce

MacKay and Flood (1969) stored red clover seed with different initial moisture contents varying from 6 to 19% sealed in polyethylene bags and showed that the higher the moisture content, the quicker deterioration occured. Evans (1957) concluded that moisture content of red clover seed is the most important single factor governing its longevity.

Although it is very important to reduce seed moisture content to a safe level for storage, it is also necessary to be aware of possible adverse effects of low moisture content. Very dry seed are susceptible to mechanical breakage or fracturing of essential seed parts, which make the seed vulnerable to fungal attack, and reducesstorage potential. The safe moisture content in a given seed kind is determined by the length of storage required and the storage environment provided. A moisture content in red clover of 12% or less is considered as a safe moisture content for packing under commercial storage conditions (Owen, 1956).

5) Viability and vigour

It is generally accepted that seeds of high initial viability often resist unfavorable storage conditions better than similar seeds of low initial viability (Brett, 1952; Byrd and Delouche et al., 1971; Ching et al., 1960). Sometimes even small differences in germination among seed lots can result in big difference in longevity (Ellis et al., 1982). In a study of forage legumes and grasses (Rincker, 1979; 1980), reported that forage yield would not be affected following long subfreezing storage if the seeds were of high viability when initially stored.

Seed lots may be of the same species, variety, chronological age and germination capacity, but yet differ in viability after storage under identical conditions. This is caused by difference in seed vigour (Delouche and Baskin, 1973). The decline in vigour and viability of seeds is sometimes illustrated by the sigmoid survival curves (Justice and Bass, 1978); the viability curve initially gradually decreases, is followed by a sharp decline, and finally a gradual loss. The loss in seed vigour essentially parallels that of viability, but at lower levels. The rate at which seeds will decline in vigour or viability depends on several factors, including genetic constitution of the species or cultivar, condition of the seed, storage conditions, uniformity of seed lot, and storage moulds (Justice and Bass, 1978).

2.2.2.2 Storage environment

Many environmental factors during storage may influence longevity; these include temperature, relative humidity, light, radiation, oxygen and carbon dioxide. Of all these factors, temperature and relative humidity are recognized as being of prime importance.

1) Temperature

Temperature is one of two important factors affecting seed storage life; generally it is considered that the cooler the temperature the more slowly seed viability declines. The role of high temperature in speeding seed deterioration is not quite clear. It is generally assumed that temperature affects the rates of biochemical processes in seeds, and the high seed respiration at high temperature is related in some way to rapid loss in germination (Harrington, in Kozlowski, 1972). In red clover, Davies (1956) reported

that seed stored in cold storage maintained germination over 7 years, but at laboratory temperature, red clover began to lose its germinating capacity after three years, decreasing to less than 10% in 5 years.

2) Relative humidity

The main way in which the relative humidity affects seed longevity is through governing seed moisture content. Since seeds are hygroscopic, their moisture content comes to equilibrium with the relative humidity of the atmosphere around them. Seed moisture content at equilibrium with a given relative humidity is dependent on temperature,the species, the proportion between the volume of the air and the seeds, and the hysteresis effect (Harrington, in Kozlowski, 1972; Justice and Bass, 1978). At cooler temperature the equilibrium moisture content will be slightly higher at the same relative humidity. This is because the water molecules have less energy at the cooler temperature and more molecules are absorbed on the macromolecules and capillary surfaces of the seed. The difference in equilibrium moisture can be up to 1% with extremes in storage temperature. Equilibrium moisture contents for some small seeded legumes are summarized in Table 2.2.

Table 2-2 The equilibrium moisture content of alsike, red and white clover seed at different relative humidities and approximately 25 °C (Harrington, 1968) and 23 °C (Dexter, 1957)

Species	Approximate temperature (°C)	Moisture content (%) at indicated relative humidity (%)						
		15	30	45	70	75	80	85
Alsike clover	25	6.1	7.9	9.7				
	23				9.3	--	15.9	18.9
Red clover	25	5.7	7.6	9.4				
	23				9.1	11.2	15.6	18.7
White clover	25	5.9	7.8	9.7				
	23				8.7	10.9	15.4	18.0

2.2.2.3 Storage fungi

Storage fungi may have deleterious effects on seed longevity, which have been reviewed by several authors (e.g. Christensen 1973; Christensen and Kaufmann, 1965; 1969; 1974; Christensen et al., 1969; Powell et al. 1984).

1) Common storage fungi

Seeds may become contaminated by storage fungi before harvest, but invasion does not usually occur until the seeds are in store. The common storage fungi reported in all crops comprise about 10-15 species of Aspergillus, of which only five or six are at all common until deterioration is fairly well advanced, plus several species of Penicillium. The main groups of Aspergillus includes A. restrictus, A. glaucus, A. candidus, A. ochraceus and A. flavus

2) Conditions required for growth of storage fungi

Factors influencing the growth of storage fungi (Christensen and Kaufmann, 1969) include : (1) seed moisture content (which is associated with relative humidity), (2) the storage temperature, (3) the length of the time the seed is stored, (4) the degree to which the seed already has been invaded by storage fungi before storage, (5) the amount of foreign material present in the seed lot, and (6) the activities of insects and mites. Each of these is closely related to most of the others; relative humidity, and hence seed moisture content, and storage temperature are the most important (Christensen and Kaufmann, 1969; Christensen, and Kaufmann, 1974; Powell, et al., 1984b).

a) Seed moisture content

For each of the common species of storage fungi there is a minimum seed moisture content below which it cannot grow. The most drought-resistant of the storage fungi (Aspergillus restrictus) cannot grow at moisture contents below those in equilibrium with a relative humidity of approximately 65%. Any seeds whose moisture content is below this (see Table 2.3) should therefore be safe from invasion by storage fungi, regardless of the other conditions of storage (Christensen and Kaufmann, 1974).

Table 2-3 Minimum relative humidity for the growth of common storage fungi at their optimum temperature for growth (26 - 30 °C)*

Fungus	Minimum relative humidity (%)
Aspergillus halophilicus	68
A. restrictus	70
A. glaucus	73
A. candidus, A. ochraceus	80
A. flavus	85
Penicillium, depending on species	80 - 90

* Data adapted from Christensen and Kaufmann (1974).

Christensen and Kaufmann (1974) concluded that there is a more or less regular ecological succession of fungal invasion: first a slow or moderately rapid increase in A. restrictus and A. glaucus in the first stage of deterioration, followed by A. candidus and A. flavus. By the time that 5 to 10% of the seeds have been invaded by A. candidus or A. flavus, spoilage is well under way and heating will shortly occur.

b) Temperature

Storage fungi grow most rapidly at about 30-32 °C and their growth rate decreases as temperature decreases. Increased storage temperature has been reported as being associated with high levels of infection by storage fungi on soybean (Dorworth and Christensen, 1959), cowpea (Onesirosan,1983), peas (Field and King, 1962) and beans (Lopez and Christensen,1962). The approximate minimum, optimum, and maximum temperatures for growth of storage fungi are summarized in Table 2.4

Table 2-4 Approximate minimum, optimum, and maximum temperatures for growth of common storage fungi

Fungus	Temperature for growth (°C)		
	Minimum	Optimum	Maximum
Aspergillus restrictus	5-10	30-35	40-45
A. glaucus	0-5	30-35	40-45
A. candidus	10-15	45-50	50-55
A. flavus	10-15	40-45	54-50
Penicillum	-5-0	20-25	35-40

* Data adapted from Christensen and Kaufmann (1974).

3) Effects of fungal invasion on seed quality

Invasion of seeds by storage fungi can result in: a) decreases in germinability, b) discoloration, c) production of mycotoxins, d) heating, e) mustiness, and, finally, f) total decay (Christensen and Kaufmann, 1974). Among those, the most significant effect is to reduce seed viability (Christensen, 1974; Powell, et al., 1984b). Fields and King (1962) demonstrated that peas free of storage fungi retained a germination of 98% throughout the period of the test, whereas the germination of seeds that had been inoculated with various storage fungi was reduced to zero.

2.2.3 Predicting the relative storability of seed lots

2.2.3.1 Standard germination and seed relative storability

It is important for the seedsman to make correct decisions as to which seed lots among those held in store should be marketed or sown first, and which can be safely held over a longer period, and for how long. The judgment as to the relative storability in many situations is based on the results of a standard germination test. A seed lot that germinates 70% will generally not store as well as another one of the same seed kind that germinates 95%. However, germination of most lots is often quite similar -- all lots may germinate between 85 to 90%;, for example in Table 2.5, seed lots within

each species had comparable initial germination percentages but actual storability of the individual lots ranged from excellent to very poor. Obviously, germination percentage of the lots at the time they were placed in storage provided essentially no information on which to base decisions regarding their suitability for storage.

Table 2-5 Comparison of germination percentage among seed lots of two herbage species under the same storage conditions

Species	Lot	Initial germination (%)	Period of open storage (months)* 6	12	18	42
Tall fescue	A	90	91	90	-	-
	B	91	90	73	-	-
	C	90	84	58	-	-
	D	88	74	24	-	-
	E	88	58	6	-	-
Crimson clover	A	90	90	92	88	78
	B	90	90	89	82	60
	C	94	92	84	60	28
	D	88	81	60	49	12
	E	88	76	48	20	0

* Seeds stored in non - conditioned warehouse at State College, Mississippi; data adapted from Delouche and Baskin (1973).

2.2.3.2 Seed vigour and relative storability

Seed field performance and seed storability are two important and reciprocal properties of seedlots. The field performance and storability decreases as the level of deterioration increases (Delouche and Baskin, 1973); therefore, theoretically, vigour test methods are suitable for measuring or predicting seed storability. Helmer et al. (1962) stated that seed vigour, properly evaluated, is not only a measure of the

capacity of seed to survive and emerge under adverse field conditions, but also a measure of the storability of seed, i.e. the keeping quality of seed. Seeds low in vigour are as susceptible to adverse storage conditions as they are to adverse field conditions. Although the literature relating vigour tests to storability is not as extensive as that related to field performance, different kinds of vigour methods have been experimentally used for predicting the relative storability of seed lots.

Vigour test methods used for predicting seed lots' storability are seedling growth and evaluation (Delouche and Baskin, 1973; Egli et al. 1979), controlled deterioration (Powell and Matthews, 1984a, b), accelerated aging (AA) (Byrd and Delouche, 1971; Baskin and Viera, 1980), cold test (Delouche and Baskin, 1973; Grabe, 1965), tetrazolium (Byrd and Delouche, 1971), conductivity (Helmer et al., 1962; Ching, 1972), respiration (Delouche,1973) and glutamic acid decarboxylase activity test (Delouche and Baskin, 1973; Grabe,1965).

Almost all the vigour methods used have been valuable in predicting the relative storability of seed lots under both manipulated conditions (Byrd and Delouche, 1971; Delouche, 1965b) or open storage conditions (Baskin et al. 1980; Delouche and Baskin, 1973). Experiments which involved comparing the different methods showed that the AA test was more accurate and effective than others (Delouche and Baskin 1973; Egli et al., 1979), but the others also showed value in some cases. For example, the optimum accelerated aging conditions for some herbage crops are listed in Table 2.6. Byrd et al.(1971) demonstrated that the effectiveness of any test is generally limited to a given time-storage environment. For example, if storage conditions are good and /or the storage period is short, the standard germination test results are effective; however, if storage conditions are not optimum and /or the storage interval is long, the AA, heat treatment and cold test are much more effective and sensitive indices of storage potential. The controlled deterioration test has been reported to accurately predict the relative storability in a number of small vegetable seeds, both under optimum and adverse storage conditions (Powell and Matthews, 1984a; b). However, it has not been used on small herbage legume seeds.

Table 2-6 Optimum accelerated aging conditions and treatment times for predicting seed relative storability of some small seeded forage legumes*

Seed kind	Accelerated aging test		Modified aging test
	Temperature (°C) at 100% RH	Time (Hours)	Time (Weeks) at 30 °C and 75% RH
Alfalfa	42	84	6
Crimson clover	40	72	9
Lespediza	40	72	6
Red clover	40	72	9

* Data from Delouche and Baskin (1973).

2.3 Red clover cv. Grasslands Pawera

2.3.1 Taxonomy, origin and distribution

Red clover belongs to the genus Trifolium L, the subfamily Papilionoideae, and the family Leguminosae. The cultivar Grasslands Pawera was bred from tetraploid lines obtained by colchicine treatment of late-flowing New Zealand Grassland Turoa and Swedish red clovers (Anderson , 1973a). It was first certified in New Zealand in 1974, and since then has been distributed to many countries as an important pasture crop e.g. Australia, England, and USA.

2.3.2 Importance in agriculture

Pawera can be used to establish temporary or permanent pastures, particularly with annual or biennial grasses for grazing or conservation. It establishes rapidly in most areas with spring or autumn sowings (Anonymous, 1982).

Pawera has high annual herbage yield (15-10% more than Turoa), grows vigorously from mid-spring to late autumn, with a peak in midsummer. In favorable conditions it

is more persistent than other red clovers i.e. Hamua or Turoa. Seed production is reasonably good if pollinators are numerous and harvesting conditions favorable. Seed yields as high as 300-400 kg/ha. have been obtained (Anonymous, 1982).

Pawera plants are highly nutritious, very acceptable to livestock, and produce excellent hay or silage, either as a pure crop or with grasses.

Pawera is deep rooted, late flowering, has a good measure of resistance to diseases and nematodes, has an erect growth habit which enables it to efficiently utilize sunlight for growth (Anderson, 1973a,b), has good drought tolerance and thus high potential summer growth in summer-dry regions (Hay and Ryan, 1983). It also has better ability to over winter and to take up certain major elements from soil than the diploid red clovers (Anderson, 1973b).

2.3.3 Seed

Pawera has a relative large seed size, and a seed weight of 3 to 3.5g/1000 has been reported (Anonymous, 1982). Hampton et al. (1987) found that the optimum temperature for Pawera seed germination was 20 ^{o}C; germination rate slowed and the number of days to the start of growth increased as temperature moved away from the optimum. However, the final percentage germination did not differ significantly within the temperature range 5 to 20 ^{o}C.

CHAPTER 3

MATERIALS AND METHODS

3.1 Preliminary experiment

Samples of 17 certified herbage legume seed lots including 8 lots of white clover cv. Grasslands Huia, 4 lots of red clover cv. Grasslands Pawera and 5 lots of lucerne cv. Wairau were supplied by the Official Seed Testing Station. Random samples of each of the 17 lots were taken by the quartering subdivision method (ISTA, 1985), since the amount of seed of each lot was limited. The seeds were then tested for determination of moisture content, standard germination and thousand-seed weight (ISTA, 1985). Four lots from each species were vigour tested using the accelerated aging method (ISTA, 1987).

3.1.1 Moisture content

Two 5g replicates of each seed lot were weighed, placed in aluminium containers and dried using the high temperature oven method (ISTA, 1985), i.e. 130 ^{o}C for 1h. The seeds were then cooled in a desiccator for about 30 minutes before reweighing. Moisture content was calculated on a wet-weight basis and expressed as a percentage (ISTA, 1985).

3.1.2 Standard germination

The top of paper method (ISTA, 1985; Johnstone, 1989) was used. Moist towelling was placed on a flat tray and covered by a sheet of white filter paper on which small germination blotting pads were placed. Samples of 2 x 50 seeds for each lot were positioned on each pad which was then covered by a plastic cover. Trays were then placed in a germination cabinet at 20 ^{o}C for 10 days. The first count was made at 4 days, and at 10 days the number of normal seedlings, abnormal seedlings, hard, fresh ungerminated and dead seeds were counted and the results were expressed as a percentage (ISTA, 1985).

3.1.3 Thousand seed weight

The weight of 1000 seeds was calculated by obtaining the mean weight of 4 x 100 seeds for each lot, and multiplying by ten.

3.1.4 Accelerated aging

Seed samples were subjected to a modified 'jar' accelerated aging method (ISTA, 1987). One hundred seeds were placed in a muslin cloth bag and tied with a rubber band. Wire mesh stands were used to support the seed 3 cm above the surface of the 100 ml water in the bottom of the jar. The jars had a diameter of 8.5 cm and were 16 cm high. The system used maintained near 100% relative humidity (RH). One hundred 100 seeds from each of 12 seed lots were placed in the same jar. Six jars were used for the different times of treatment i.e., 16, 24, 48, 64, and 72 h, and all were aged at 40 (± 0.5) ^{o}C. The seeds after aging were given a standard germination test (see Section 3.1.2, except that only 4 x 25 seeds per sample were used).

3.2 Main experiment

Seven seed lots of certified Pawera red clover (named lots 1, 2, 3, 4, 5, 6 and 7 respectively) were selected because they had a germination percentage of 85% or greater, were the same certification class, and had all been harvested in 1988. These were supplied by Challenge Seeds Ltd. Christchurch. The seeds were kept at 5 ^{o}C at the Seed Technology Centre, Massey University before each test was conducted. All 7 lots were used for the laboratory quality assessments (with the exception that only 4 lots were used for the germination rate test), 6 lots were selected for field performance trials, and 4 lots were chosen for the storage test (although all 7 lots were stored at ambient conditions). The period of experimentation lasted from June 1988 to July 1989. Random samples were taken from each lot, using the Nobbe trier sampling method (ISTA, 1985) to take about 1 kg seeds from each seed bag. The sample was then further divided using the quartering subdividing method (ISTA, 1985) to suitable sample amounts for each of the following tests:

3.2.1 Laboratory quality assessment

3.2.1.1 Moisture content

Refer to section 3.1.1.

3.2.1.2 1000-seed weight

Refer to section 3.1.1.

3.2.1.3 Standard germination

The test was similar to the preliminary germination test (Section 3.1.2) except that (1): the number of seeds tested was increased to 4 x 50 seeds for each lot, and (2): first and intermediate counts were also employed by removing and counting those normal seedlings which were at least 1.5 cm long at 4 and 7 days after planting respectively.

3.2.1.4 Mechanical damage

Mechanically damaged seeds were separated from a sample of 4 x 100 seeds in each lot by visual observation. The percentage of damaged seed was then calculated.

3.2.1.5 Imibibition rate

Four replicates of 100 seeds from each lot were placed between 2 layers of saturated, blue germination blotters (the blotter was 9 x 9 cm, and 30 ml water was added). The blotters were then enclosed in plastic boxes and placed in a 20 ^{o}C germinator. After 1, 2, 4, 6, 8, and 24 hours, the seeds that had become swollen were counted and taken out. Seeds which remained firm after 24 hours were discarded. Swollen seeds were identified by their larger size, lighter colour, glossy appearance and softer, spongy texture as compared to firm seeds. The percentage of swollen seeds removed after each time interval was calculated.

3.2.1.6 Storage fungi

High salt potato dextrose agar (PDA) (Hampton 1988; Karen Johnstone personal communication) was used for detecting storage fungi. Thirty nine g PDA and 75 g

NaCl were suspended in 1 litre of tap water and dissolved by microwaving at high power for 5 minutes. The solution was then autoclaved for 15 minutes at 120 ^{o}C. Before the cooling agar solution was poured into the petri dishes, streptomycin at a concentration of 100 units/ml (10 ml per 100 ml agar solution) was added to flasks. Seeds were placed in a muslin cloth bag, immersed in a 1% Janola solution for 5 minutes, then rinsed under tap water for another 5 minutes. Five replicates of 20 seeds for each lot were placed on the surface of the agar in each petri dish under sterile conditions and dishes were incubated at 25 ^{o}C. After 4, 6, 8, and 10 days, microscopic examination was made to determined percentage of storage fungi. The percentage of seeds invaded by the storage fungi and the number of each kind of storage fungi were determined.

3.2.1.7 Electrical conductivity

Four replicates of 0.5 g seeds from each lot were soaked in 100 ml of distilled water in 125 ml flasks. The flasks were stirred to remove air bubbles and floating seed, then covered by laboratory film and held at 20 ^{o}C. After the predetermined soaking time i.e., 2, 4, 6, 8, and 24 h, the leachates were mixed well by stirring the flasks, then the conductivity of the soak water was measured by using a Radiometer CDM - 83 conductivity meter. The flasks were recovered between each measurement. The results were expressed in microsiemens per gram of seeds (uS/cm g) (ISTA, 1987).

3.2.1.8 Accelerated aging test

The test was similar to that described in section 3.1.4, except for the following differences:

(1): Seed samples: 0.8 g seeds from each lot (enough to provide the 4 x 50 seeds required in the germination test) were packed in a muslin bag. There were 7 bags placed in each jar.

(2): Aging time: 1, 2, 3, 4, and 5 days, respectively.

(3): Pretreatment: micro-organisms present on the seed surface may influence the aging result, and so surface sterilized seeds were compared with non-surface sterilized seeds. Non-surface sterilized seeds in their muslin bags were directly placed on the wire mesh in the aging jar. A further set of seeds were surface sterilized by the following steps: a) seeds were packed within the muslin bags, b) soaked in 1% Janola solution for 5 minutes, c) rinsed in running tap water for 5 minutes, d) seeds were

removed from the bags, placed on top of 2 layers of paper toweling, then dried at laboratory temperature for about 2 hours until they had dried back to their original weights, and e) repacked on the muslin bags and placed on the wire mesh in the jar.

3.2.1.9 Controlled deterioration

Samples of 0.5 g seeds of known initial moisture content (Section 3.2.1.1) were placed in aluminium bags. There were 5 samples for each of the 7 lots to provide 5 levels of final required moisture contents i.e., 16, 18, 20, 22, and 24%. The required moisture contents were reached by the addition of water, calculated from the following formula (Coolbear, 1988 c):

$$\left[\frac{100 - MCo}{100 - MCr} \times W\right] - W = V \ (cm^3)$$

Where MCo = original moisture content of seeds.
MCr = required moisture content of seeds.
W = weight of seeds (g).
V = water added.

After adding the water the aluminium foil bag was sealed tightly using a Ribbon Sealer and placed at 10 ^{o}C overnight to allow the seeds slow and even imbibition. The sealed packets were then moved to 45 ($\pm$ 0.5)^{o}C and incubated for a further 24 hours. The standard germination test as described in section 3.2.1.3 was conducted on all aged seed samples. Both normal seedlings and radicle appearance were assessed.

3.2.1.10 Germination rate

Germination rate was determined at 5 temperatures including constant temperatures of 5, 10, 15, and 20 ($\pm$ 0.5) ^{o}C, and a fluctuating temperature of 5/10 ($\pm$ 0.5) ^{o}C (16 / 8 h per day). Four replicates of 50 seeds for each of 4 seed lots (lot 1, 2, 3, and 4) were used in each temperature treatment. The 50 seeds were placed on the top of 2 layers of germination blotters which had been moistened with 20 ml of tap water. The blotters were then enclosed in a plastic box. All boxes in each treatment were placed completely randomly in a germinator in the dark. Germinated normal seedlings which were at least 1.5 cm long were counted and removed daily ($\pm$ 2 h) during the test

period. The tests were continued until almost no further seed germination occurred; these times were: 8 days at 20 ^{o}C, 9 days at 15 ^{o}C, 12 days at 10 ^{o}C, and 17 days at 5 and 5/10 ^{o}C. The germination rate was calculated by using the following formula (Maguire, 1962):

$$\text{Rate} = \frac{\text{No. of normal seedling}}{\text{Days to first count}} + \ldots\ldots + \frac{\text{No. of normal seedlings}}{\text{Days to final count}}$$

3.2.2 Field performance trial

The plots were located beside the Seed Technology Centre, Massey University. The soil was a ToKomaru silt loam soil (Hampton, personal communication). It is formed on faily thick deposits of loess of fine sand loam texture, and has an average rainfall ranging from 890 to 1140 mm. There is a virtural absence of coarse sand in the soil, but clay increased from 20% in the 0 to 11 cm horizon to a maximum of 29% at 43 to 58 cm (Cowie, 1978). Soil chemical analysis showed that the topsoil is low to very low in available phosphorus, low in exchangable calcium and potassium, and medium in exchangable magnesium. The main requirements of ToKomaru silt loam are topdressing of lime, phosphate and potash, and drainage. With adequate topdressing and drainage, high producing ryegrass-white clover pastures can be maintained (Cowie, 1988).

All the field trials were conducted using a randomized complete block design with 4 replicates of 50 seeds from each of 6 lots (lots 1, 2, 3, 4, 5, and 6) (lot 7 was not used because it was significantly lower in germination percentage than others). Seeds were sown by hand at a depth of about 1 cm, the spacing being 3 cm between seeds within the row and 15 cm between the rows. There were 3 sowing dates in the spring of 1988, and 5 sowing dates in the autumn of 1989. The dates were 23/Sep., 18/Oct., and 13/Nov. in 1988, 21/Mar., 5/Ap ., 20/Apr., 9/May, and 20/May in 1989.
The measurements carried out at each sowings included the following:

3.2.2.1 Final emergence and emergence rate

Seedlings were counted as soon as the cotyledons broke free of the soil surface. Counts were made on the first and second days following emergence, and then every other day until there were no further increases in seedling numbers. The final

emergence was expressed as the percentage emerged seeds. The emergence rate was calculated using the same formula as described in the germination rate test (Section 3.2.1.10).

3.2.2.2 Seedling length and dry weight

In order to avoid the different density effects, seedlings were thinned to 30 seedlings per row immediately after the final emergence count for the trials conducted in the spring. No thinning was conducted in the autumn trials since the seedlings were small and could not cause density effects. The seedling length and dry weight were measured after 8 weeks in the spring and 5 weeks in the autumn. Ten seedlings from each row were randomly harvested, the length was measured on individual seedlings, and the mean length was calculated. Each lot of 10 seedlings was put into a paper bag and dried in a 65 ^{o}C oven for 4 days, then weighed and expressed as dry weight per seedling.

3.2.3 Storage test

Seeds were stored under the following temperate climatic conditions:

(1) Ambient conditions

About 200 g seeds for each of the 7 lots were packed in a muslin bag and stored in a cabinet in the seed processing room at the Seed Technology Centre of Massey University.

(2) 20 ^{o}C, 45% RH.

(3) 20 ^{o}C, 60% RH.

(4) 20 ^{o}C, 75% RH.

(5) 20 ^{o}C, 90% RH.

Under conditions of (2), (3), (4), and (5), about 16 g seeds for each of 4 seed lots (lots 1, 2, 3, 4) were packed in muslin bags, and 4 bags (1 from each lot) were tied together with a rubber band and stored in the same preserving jar on the top of a wire stand, similar to the accelerated aging test. The jar was sealed by a screw cover. The desired RH inside the jar was reached by adding 100 ml of the appropriate water and glycerine solutions (Table 3.1). Jars were stored in a 20 ^{o}C room. The following measurements were conducted after storage for 1, 2, 3, 5, 7, 9, and 11 months on 4 seed lots.

(1) Standard germination test

Same as the test described in section 3.2.1.3, including 4-days, 7-days, and final counts.

(2) Moisture content

See section 3.2.1.1.

(3) Storage fungi

See section 3.2.1.6.

(4) Conductivity

See section 3.2.1.7 except 3 x 0.5 g seeds were tested for each lot.

(5) Others

In order to determine seed quality changes from spring to the autumn, the standard germination, conductivity and field emergence test (21 / Mar. 1989) were conducted on each of 7 lots stored both under ambient and 5 ^{o}C conditions for 7 months.

3.3 Statistical analyses

An analysis of variance was performed on the results from each test (except the moisture content and some tests in the preliminary experiment) to compare the different quality of individual seed lots. The ranking of lots in most cases was determined by Duncan's multiple range test, or the least significant difference (LSD) as appropriate. Simple correlation coefficients were calculated to evaluate the association between the results of each laboratory test and the seed performance tests under field and storage conditions. Regression analysis of some vigour test results was also used to predict field emergence and storability. Main data analyses were done by using the computer program SAS available at Massey University.

Table 3-1 The proportion of water and glycerine required to maintain the different levels of RH at 20 ^{o}C (modified from Hill 1988)

RH at 20 ^{o}C (%)	Water (ml)	Glycerine (ml)
45	5	95
60	31	69
75	54	46
90	92	8

CHAPTER 4

LABORATORY QUALITY ASSESSMENTS

4.1 Preliminary experiment

4.1.1 Seed moisture content

Seed moisture content of the 17 lots was very similar, with an average of 6.8% in lucerne 7.5% in red clover and 7.4% in white clover (Table 4-1).

4.1.2 Thousand seed weight

Mean thousand seed weight (TSW) of red clover was highest (3.2g), followed by lucerne (2.3g) and then white clover (0.7g) (Table 4-1).

4.1.3 Standard germination

The lowest germination percentage recorded was 86%, and the average was 91% in both lucerne and red clover, and 90% in white clover (Table 4-1). There were only slight differences in abnormal seedlings and hard seeds between the seed lots in each species, and only 3 seed lots contained dead seeds.

4.1.4 Seed vigour

Germination after 72 h accelerated aging (AA) showed marked differences between the seed lots within each species, in contrast to the standard germination (SG) results. For instance, the difference in the percentage of normal seedlings between lucerne seed lots 2 and 3 was only 1% in the SG test, but 45% after AA. Similar results were also demonstrated between the lots of red clover and white clover (Table 4-1). These results indicate that vigour differences clearly exist between seed lots in each of the three species.

Table 4-1 Seed moisture content (SMC), 1000-seed weight (TSW), and percentage germination before (SG) and after 72-hour accelerated aging (AA) in 17 lucerne, red clover and white clover seed lots

Species	Lot	SMC (%)	TSW (g)	SG (%)				AA (%)
				NS*	AS	HS	DS	NS
Lucerne	1	6.6	2.2	90	5	2	0	43
	2	6.8	2.4	94	3	3	0	76
	3	7.2	2.5	93	4	4	0	31
	4	6.6	2.3	91	1	8	0	76
	5	7.0	2.3	86	12	2	0	57
Red clover	1	7.5	3.6	87	8	5	0	25
	2	7.8	3.2	88	9	2	1	4
	3	7.5	3.1	93	5	1	0	35
	4	7.3	3.0	95	5	0	0	71
White clover	1	7.3	0.6	87	10	3	0	40
	2	7.7	0.7	91	6	3	0	35
	3	7.4	0.7	87	8	4	0	32
	4	6.1	0.7	91	7	0	1	15
	5	7.6	0.7	88	8	3	0	56
	6	7.3	0.7	87	5	8	0	31
	7	7.8	0.7	91	5	3	1	38
	8	8.0	0.7	94	4	2	0	31

* NS: normal seedlings, AS: abnormal seedlings, HS: hard seed, DS: dead seed

The effects of different periods of AA on seed lot vigour for each species are illustrated in Figure 4-1 and 4-2 respectively. Vigour generally decreased as the aging period increased; the loss was slight at the beginning of aging, then increased sharply after 48 h for all three species (Figures 4-1, 4-2). There was no obvious difference in vigour between the species during the first 48 h, e.g. the average change of germination in lots was: from 90 to 86% in lucerne (Figure 4-1), from 91 to 88% in red clover (Figure 4-2A), and from 90 to 80% in white clover (Figure 4-2B). However, marked differences among the species appeared after 48 h. Between 48 and 72 h, the loss of germination was quickest in red clover (average from 88 to 34%), followed by white clover (from 80 to 39%) and then lucerne (from 86 to 63%) (Figures 4-1, 4-2).

The response of seed lots to different aging period tends to show that as the aging period increased, vigour differences between the lots were further distinguished. For instance, the vigour between 4 lots of lucerne was not significantly different ($P < 0.05$) at 16 and 24 h of aging; at 40 and 48 h lot 2 was significantly lower ($P < 0.05$) than lot 4; and at 72 h, lot 2 was also lower ($P < 0.05$) than lot 5, which in turn was lower ($P < 0.05$) than lots 4 and 1 (Figure 4-1). Similar tendencies were also observed in red clover (Figure 4-2A), and white clover, except between 24 and 48 h (Figure 4-2B).

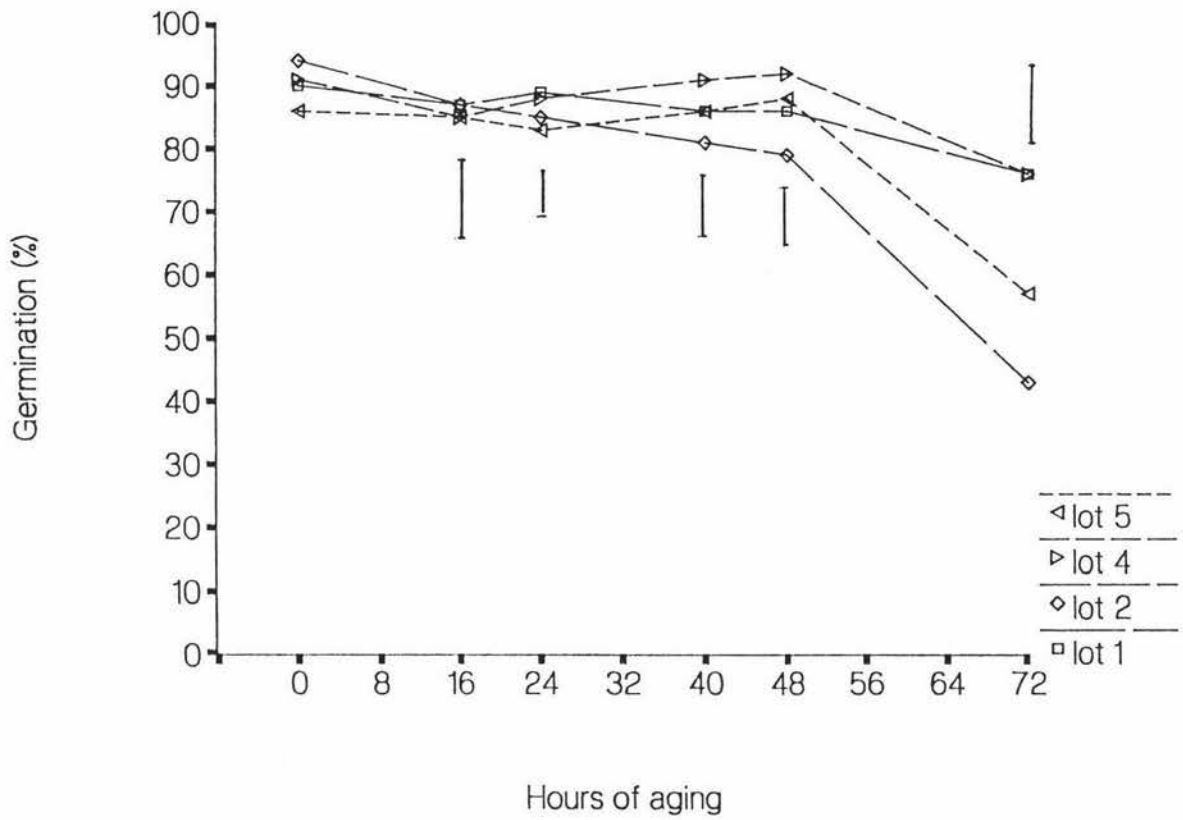

Figure 4-1 Standard germination after different periods of accelerated aging of 4 lucerne seed lots. Vertical bars represent LSD at P ≤ 0.05.

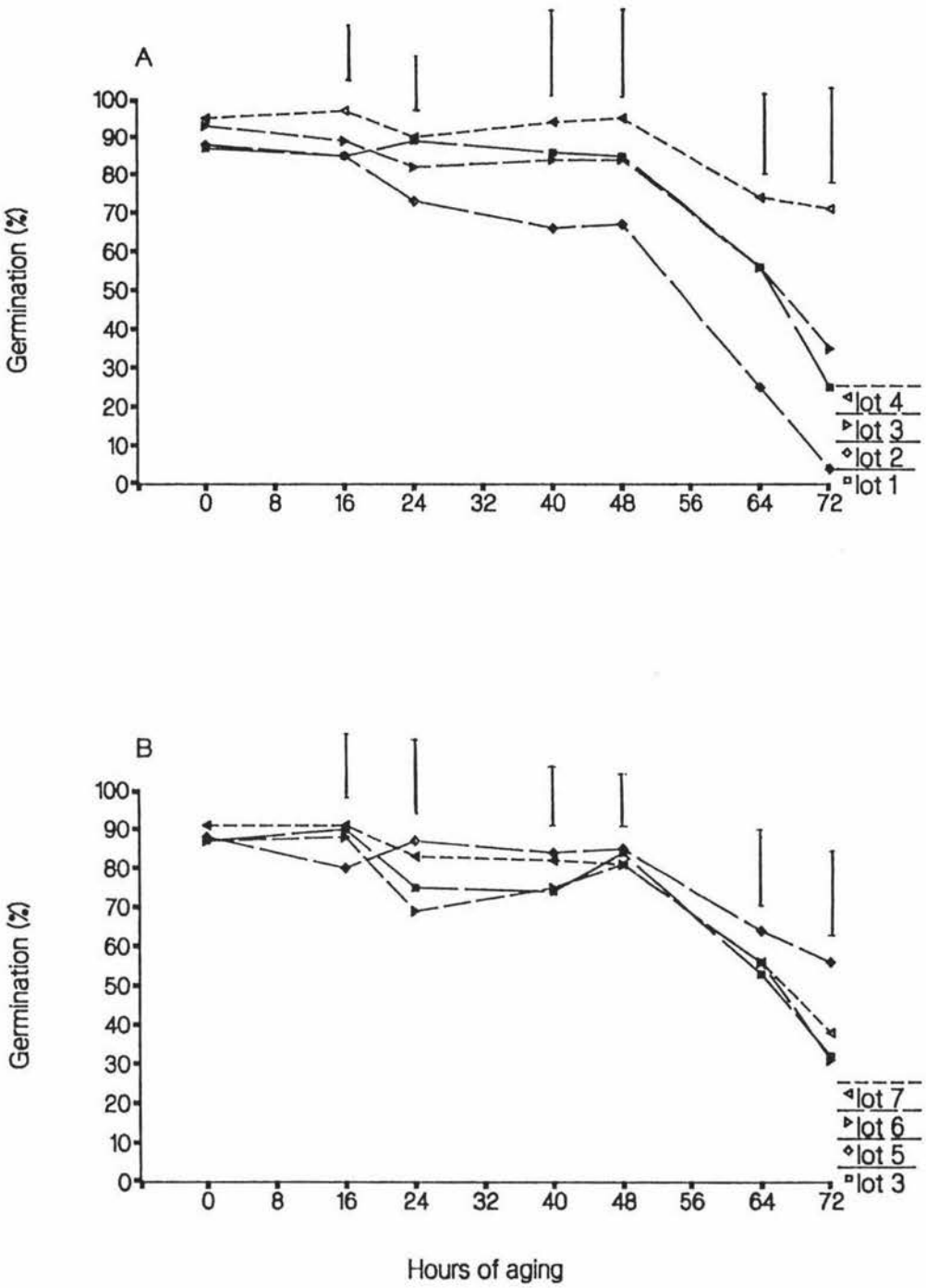

Figure 4-2 Standard germination after different periods of accelerated aging in red clover (A), and white clover (B). Vertical bars represent LSD at $P \leq 0.05$.

4.2 Main experiment

4.2.1 Seed moisture content

Among the 7 seed lots of red clover cv. Grassland Pawera tested, seed moisture content (SMC) ranged from 10.9% in lot 4 to 12.4 in lots 3 and 7 (Table 4-2).

4.2.2. Thousand seed weight

Average thousand seed weight was 3.8g, but varied from 3.2g (lot 2) to 4.1g (lot 1) (Table 4-2).

4.2.3 Mechanically damaged seed

The mechanically damaged seed content of lots 1 (5.8%) and 7 (5.0%) was significantly higher ($P < 0.05$) than that of the other lots (Table 4-2).

4.2.4 Storage fungi

The incidence of storage fungi (SF) was low, with an average of 1.4%. No significant differences was recorded between seed lots (Table 4-2). The main species of SF identified were Aspergillus glaucus and a Penicillium sp.

4.2.5 Standard germination

The mean germination (% normal seedlings = NS) of the 7 lots was 89%, ranging from 82% (lot 7) to 92% (lot 5). There were no significant differences ($P < 0.05$) in NS and abnormal seedlings (AS) except for lot 7 which was lower ($P < 0.05$) in NS% and was higher ($P < 0.05$) in AS%. However, there was some variation between seed lots in the 4-day and 7-day counts, and lot 1 and 7 had significantly lower counts than seed lots 3, 5, and 6 (Table 4-2). The main category of AS was a damaged root system, the symptoms being shortened and split roots. The percentages of hard seed (HS) and dead seed (DS) were relatively low in all lots although the HS% in lot 4 (4%) and DS% in lot 3 (5%) was higher than the others (Table 4-2).

4.2.6 Imbibition rate

After 6h of imbibition, the percentage of swollen seeds ranged from 93 to 98% (Table 4-2). The lots with the lowest germination and highest percentage of mechanically damaged seed (i.e. lots 1 and 7) imbibed more quickly ($P < 0.05$) than other lots in the first 4 h, but more slowly during the next 2 h. The reverse occurred with other lots (Table 4-2, Figure 4-3). Any mechanically damaged seeds were fully imbibed within the first 2 h. The imbibition rate over time is presented in Figure 4-3.

Table 4-2 Results of tests for seed moisture content (SMC), 1000-seed weight (TSW), mechanical damage (MD), storage fungi (SF), standard germination (SG) and imbibition rate (IR) in 7 red clover cv. Pawera seed lots

Test	Lot							Lot ranking*
	1	2	3	4	5	6	7	
SMC (%)	11.9	11.8	12.4	10.9	12.1	11.3	12.4	
TSW (g)	4.1	3.2	3.7	3.8	3.6	3.9	4.0	1 7 6 4 3 5 2
MD (%)	5.8	2.3	1.8	1.3	2.0	1.0	5.0	1 7 2 5 3 4 6
SF (%)	1	3	1	3	1	0	1	4 2 3 1 5 7 6
SG (%):								
SG4	70	73	78	75	82	79	65	5 6 3 4 2 1 7
SG7	80	87	90	89	93	90	79	5 6 3 4 2 1 7
NS	87	90	90	90	92	90	82	5 2 3 4 6 1 7
AS	9	7	5	6	6	8	16	7 1 6 2 4 5 3
HS	2	3	1	4	1	2	1	4 2 1 6 5 3 7
DS	3	0	5	0	2	1	2	3 1 5 7 6 2 4
IR (%):								
IR4	45	25	24	28	29	39	63	7 1 6 5 4 2 3
IR4-6	49	72	73	66	69	59	30	3 2 5 4 6 1 7
IR6	94	97	97	94	98	98	93	

SG4: 4-day count of SG, SG7: 7-day count of SG, NS: final count of SG
AS: abnormal seedlings, HS: hard seed, DS: dead seed
IR4: IR in first 4 h, IR4-6: IR between 4 and 6h, IR6: IR in first 6h
* The lots with the same line are not significantly different at 5% level in Duncan's multiple range test.

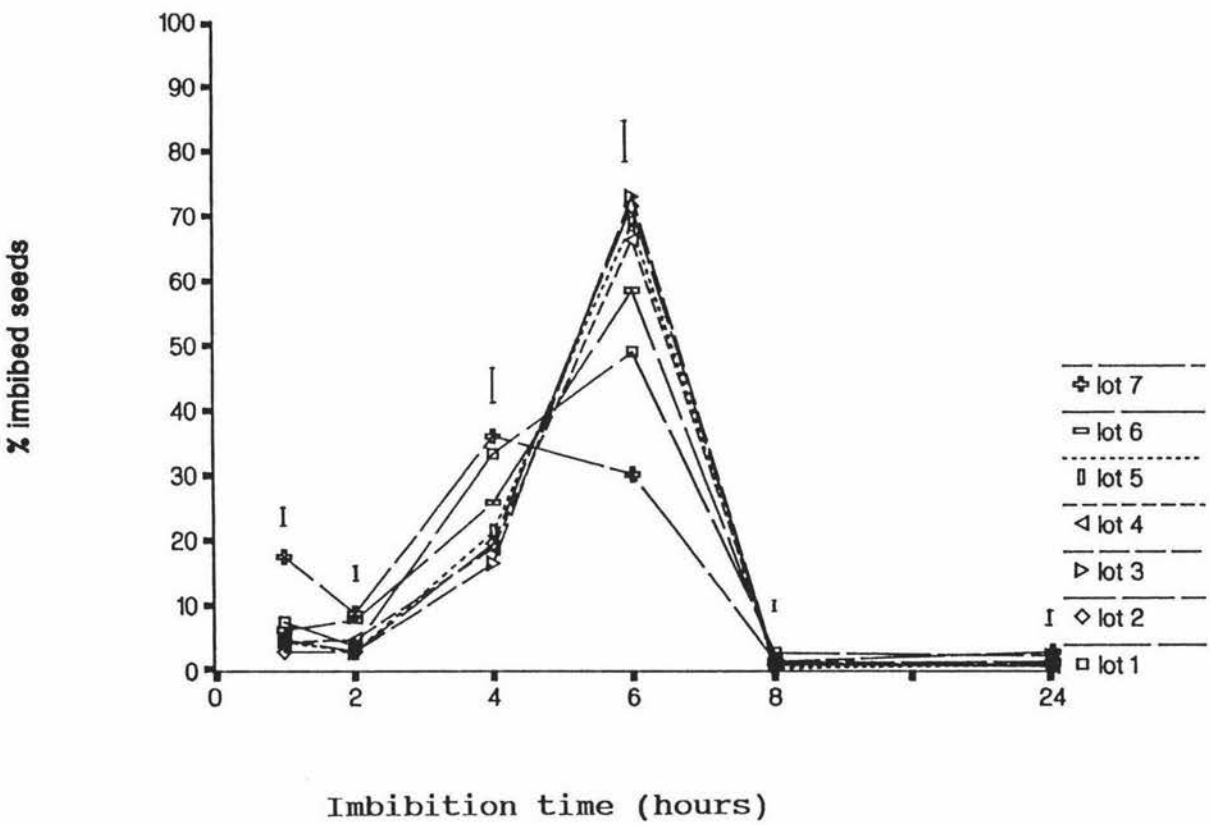

Figure 4-3 Imbibition (rate per 2 h) of 7 red clover cv. Pawera seed lots. Vertical bars represent LSD at $P \leq 0.05$.

4.2.7 Seed vigour

4.2.7.1 Conductivity

Significant differences in conductivity were recorded between seed lots (Figure 4-4). After 24h soaking, the conductivity of lot 4 was greater ($P < 0.05$) than that of all other lots, while that of lots 2 and 6 was lower ($P < 0.05$) than 3, 5 and 7. The seed lot conductivity differences were consistent with increasing soaking time, particularly during the period from 6 to 24 h. For example, the results for 6 or 8 h soak were highly correlated ($r = 0.929***$, $r = 0.929***$, $P < 0.001$ resp.) to the 24 h soak results. Conductivity increased rapidly during the first 8 h of soaking, but after that the rate slowed (Figure 4-4).

4.2.7.2 Accelerated aging

Accelerated aging (AA) separated the seed lots into a low vigour group i.e. lots 1, 3 and 7, and a high vigour group i.e. lots 2, 4, 5 and 6 during the first 3 days of aging (Table 4-3). Increasing the aging period from 0 to 3 days tended to further separate the seed lots (Table 4-3). Germination after 4-day AA was less than 26% in all lots, and the lot ranking differed compared to the other AA tests (Table 4-3). 5-day AA killed all the seeds. As the aging period increased, more mould developed on the seeds, particularly after 3 days, which created difficulties for seedling evaluation.

Surface sterilization of seeds prior to AA (AASS) did not change the seed lot ranking markedly or consistently (Table 4-3), with the exception that germinations were significantly lower in some of the AASS lots (lots 2, 4 and 7) after a relatively longer period (e.g. 2 to 4 days) of aging (Table 4-4).

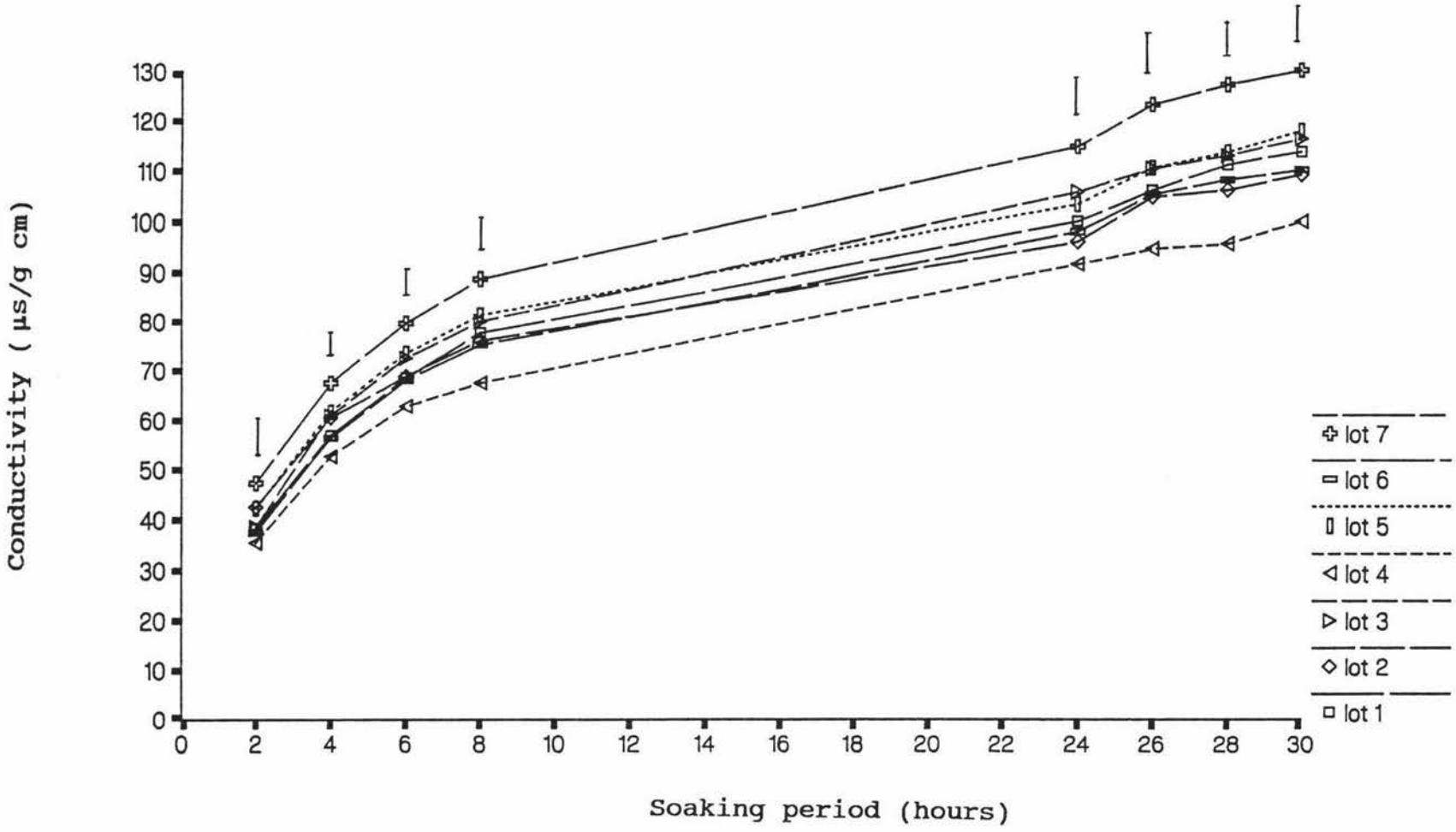

Figure 4-4 Conductivity of 7 red clover cv. Pawera seed lots after different periods of soaking. Vertical bars represent LSD at $P \leq 0.05$.

Table 4-3 Germination percentage (%) after different periods of accelerated aging in 7 red clover cv. Pawera seed lots

Test	Lot							Lot ranking*
	1	2	3	4	5	6	7	
0-day(SG)	87	90	90	90	92	90	82	5 2 4 3 6 1 7
1-day AA	84	86	60	87	86	85	57	4 5 2 6 1 3 7
2-day AA	59	82	50	76	74	73	41	2 4 5 6 1 3 7
3-day AA	23	63	8	42	60	39	4	2 5 4 6 1 3 7
4-day AA	2	21	2	26	11	1	2	4 2 5 1 7 3 6
0-day AASS	81	93	92	94	95	91	76	5 4 2 3 6 1 7
1-day AASS	77	87	64	81	83	89	51	4 5 2 6 1 3 7
2-day AASS	62	73	51	80	76	78	29	4 6 5 2 1 3 7
3-day AASS	22	46	19	43	36	33	6	2 4 5 6 1 3 7
4-day AASS	2	4	2	3	0	3	1	2 4 6 1 3 7 5

AA: Accelerated aging AASS: AA of surface sterilized seeds

*: See Table 4-2.

Table 4-4 Effects of surface sterilization on germination after different periods of accelerated aging in 7 red clover cv. Pawera seed lots

Aging time	Lot*						
	1	2	3	4	5	6	7
0-day	--	--	--	--	--	--	--
1-day	--	--	--	--	--	↑	--
2-day	--	↓	--	--	--	--	↓
3-day	x	↓	↑	--	↓	x	x
4-day	x	↓	--	↓	↓	x	x

--: no effect ↑ : increase ↓ : decrease x: no comparison

*: The comparison is based on Duncan's multiple range test at P=0.05

4.2.7.3 Controlled deterioration (CD)

Significant differences in vigour were also observed amongst the seed lots following the CD test (Table 4-5, Plates 1, 2). The vigour ranking was similar to the AA and AASS test results, i.e. the vigour of lots 1, 3 and 7 was lower than that of the other 4 lots. Germination in lots 1, 3 and 7 was significantly lower ($P < 0.05$) than lots 2 and 4 in all of the ten measurements, and lower ($P < 0.05$) than lots 5 and 6 in most of the measurements (Table 4-5).

The germination percentage declined markedly as the SMC increased in all the lots (Table 4-5). The decline was quicker in low vigour lots (e.g. lot 3) than in high vigour lots (e.g lot 4), particularly as the SMC increased from the control (average 11.8%) to 20%. For example, when the SMC was increased from 16% to 18 %, the germination decline was 26% in lot 3, but only 15 % in lot 4. Similar results was also found for radicle appearance and between the other low and high vigour lots (Table 4-5). As the SMC increased from 22 to 24%, changes in the germination became irregular (Table 4-5).

The significance of lot ranking for the normal seedling and radicle appearance results was similar, although the lot order sometimes differed (Table 4-5).

Table 4-5 Germination percentage measured for both normal seedlings and radicle appearance after controlled deterioration in 7 red clover cv. Pawera seed lots

Test	Lot							Lot ranking*
	1	2	3	4	5	6	7	
SG (control SMC)	87	90	90	90	93	90	82	
Normal seedlings (%)								
16% SMC	50	75	48	74	78	78	39	6 5 2 4 1 3 7
18% SMC	31	61	22	59	54	55	21	2 4 6 5 1 3 7
20% SMC	11	43	10	51	44	27	9	4 5 2 6 1 3 7
22% SMC	10	24	20	28	25	24	3	4 5 6 2 3 1 7
24% SMC	12	21	9	22	14	21	3	4 2 6 5 1 3 7
Radicle appearance (%)								
16% SMC	65	86	74	86	89	90	56	6 5 4 2 3 1 7
18% SMC	44	82	44	78	71	79	40	2 6 4 5 1 3 7
20% SMC	28	65	24	74	65	48	18	4 2 5 6 1 3 7
22% SMC	20	43	35	49	45	41	10	4 5 2 6 3 1 7
24% SMC	25	43	25	41	29	44	6	6 2 4 5 3 1 7

SMC: seed moisture content before accelerated aging

* See table 4-2.

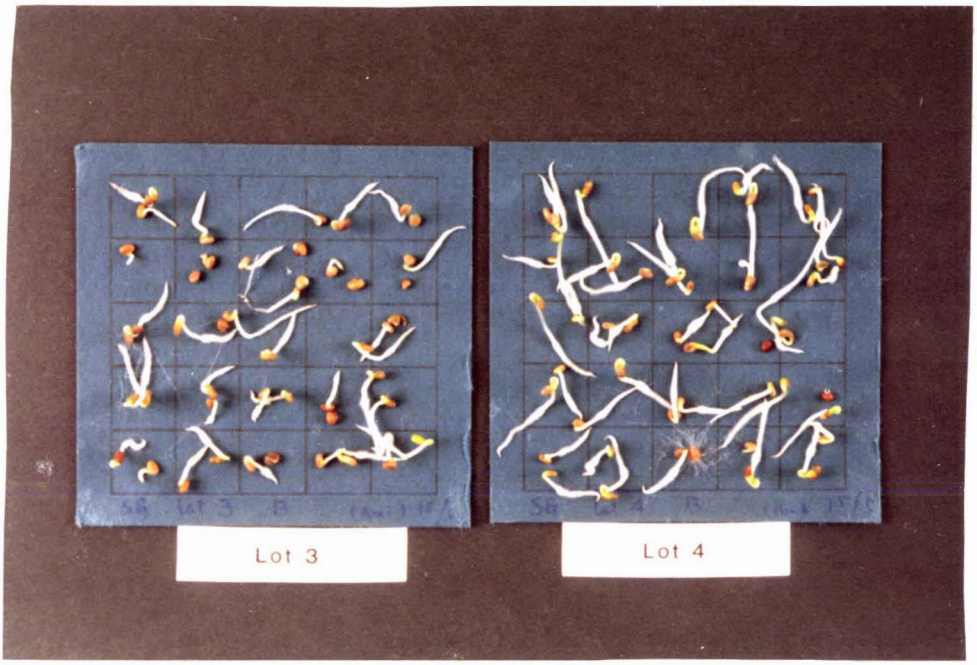

Plate 1. Standard germination (4-day count) for seed lots 3 and 4

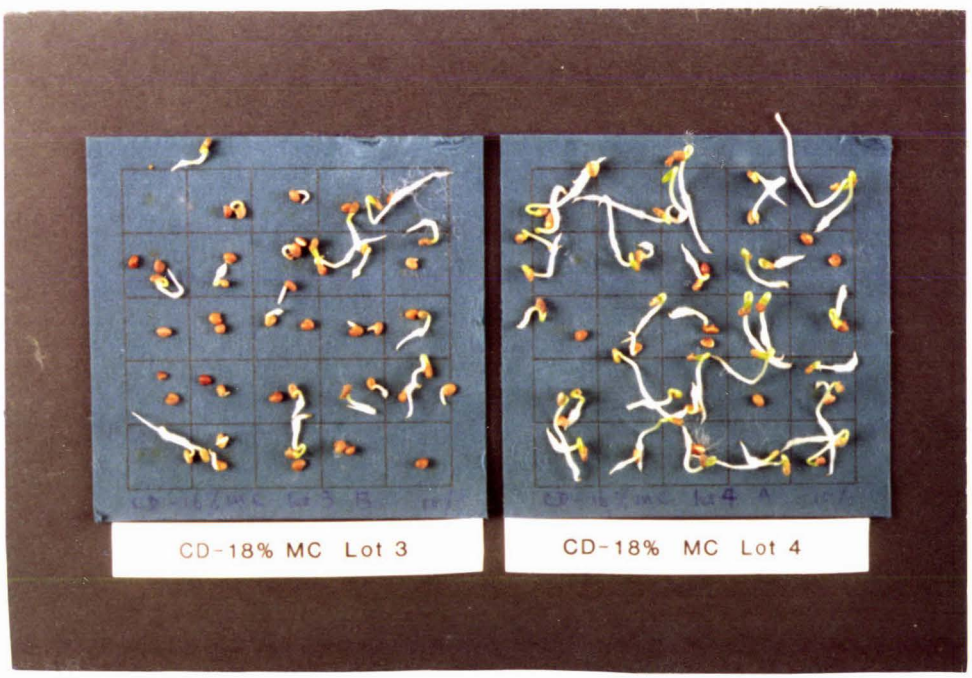

Plate 2. Germination (4-day count) following controlled deterioration at 18 % seed moisture content

4.2.7.4 Germination rate (GR)

At low temperatures (5 ^{o} and 5/10 ^{o}C), the germination rate of seed lots 2 and 4 (high vigour) was significantly greater than that of lots 1 and 3 (low vigour). However as temperature increased, seed lot germination rate differences disappeared.

Table 4-6 Germination rate (GR) of 4 red clover cv. Pawera seed lots at different temperatures

Test		Lot				Lot ranking*
		1	2	3	4	
GR:	5 ^{o}C	4.95	6.37	5.33	6.43	4 2 3 1
	5/10 ^{o}C	6.44	7.58	6.33	7.48	2 4 1 3
	10 ^{o}C	8.81	10.38	10.13	9.60	2 3 4 1
	15 ^{o}C	15.50	17.63	16.73	16.35	2 3 4 1
	20 ^{o}C	24.56	26.64	26.35	25.36	2 3 4 1
SG:		87	90	90	90	2 3 4 1

* See table 4-2.

4.2.8 Relationship between standard germination, vigour and several seed physical characteristics

Amongst the laboratory quality tests, both germination and seed vigour were negatively affected by thousand seed weight, mechanical damage and 4h imbibition rate, and were positively influenced by the imbibition rate between 4 and 6 h (Table 4-7). The influence of imbibition rate on standard germination was much greater than on seed vigour (Table 4-7).

Table 4-7 Correlation coefficient of standard germination (SG), seed vigour (AA2, CD16) with thousand seed weight (TSW), mechanical damage (MD) and imbibition rate (IR4, IR4-6) in 7 seed lots

	TSW	MD	IR4	IR4-6
SG	-0.544	-0.771*	-0.914**	0.936**
AA2	-0.596	-0.596	-0.648	0.657
CD16	-0.527	-0.732+	-0.609	0.638

+, * ** significant at 10%, 5% and 1% probability level respectively.

IR4, IR4-6: See Table 4-2.

AA2: 2-day AA.

CD16: CD with 16% SMC.

CHAPTER 5

QUALITY CHANGES DURING STORAGE

5.1 Seed moisture content (SMC)

Prior to storage, SMC of the 4 seed lots ranged from 10.9% to 12.4%. Storage for 1 month under controlled conditions produced SMC's of 6.6% at 45% RH, 9.7% at 60% RH, 14.0% at 75% RH and 27% at 90% RH, and 9.1% under ambient conditions (Figure 5-1). There were no significant difference in SMC between seed lots (Appendix 1).

SMC reached equilibrium after 1 month in all but the 90% RH treatment. AT 90% RH, SMC continued to increase, and by 2 months of storage, the seed was dead (Figure 5-1). SMC under ambient conditions varied slightly (from 7.1% to 9.9% with an average of 8.8%) as storage conditions changed with time (Figure 5-1).

5.2 Storage fungi content (SFC)

SFC did not differ significantly between seed lots under most storage regimes (Appendix 2). Seed stored at 45% RH, 60% RH and under ambient conditions (with SMC from 6.6 to 9.9%) maintained the original low SFC over the 11 months, with the average for the lots ranging from 1 to 4% (Figure 5-2, Appendix 2). However, a high SFC occurred at 75% and 90% RH (with SMC from 14.1 to 30.6%). After 1 month of storage under these two high RH conditions, the SFC was significantly increased ($P < 0.05$) and continued to increase as the storage period increased. For instance, the SFC at 75% RH was 4% after 1 month, 35% after 2 months and 100% after 11 months of storage. The main storage fungi were Aspergillus glaucus at 75% RH, and a Penicillium sp. at 90% RH. Table 5-1, Plates 3, 4).

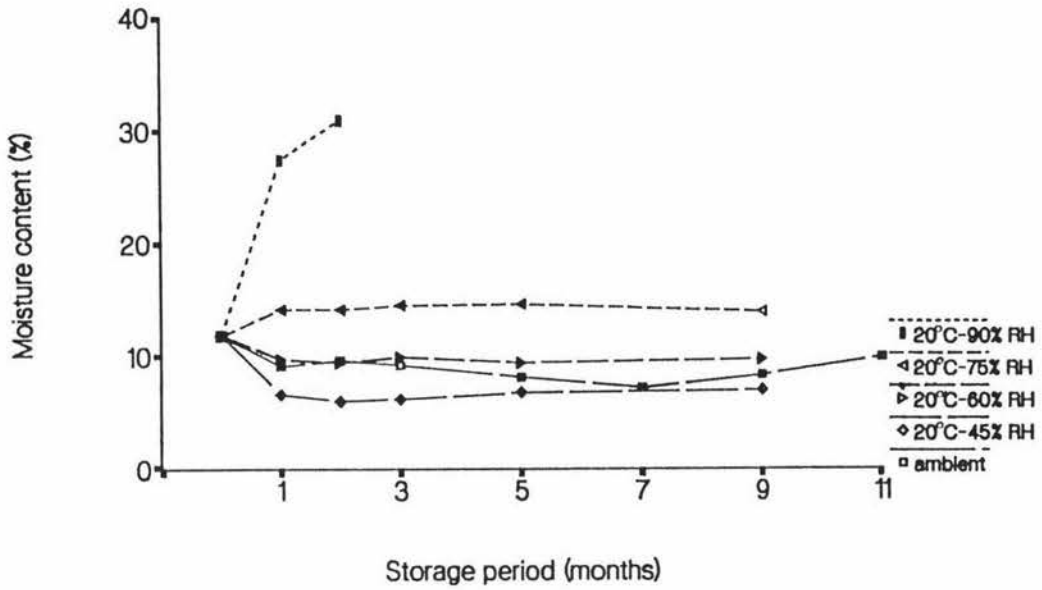

Figure 5-1 Moisture content of Pawera seed lots after storage at different relative humidities (RH).

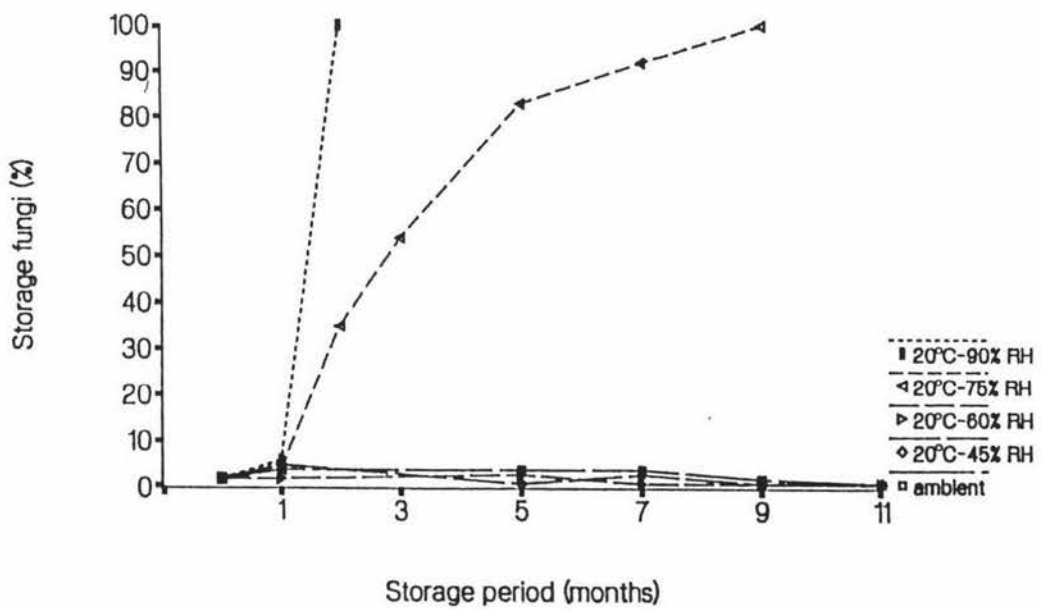

Figure 5-2 Storage fungi content of Pawera seed lots after storage at different relative humidities (RH).

Table 5-1 Incidence (%) of main storage fungi in Pawera seed lots stored for 11 months at different relative humidities

Storage condition	Fungal species	Storage period (months) 0	1	2	3	5	7	9	11
20 °C-45% RH	A. glaucus	1a+	1a	--	--	0a	0a	0a	0a
	A. flavus	1a	1a	--	--	0a	0a	0a	0a
	P. sp.	0a	3a	--	--	1a	1a	1a	1a
20 °C-60% RH	A. glaucus	1a	0a	--	--	2a	0a	0a	0a
	A. flavus	1a	0a	--	--	0a	0a	0a	
	P. sp.	0a	2a	--	--	3a	1a	1a	1a
Ambient	A. glaucus	1a	0a	--	--	2a	1a	1a	0a
	A. flavus	1a	1a	--	--	0a	1a	0a	0a
	P. sp.	0a	3a	--	--	2a	2a	1a	1a
20 °C-75% RH	A. glaucus	1a	0a	28b	53c	83d	92e	100e	
	A. flavus	1a	1a	2a	0a	0a	0a	0a	
	P. sp.	0a	3a	5a	1	0a	0a	0a	
20 °C-90% RH	A. glaucus	1a	3a	0a					
	A. flavus	1a	1a	0a					
	P. sp.	0a	a3	100b					

A: Aspergillus P: Penicillium

+ For each species at each storage condition, means within any line followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's multiple range test.

Plate 3. Seed infected with a Aspergillus glaucus following storage at 20 ^{O}C-75% RH for 5 months

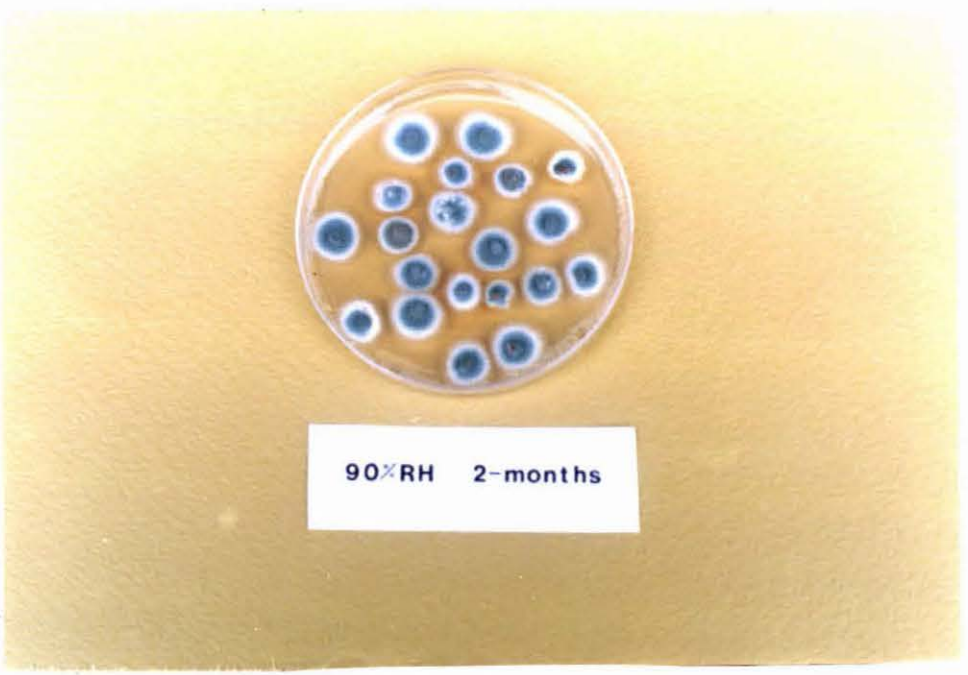

Plate 4. Seed infected with a Penicillium sp. following storage at 20 ^{O}C-90% RH for 2 months

5.3 Standard germination

5.3.1 Normal seedlings

The percentage of normal seedlings (NS or SG) did not differ significantly ($P < 0.05$) between seed lots before storage (0 month). However, significant differences occurred during storage, beginning after 1 months storage at all RHs (Figures 5-3, 5-4). With a few exceptions, the results very consistently indicate that the lots which had a high initial vigour i.e. lots 2 and 4 (Chapter 4) maintained a higher germination during storage than those with initial low vigour i.e. lots 1 and 3 (Figures 5-3, 5-4).

The germination response to the storage periods reflected both the seed lot vigour and the storage conditions. Under dry and sub-dry conditions i.e. 45% RH, 60% RH and ambient, lots 2 and 4 maintained a germination around 90% over the storage period, but the germination in lots 1 and 3 gradually declined as the storage period increased (Figure 5-3). There was a significant decline ($P < 0.05$) after 9 months storage in lot 1 at 45% RH, after 7 months in lot 3 at 60% RH, and after 5 months in both lots 1 and 3 at ambient conditions. Under high relative humidities, i.e. 75% RH and 90% RH, the germination of all seed fell rapidly as the storage period increased (Figure 5-4). The loss of germination in lot 3 was slightly quicker than lot 2 or 4 during the first 3 months at 75% RH, and the first month at 90% RH, but not at other periods (Figure 5-4 A, B). At 75% RH, the loss of germination was slow in the first 2 months, fast from 2 to 7 months, and after then it slowed again (Figure 5-4A). At 90% RH, the loss was very quick over the total 2 months period, the average germination was only 51% after the first month, and only 1% after 2 months storage (Figure 5-4 B).

The germination response to the different storage conditions varied between seed lots and storage periods (Table 5-2). After 1 month, the germination of all lots at 90% RH was significantly lower ($P < 0.05$) than that of the other conditions; after 2 months at 75% RH for lots 3 and 4, or 3 months for lots 1 and 2, germination was significantly lower than at 45% RH, 60% RH and ambient conditions. The germination at ambient and 60% RH was lower than at 45% RH as the storage period increased e.g. after 7 months; this was more obvious in lot 3 than in the other lots (Table 5-2).

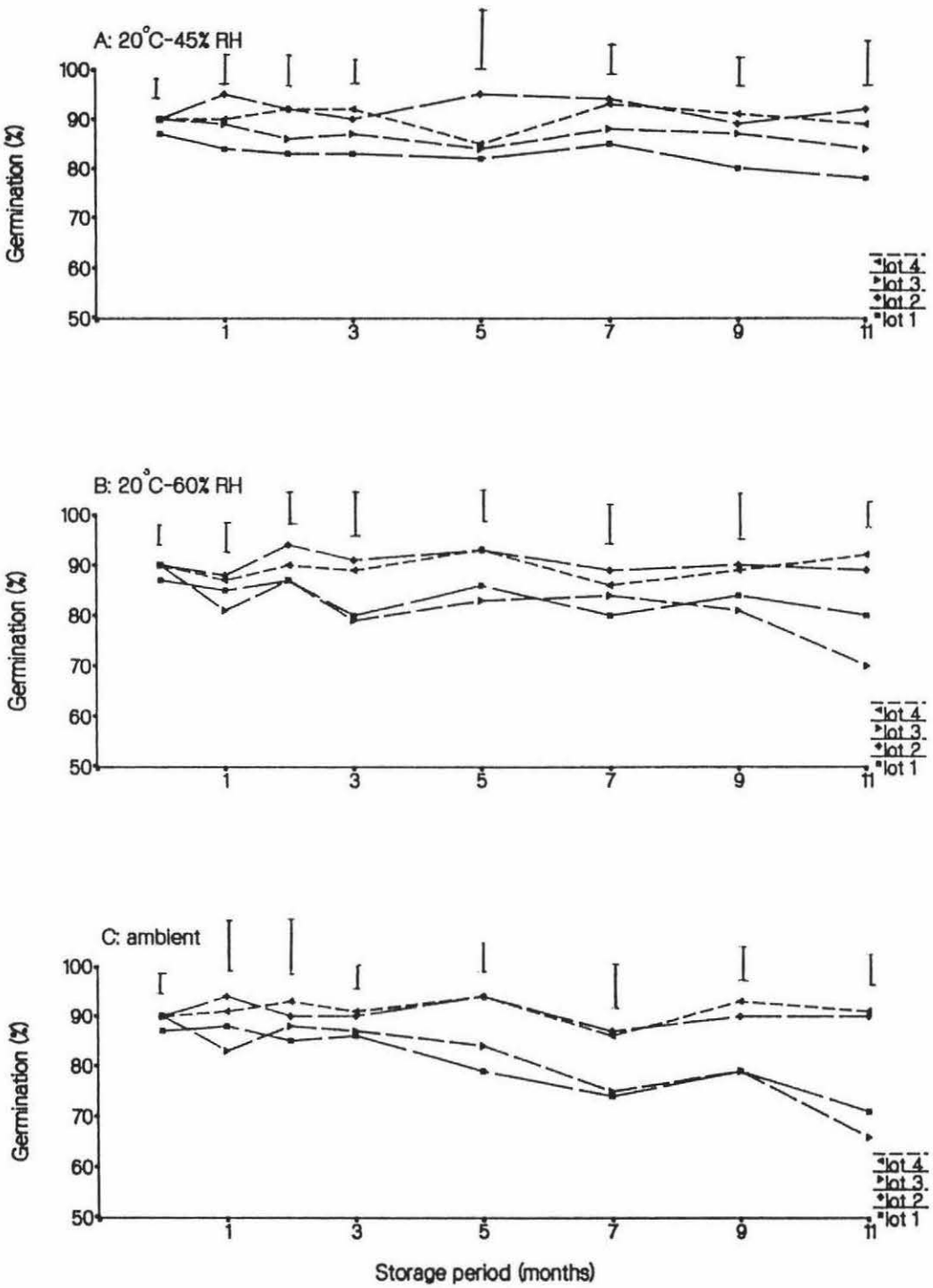

Figure 5-3 Standard germination of 4 Pawera seed lots stored under conditions of 20°C-45% RH (A), 20°C-60% RH (B), and ambient (C). Vertical bars represent LSD at $P \leq 0.05$.

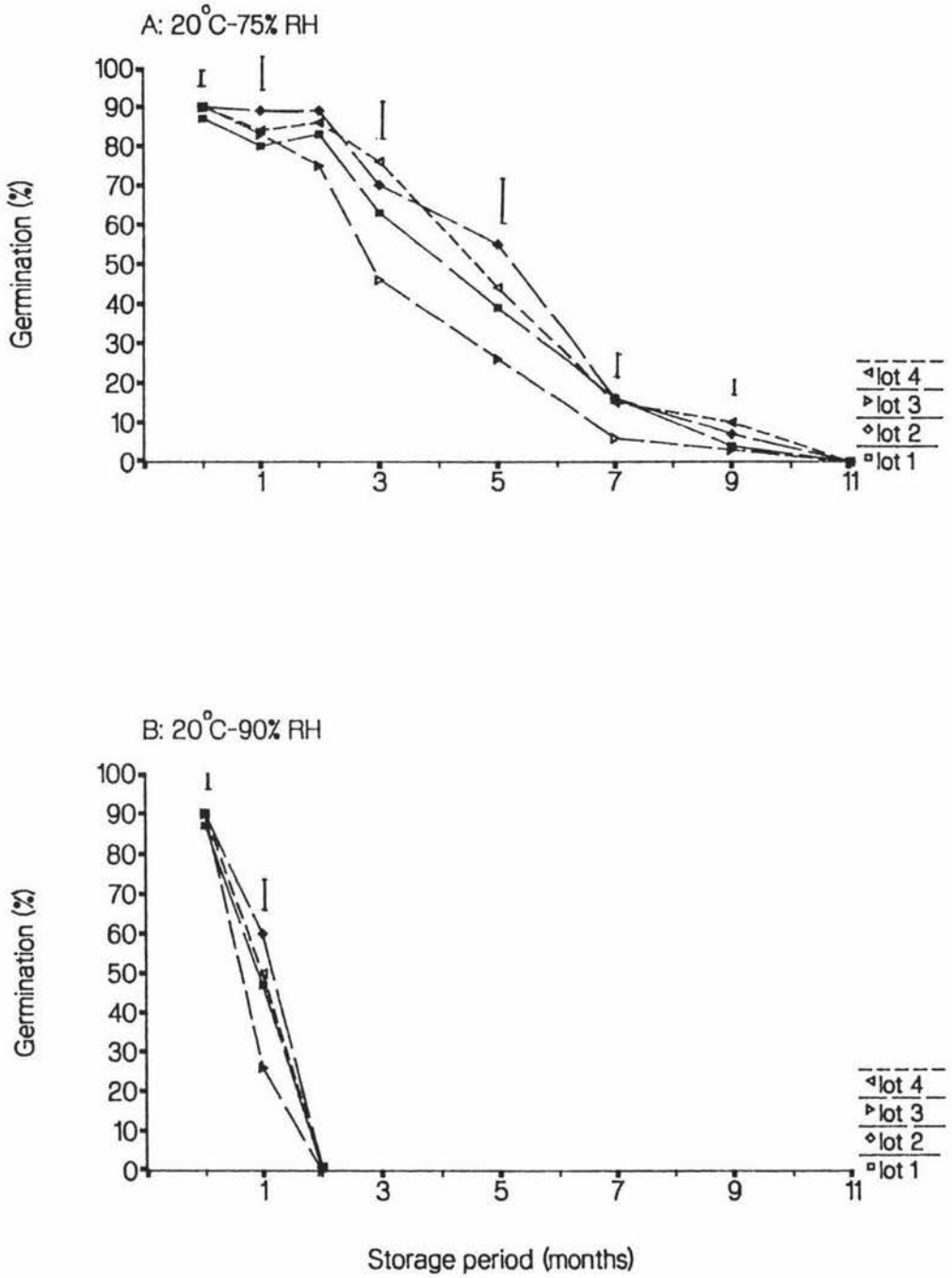

Figure 5-4 Standard germination of 4 Pawera seed lots stored under conditions of 20°C-75% RH (A), 20°C-90% RH (B). Vertical bars represent LSD at $P \leq 0.05$.

Table 5-2 Normal seedling s(%) following germination of Pawera seed lots stored for 11 months at different relative humidities

Lot	Storage condition	Storage period (months) 1	2	3	5	7	9	11
Lot 1	45% RH	84a*	83a	83a	82a	85a	80a	78a
	60% RH	85a	87a	80a	86a	80ab	84a	80a
	ambient	88a	85a	86a	79a	74b	79a	71a
	75% RH	80a	83a	63b	39b	16c	4b	0b
	90% RH	47b	1b					
Lot 2	45% RH	95a	87a	90a	96a	94a	90a	92a
	60% RH	88a	94a	91a	93a	89ab	90a	89a
	ambient	94a	90a	90a	94a	87b	90a	90a
	75% RH	89a	89a	70b	55b	15c	7b	0b
	90% RH	60b	1b					
Lot 3	45% RH	89a	86a	87a	84a	88a	87a	84a
	60% RH	81a	85a	80a	83a	84a	81b	70b
	ambient	83a	88a	87a	84a	76b	78b	66b
	75% RH	83a	75b	46b	26b	6c	3c	0c
	90% RH	26b	0c					
Lot 4	45% RH	90a	92a	92a	86a	93a	91a	89a
	60% RH	87a	90ab	89a	93a	86b	89a	92a
	ambient	91a	93a	91a	95a	86b	83a	91a
	75% RH	87a	86b	76b	44b	15c	10b	0b
	90% RH	50b	5c					

* For each seed lot, means within any column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's multiple range test.

5.3.2 Four-day count

The changes in the percentage germination after four days (SG4) were similar to the percentage of normal seedlings at the final count (Figures 5-3, 5-4), both between seed lots and storage conditions, or storage periods (Figures 5-5, 5-6). However, there were several exceptions: first, the SG4 of lot 4 was significantly lower ($P < 0.05$) than that of lot 2 at 45% RH (Figure 5-5A), although both lots had similar final germinations (Figure 5-3A); second, the SG4 under dry condition (i.e. 45% RH) was significantly lower than that at higher humidities (i.e. 60% RH and ambient) for the first few months (Figure 5-5 A,B,C; Plate 5); third, the variation in results as indicated by the LSD in SG4 (Figures 5-5, 5-6) was higher than that for standard germination (Figures 5-3, 5-4).

5.3.3 Abnormal seedlings (AS)

The AS% of the 4 seed lots did not differ significantly ($P < 0.05$) at 45% RH and ambient conditions for the first 3 months, and at 60% RH and 75% RH for the first month. Significant differences, however occurred in the remaining storage conditions and periods (Table 5-3). Amongst the differences, the AS% of lot 1 or 3 was usually higher than that of lot 2 or 4 ($P < 0.05$).

5.3.4 Hard seed (HS)

The HS% was less than 5% and with a few exceptions, there were no significant differences ($P < 0.05$) between seed lots, storage periods or conditions (Table 5-4).

5.3.5 Dead seed (DS)

Significant differences in the DS% between seed lots existed in most of the tests, with the exceptions of seed stored at 45% RH for 3 months, and at 60% RH for 5 and 9 months (Table 5-5). In general, the DS% of lot 1 or 3 was higher ($P < 0.05$) than that of lot 2 or 4 (Table 5-5). The DS in lots stored at 45% and 60% RH, and in lots 2 and 4 at ambient was not affected by different storage periods, while the DS% in lots 1 and 3 at ambient conditions, and in all lots at 75% RH and 90% RH declined significantly as the storage periods increased (Table 5-5).

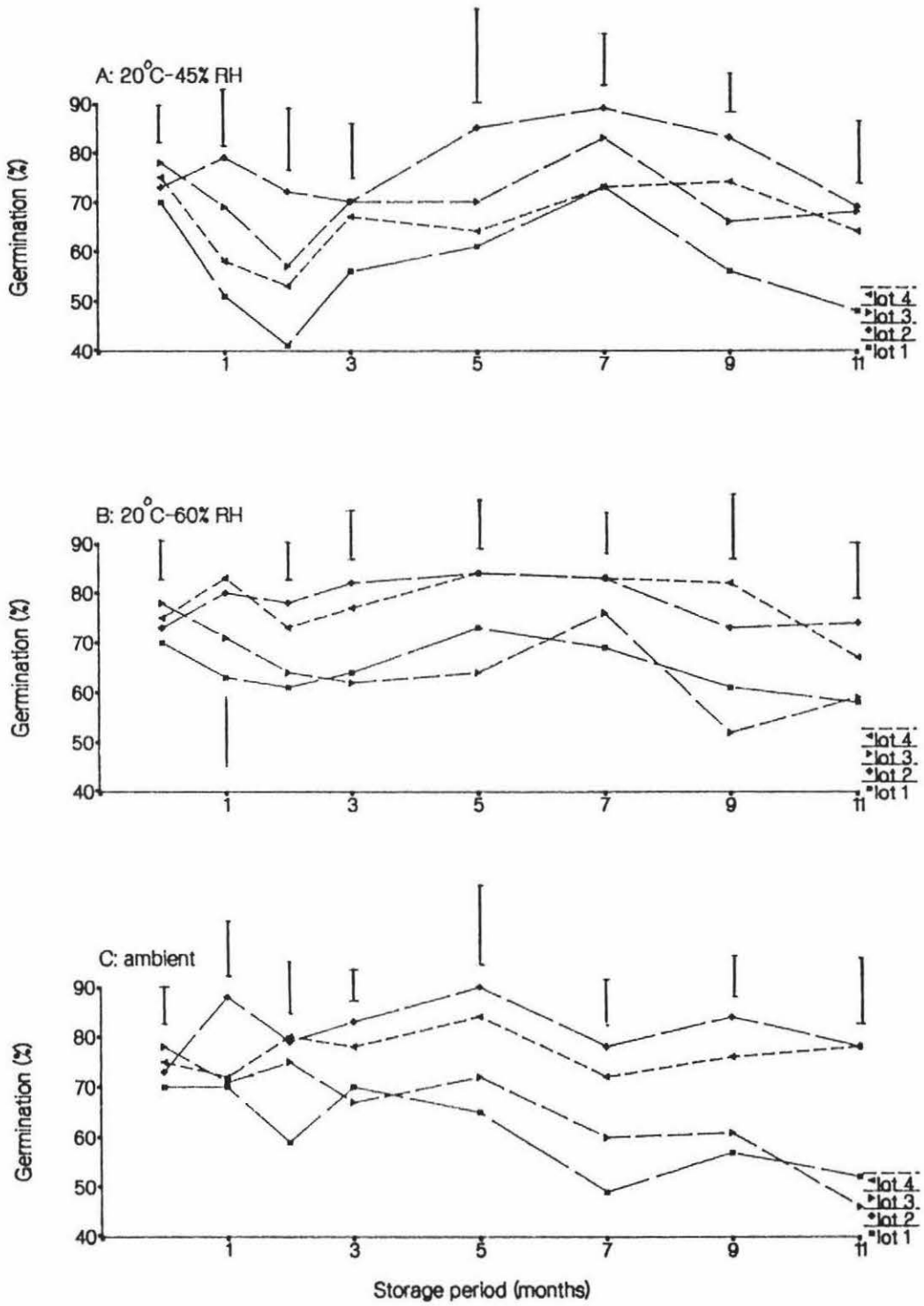

Figure 5-5 Four-day count of standard germination of 4 Pawera seed lots stored under conditions of 20°C-45% RH (A), 20°C-60% RH (B), and ambient (C). Vertical bars represent LSD at $P \leq 0.05$.

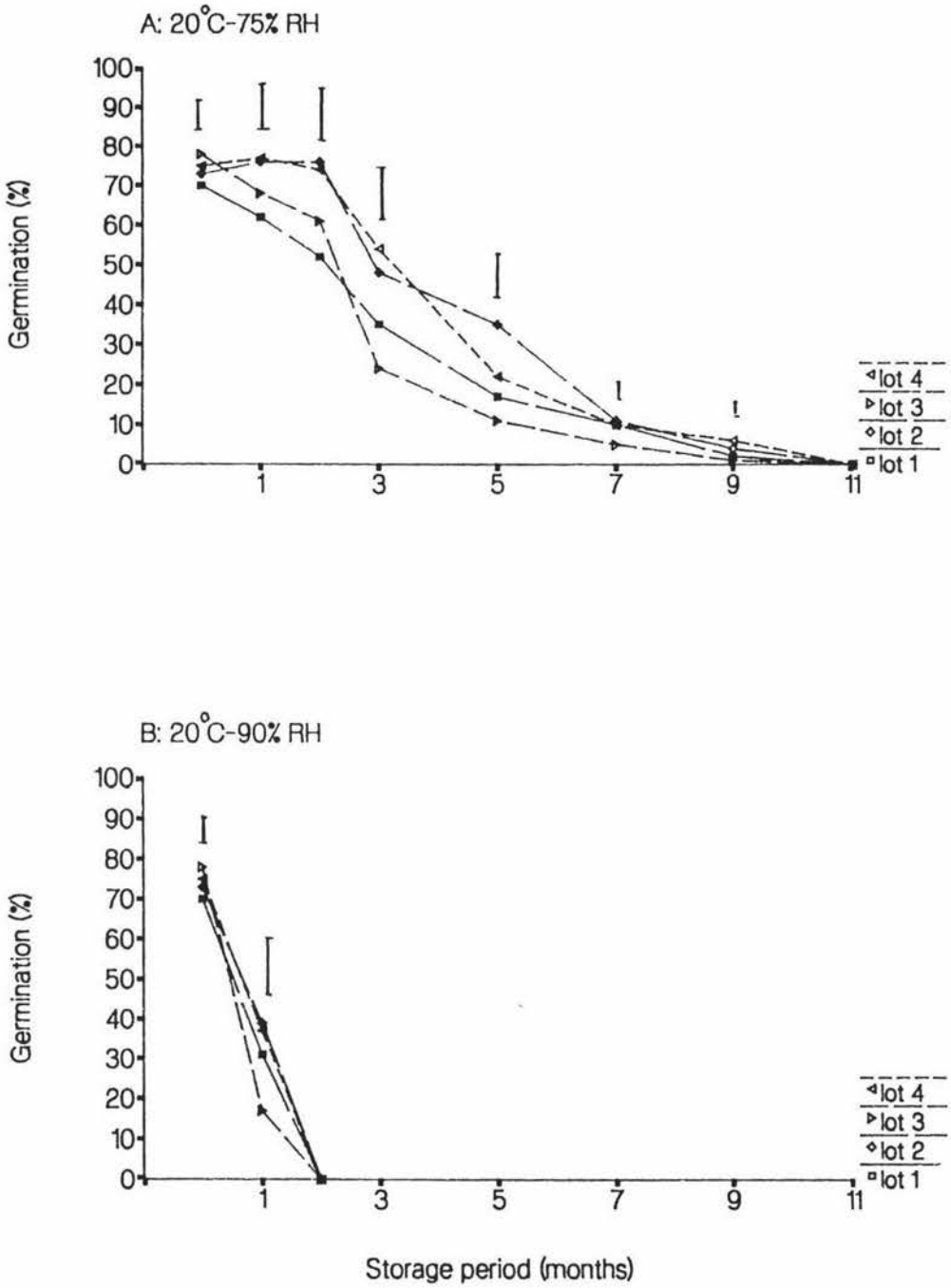

Figure 5-6 Four-day count of standard germination of 4 Pawera seed lots stored under conditions of 20°C-75% RH (A), 20°C-90% RH (B). Vertical bars represent LSD at $P \leq 0.05$.

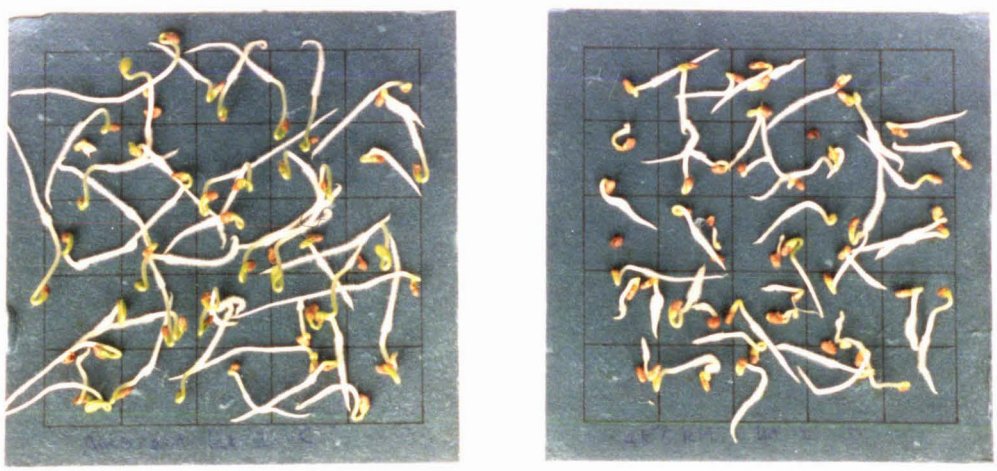

Plate 5. Germination (4-day count) after storage under ambient conditions and at 20 ^{o}C-45% RH for 3 months

Table 5-3 Abnormal seedlings (%) after germination of 4 Pawera seed lots stored for 11 months at different relative humidities

Storage condition	Lot	Storage period (months)						
		1	2	3	5	7	9	11
20 °C-45% RH	1	8*	10a	8a	8a	7a	11a	12a
	2	5a	10a	6a	2a	3b	9ab	4c
	3	4a	6a	8a	9b	6ab	8bc	10ab
	4	5a	4a	5a	11a	2b	4c	6bd
20 °C-60% RH	1	7a	9ab	10ab	8ab	10a	7ab	5b
	2	9a	4c	5b	4b	9a	6bc	4b
	3	11a	10a	14a	12a	12a	10a	14a
	4	6a	6bc	6b	5b	6b	4c	6b
ambient	1	9a	8a	7a	15a	11a	8b	12ab
	2	5a	7a	6a	3b	7b	7b	7b
	3	10a	8a	6a	10ab	12a	15a	17a
	4	5a	3a	5a	4b	9ab	4b	6b
20 °C-75 RH	1	12a	10b	21b	34a	20bc	11b	
	2	8a	8b	21b	26b	36a	17a	
	3	13a	17a	30a	26b	19c	6b	
	4	7a	7b	5c	23b	32ab	10b	
20 °C-90% RH	1	10b	0b					
	2	19a	6a					
	3	12b	1b					
	4	21a	2ab					

* For each storage condition, means within any column followed by the same letter do not differ significantly at the 5% level of probability according to Duncan's multiple range test.

Table 5-4 Hard seed (%) after germination of 4 Pawera seed lots stored for 11 months at different relative humidities

Storage condition	Lot	Storage period (months) 1	2	3	5	7	9	11
20 °C-45% RH	1	0a	1a	4a	3a	4a	2a	3a
	2	1a	3a	3a	1a	2a	0a	2bc
	3	1a	2a	1a	1a	1a	0a	0c
	4	4a	3a	1a	4a	3a	0a	3ab
20 °C-60% RH	1	3ab	3a	3a	1a	4a	5a	3a
	2	2bc	1a	4a	1a	2bc	4a	3a
	3	1c	2a	1a	1a	1c	2a	1a
	4	4a	3a	2a	2a	3ab	5a	3a
ambient	1	3a	2a	3a	3a	2a	2a	2a
	2	1a	4a	3a	2a	2a	1a	1a
	3	3a	1a	1a	1a	2a	2a	0a
	4	3a	3a	3a	1a	1a	0a	1a
20 °C-75 RH	1	1a	2a	1b	1a	0a	0b	1a
	2	2a	2a	2ab	4a	2a	1b	0a
	3	1a	0a	0b	1a	0a	0b	1a
	4	3a	2a	4a	3a	4a	3a	1a
20 °C-90% RH	1	1a	1a					
	2	1a	1a					
	3	1a	1a					
	4	1a	1a					

* See Table 5.3.

Table 5-5 Dead seed (%) after germination of 4 Pawera seed lots stored for 11 months at different relative humidities

Storage condition	Lot	Storage period (months) 1	2	3	5	7	9	11
20 °C-45% RH	1	7a	7a	5a	8a	5ab	8a	9a
	2	0b	1c	2a	2bc	1c	2b	3b
	3	7a	6ab	4a	6ab	6a	7a	6ab
	4	2b	2bc	3a	1c	5bc	2b	3b
20 °C-60% RH	1	6a	2b	8a	5a	6a	5a	5b
	2	2b	1b	1c	2a	1b	2a	3b
	3	8a	4a	7ab	6a	5ab	2a	11a
	4	6ab	2ab	4bc	2a	6a	3a	0c
ambient	1	5a	5a	5ab	5b	14a	12a	17a
	2	1c	1c	2bc	2b	5b	3c	3b
	3	4ab	4ab	7a	7a	13a	12ab	18a
	4	2bc	2bc	1c	2b	4a	6bc	3b
20 °C-75 RH	1	8a	6a	16ab	28ab	64b	85a	99a
	2	2b	2b	8c	17c	47c	76b	100a
	3	4a	8a	24a	48a	76a	92a	99a
	4	4a	6a	9bc	36ab	50c	78b	99a
20 °C-90% RH	1	43b	98ab					
	2	21c	93c					
	3	62a	99a					
	4	28c	97bc					

* See table 5-3.

5.4 Conductivity

Measurements of the conductivity of seed steep water before and after storage were highly effective for differentiating high and low vigour seed lots (Figures 5-7, 5-8, 5-9). Conductivity of lot 3 was significantly greater ($P < 0.05$) than that of lots 2 or 4, while that of lot 1 was intermediate.

As the storage period increased, in general, the conductivity of seed with initially the poorest vigour (i.e. lot 3) gradually increased at all of the storage conditions, while for the other lots, obvious increases over the storage period only occurred under adverse conditions i.e.75% RH (except after 9 months) (Figure 5-9). At 45% RH, 60% RH and ambient, the conductivity of lots 1, 2 and 4 was also increased from 0 to 5 months; however, after 5 months there were no further significant changes at 60% RH and ambient, but a significant decline occurred after 7 months at 45% RH for all lots (Figures 5-7, 5-8).

The difference in conductivity between the highest vigour lot and lowest vigour lot tended to increase as the storage period increased under all of the conditions (Figures 5-7, 5-8, 5-9).

5.5 Quality changes from spring to autumn

The conductivity of seed stored at 5 ^{o}C did not change, and viability (via standard germination) for 6 of the 7 seed lots did not differ (except for lot 7) after storage from spring (August 1988) to autumn (April 1989). However, germination declined and conductivity increased for all 7 lots when they were stored under ambient conditions (Table 5-6). The field emergence of seed stored at 5 ^{o}C was significantly higher ($P < 0.05$) than that of seed stored under ambient conditions for 5 of the 7 seed lots, following sowing on 21st March, 1989.

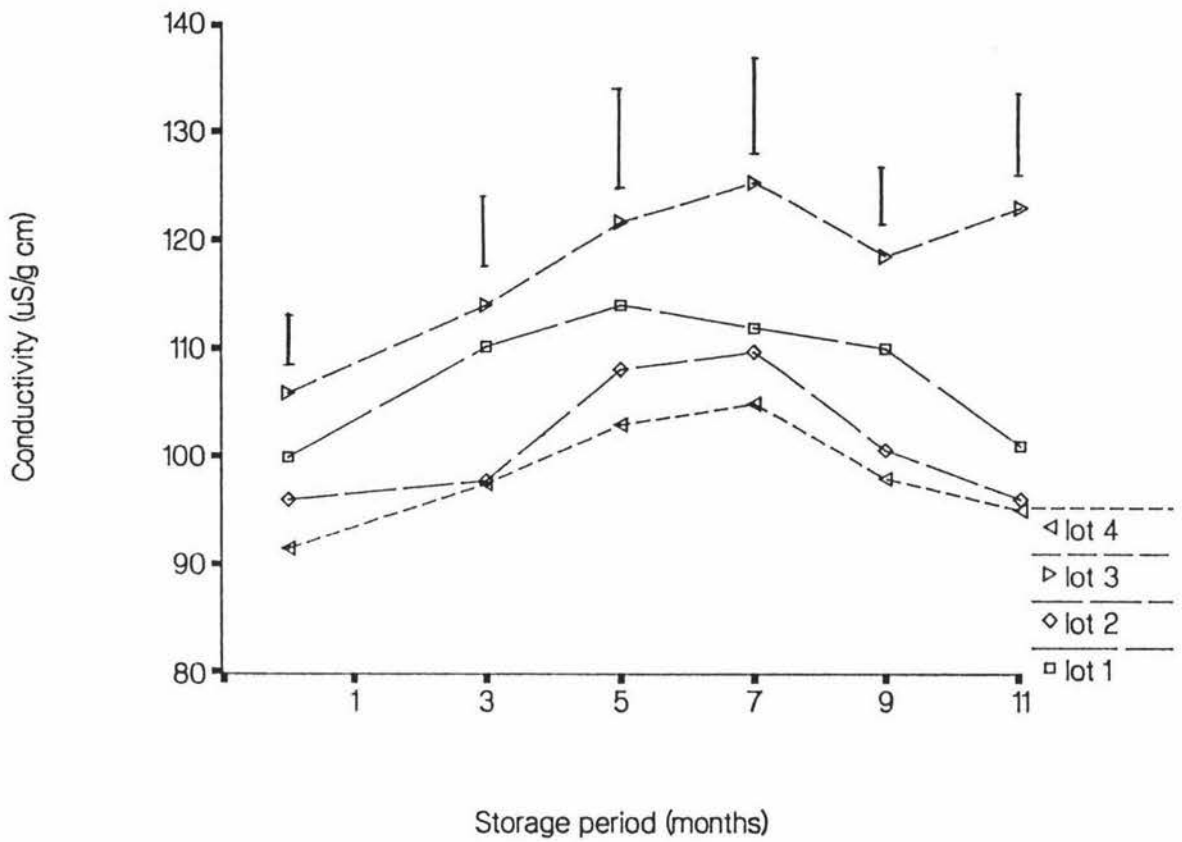

Figure 5-7 Conductivity of 4 Pawera seed lots stored at 20°C-45% RH. Vertical bars represent LSD at $P \leq 0.05$.

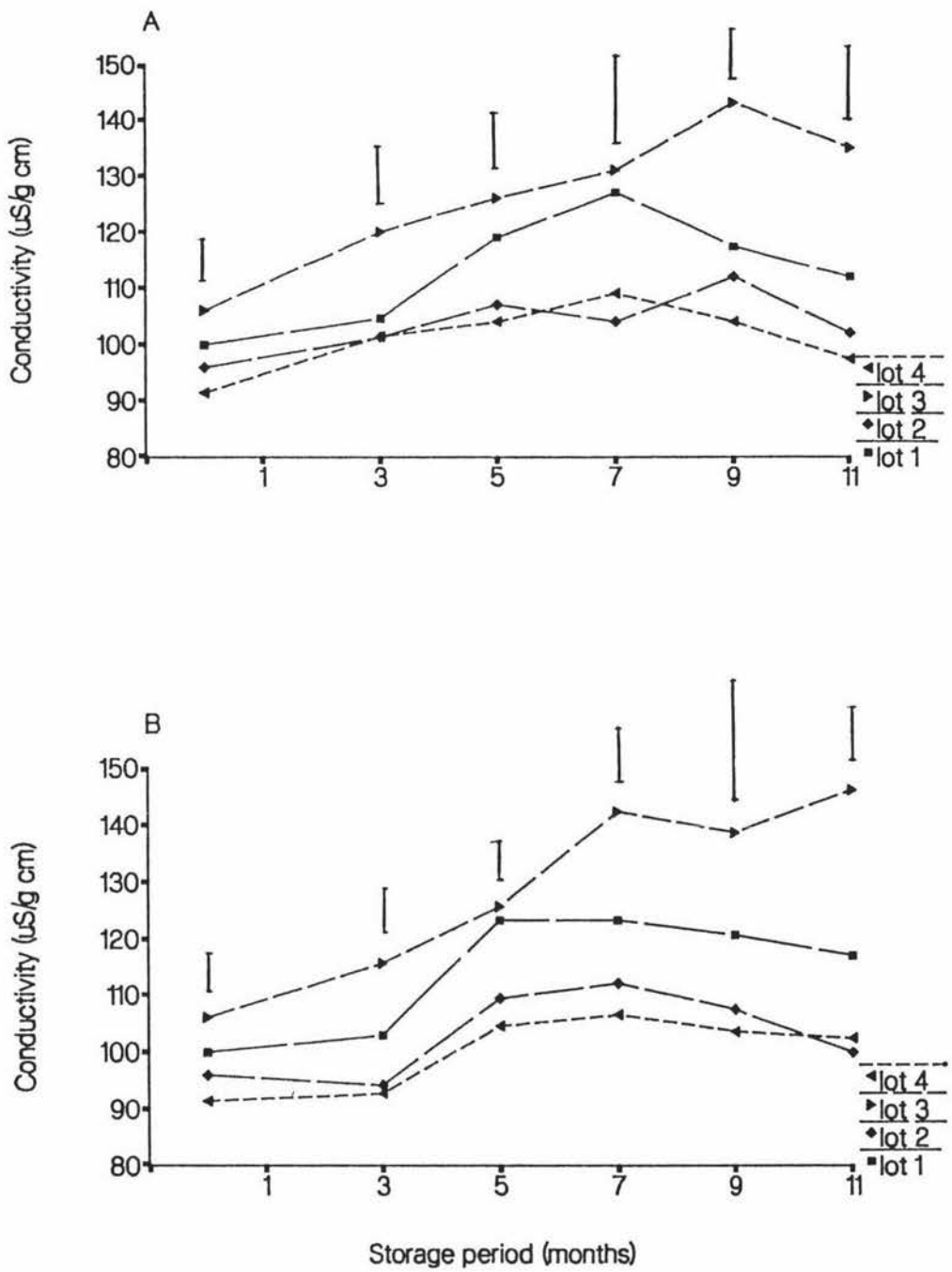

Figure 5-8 Conductivity of 4 Pawera seed lots stored at 20°C-60% RH (A), and ambient (B). Vertical bars represent LSD at $P \leq 0.05$.

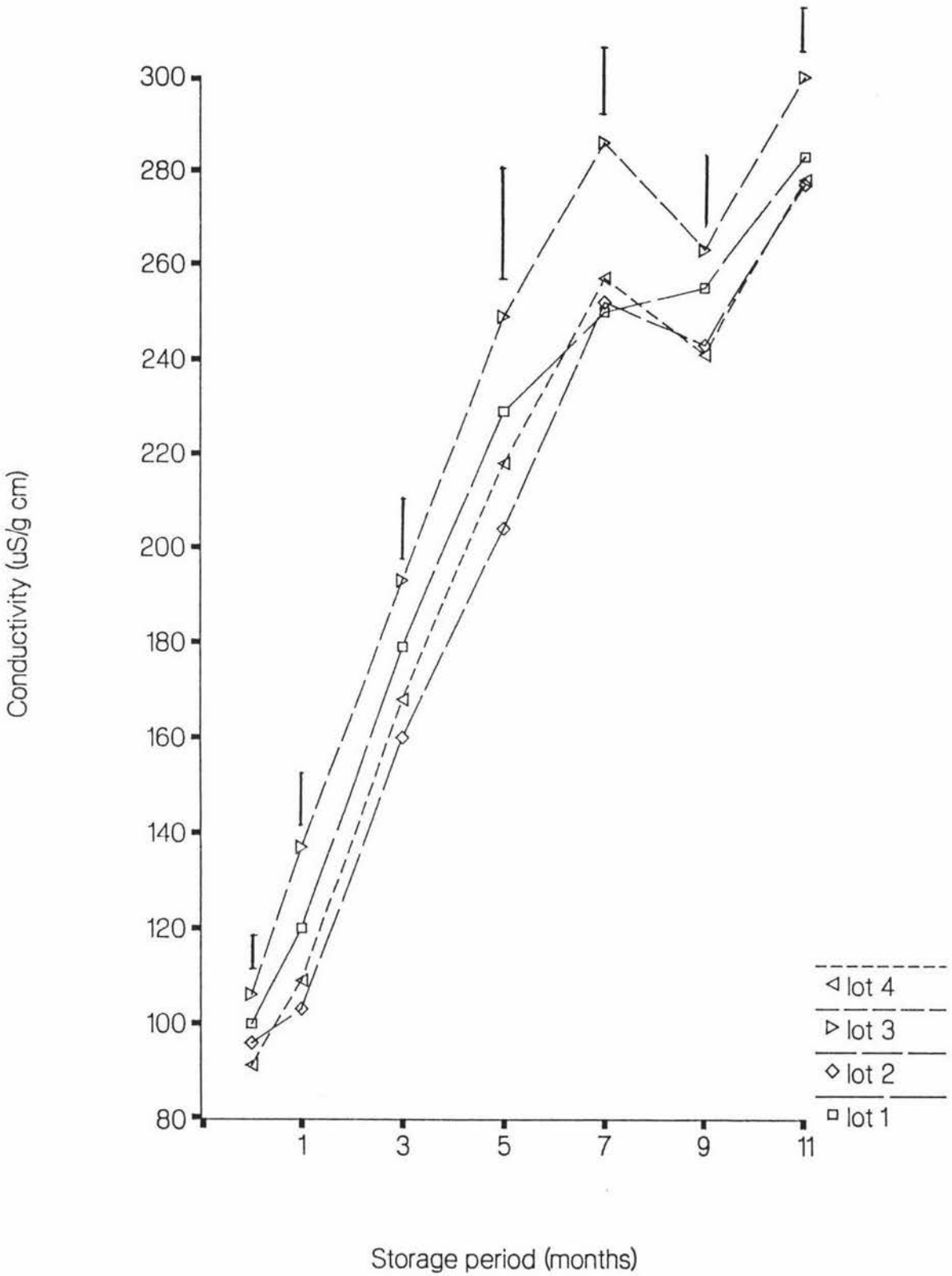

Figure 5-9 Conductivity of 4 Pawera seed lots stored at 20°C-75% RH. Vertical bars represent LSD at P ≤ 0.05.

Table 5-6 Quality changes in 7 Pawera seed lots after storage at 5 °C and under ambient conditions for 7 months

Test	Storage condition	Seed lot 1	2	3	4	5	6	7
Standard germination (%)	PS	87a*	90a	90a	90a	92a	90a	82a
	AS-5 °C	89a	90a	87ab	91a	91a	94ab	76b
	AS-ambient	74b	87a	75b	86a	83b	89b	69c
Conductivity (uS/g cm)	PS	100b	96b	106b	92b	103b	98b	115b
	AS-5 °C	102b	97b	113b	95b	101b	94b	120b
	AS-ambient	123a	112a	142a	107a	121a	108a	165a
Field emergence (%)	AS-5 °C	59a	76a	56a	78a	71a	80a	46a
	AS-ambient	52a	70a	41b	59b	53b	53b	35b

PS: Pre-storage AS-5 °C: after storage at 5 °C

AS-ambient: after storage under ambient conditions

* For each test, means within any column follwed by the same letter do not differ significantly at the 5% level of probability according to Duncan's multiple range test.

CHAPTER 6

RELATIONSHIP BETWEEN SEED VIGOUR AND SEED PERFORMANCE

6.1 Relationship between vigour and field emergence

6.1.1 Field performance

6.1.1.1 Field emergence

Significant differences ($P < 0.05$) in field emergence between the 6 seed lots were recorded at 7 of the 8 sowings (Table 6-1); the low vigour seed lots (1 and 3) always had the lowest emergence (e.g Plate 6). The emergence of the other 4 seed lots did not differ ($P < 0.05$) at 5 of the sowings, although the ranking order was not always consistent. The average emergence of lots ranged from 55% (18 Oct.) to 78% (23 Sep.), while the emergence period varied from 13 to 25 days (Table 6-1).

6.1.1.2 Emergence rate

The lots which had lower field emergence were also slower in emergence rate (Table 6-2). For example, the lot ranking for emergence rate (Table 6-2) was very similar to that for final field emergence (Table 6-1) over the 8 sowings. The emergence rate was strongly correlated to the emergence percentage at each of the sowings [r=0.817 (20 Apr.) to 0.969 (21 Mar.), $P < 0.001$].

6.1.1.3 Seedling length and dry weight

The highest seedling length and dry weight in spring and autumn (13 Nov., and 21 Mar) (Table 6-3, Plate 7) were associated with high temperatures. Full environmental data are showed in Table 6-4.

The seedling length did not differ markedly between seed lots (Table 6-3). There were no differences in seedling dry weight between seed lots at all three spring sowings; however in autumn, as the temperature decreased (Table 6-4), the seedling dry weight of lot 3 was significantly lower than that of lots 4 and 1 at some sowings (Table 6-3).

Table 6-1 Field emergence (%) of 6 Pawera seed lots at 8 sowing dates

Sowing date	Lot						Mean	Lot ranking*	Mean emergence period (days)
	1	2	3	4	5	6			
23 Sep.	71	80	72	83	80	82	78	4 6 2 5 3 1	13
18 Oct.	45	58	50	63	51	60	55	4 6 2 5 3 1	25
13 Nov.	50	70	61	65	71	67	64	5 2 6 4 3 1	17
21 Mar.	59	76	56	78	71	80	70	6 4 2 5 1 3	13
5 Apr.	68	75	59	78	79	79	73	6 5 4 2 1 3	19
20 Apr.	65	77	68	89	79	84	77	4 6 5 2 3 1	14
9 May	65	73	69	75	75	85	64	6 4 5 2 3 1	14
20 May	58	67	57	79	61	72	66	4 6 2 5 1 3	19

* Seed lots sharing the same line do not differ significatly at the 5% level of probability according to Duncan's multiple range test.

Table 6-2 Field emergence rate of 6 Pawera seed lots at 8 sowing dates

Sowing date	Lot						Mean	Lot ranking*
	1	2	3	4	5	6		
23 Sep.	12.5	15.6	12.6	15.7	14.4	15.4	14.4	4 2 6 5 3 1
18 Oct.	4.6	6.2	5.4	7.1	5.7	7.1	6.0	4 6 2 5 3 1
13 Nov.	5.3	7.5	6.4	6.8	7.6	7.3	6.8	5 2 6 4 3 1
21 Mar.	8.7	11.6	7.9	11.9	11.2	12.0	10.6	6 4 2 5 1 3
5 Apr.	6.3	7.2	5.0	7.7	7.8	8.1	7.0	6 5 4 2 1 3
20 Apr.	7.8	10.7	8.3	10.9	9.9	10.4	9.7	4 2 6 5 3 1
9 May	7.8	9.3	8.2	9.4	9.4	10.8	9.1	6 4 5 2 3 1
20 May	6.3	7.7	6.2	10.4	6.7	8.8	7.7	4 6 2 5 1 3

* See Table 6-1.

Table 6-3 Seedling length and dry weight of 6 Pawera seed lots at 8 sowing dates#

Sowing date	Character	Lot 1	2	3	4	5	6	Mean	Lot ranking*
23/Sep.	SL	24.0	23.0	22.5	22.3	22.3	21.8	22.6	1 2 3 5 4 6
	SDW	500	420	400	400	410	450	430	1 6 2 5 3 4
18/Oct.	SL	29.4	28.3	27.2	27.7	27.7	29.9	28.4	6 1 2 5 4 3
	SDW	1060	910	810	850	990	1070	948	6 1 5 2 4 3
13/Nov.	SL	36.5	36.1	35.9	37.3	36.4	37.5	36.6	6 4 1 5 2 3
	SDW	1640	1490	1550	1840	1880	1770	1695	5 4 6 1 3 2
21/Mar.	SL	15.9	14.9	15.5	15.9	15.5	16.3	15.7	6 4 1 5 3 2
	SDW	60.0	53.0	49.0	64.0	56.0	60.0	57.0	4 1 6 5 2 3
5/Apr.	SL	13.1	12.8	11.7	13.5	12.7	12.5	12.7	4 1 2 5 6 3
	SDW	31.0	29.0	24.0	34.0	30.0	30.0	29.7	4 1 6 5 2 3
20/Apr.	SL	11.1	10.8	11.0	11.3	10.9	11.8	11.2	6 4 1 3 5 2
	SDW	19.8	22.8	21.3	26.8	25.0	23.0	23.1	4 5 6 2 3 1
9/May	SL	9.2	9.2	8.5	9.5	9.6	9.7	9.3	6 5 4 2 1 3
	SDW	10.7	9.0	8.6	9.4	9.9	9.4	9.5	1 5 4 6 2 3
20/May	SL	7.7	7.6	7.0	7.5	7.4	7.8	7.5	6 1 2 4 5 3
	SDW	7.4	5.6	6.3	7.4	6.0	7.2	6.7	1 4 6 3 5 2

#: The data were recorded 8 weeks after planting in spring (first 3 dates) and after 5 weeks in autumn.

*: See Table 6-1

SL: Seedling length (cm/seedling)

SDW: Seedling dry weight (mg/seedling)

Table 6-4 Field environment during seedling emergence and growth*

Sowing date	Environment	Week 1	2	3	4	5	6	7	8	Mean mT	Total mR
23 Sep.	T	13.1	13.3	12.9	12.5	12.7	14.1	13.6	14.2	14.1	
	R	0.6	5.9	2.3	6.7	0.2	0.4	2.2	0		2.3
18 Oct.	T	12.6	14.1	13.7	13.5	15.1	17.2	17.9	17.0	15.6	
	R	4.9	0.6	1.7	0.5	1.9	3.5	1.7	0.6		1.9
13 Nov.	T	14.3	16.0	18.5	16.8	17.8	18.8	19.9	20.1	17.8	
	R	1.9	0	5.2	0.5	0.2	0.5	6.7	0.6		2.0
21 Mar.	T	14.2	14.8	13.7	12.7	13.8				14.8	
	R	1.4	1.2	1.3	1.3	0.1					1.1
5 Apr.	T	13.8	13.0	13.6	14.4	14.0				14.5	
	R	1.4	1.3	0	5.7	7.5					3.2
20 Apr.	T	13.2	14.7	13.0	12.1	10.8				13.2	
	R	0	8.7	4.6	3.3	1.7					3.7
9 May	T	11.0	11.2	10.2	8.5	10.2				10.2	
	R	2.8	1.2	2.2	1.9	3.0					2.2
20 May	T	11.3	8.3	8.8	10.9	7.3				8.9	
	R	2.3	1.7	1.1	2.9	4.7					2.5

T: Mean daily soil (10 cm) temperature (^{o}C) for each week

R: Accumulated rainfall (mm) for each week

mT: Mean daily soil (10 cm) temperature over the total period

mR: Mean weekly rainfall.

*: The climatic data were taken from DSIR, the recording site being about 500m away from the experimental plots.

Plate 6. Field emergence of a low vigour (lot 3) and high vigour (lot 4) seed lots

Plate 7. Seedling size for a low vigour (lot 3) and high vigour (lot 4) seed lots, 5 weeks after sowing in autumn

6.1.2 Vigour tests and field emergence

6.1.2.1 Standard germination

The 4-day, 7-day and final counts of the standard germination test were not significantly ($P < 0.05$) correlated with field emergence. The exception was the 7-day and final count and the last spring sowing (Table 6-5), where soil temperature was high (average 16.3 ^{o}C over the first 3 weeks) (Table 6-4).

6.1.2.2 Seed weight

Thousand seed weight did not significantly correlate with field emergence for 7 of the 8 sowings (Table 6-5). When the data for lot 1 which had the highest seed weight but also the highest percentage damaged seedwere removed from the analysis, the correlation was no longer negative, but still not significant (Table 6-5).

6.1.2.3 Damaged seed

Mechanically damaged seed showed a negative correlation with field emergence over the 8 sowings, and a significant correlation ($P < 0.10$) was recorded at 4 of the sowings (Table 6-5).

6.1.2.4 Conductivity

Conductivity was negatively correlated to field emergence in most of the measurements, but few were significant (Table 6-6). The correlation of conductivity with field emergence was stronger at the last autumn sowing (20 May) than other sowings, as a significant correlation occurred for 6 of 7 measurements. Soaking for 24 h was a better indicator than either the shorter (Table 6-6) or the longer periods , with a significant correlation ($P < 0.10$) being recorded at 3 of 8 sowings (Table 6-6). Using 6h or 8h data to predict 24 h conductivity did not improve the relationship with field emergence.

Table 6-5 Correlation coefficient of standard germination, 1000-seed weight, and mechanical damage with field emergence in 6 Pawera seedlots at 8 sowing dates

Sowing date	Correlation coefficient with					
	Standard germination			Seed weight		Mechanical damage
	4-day	7-day	final			
23 Sep.	0.279	0.515	0.616	-0.530	(-0.207)	-0.663
18 Oct.	0.192	0.462	0.392	-0.286	(0.219)	-0.762+
13 Nov.	0.655	0.827*	0.931**	-0.725^{+}	(-0.500)	-0.820*
21 Mar.	0.271	0.406	0.434	-0.283	(0.058)	-0.595
5 Apr.	0.228	0.323	0.412	-0.152	(0.033)	-0.342
20 Apr.	0.413	0.619	0.566	-0.207	(0.345)	-0.758^{+}
9 May	0.579	0.607	0.509	-0.084	(0.480)	-0.752^{+}
20 May	0.012	0.232	0.189	-0.050	(0.303)	-0.564
Mean	0.329	0.499	0.506	-0.290	0.093	-0.657

+, *, **: Significant at 10%, 5% and 1% probability level respectively
The data in brackets indicate a correlation calculated from 5 seed lots (i.e. excluding lot 1).

Table 6-6 Correlation coefficient between conductivity results and field emergence in 6 Pawera seed lots at 8 sowing dates

sowing date	Correlation coefficient with conductivity after different hours of soaking (h)						
	2	4	6	8	24	6 - 24	8 - 24
23 Sep.	0.144	-0.212	-0.399	-0.504	-0.663	0.443	-0.559
18 Oct.	-0.318	-0.524	-0.658	-0.771^{+}	-0.760^{+}	-0.653	-0.765^{+}
13 Nov.	0.424	0.266	0.111	-0.061	-0.167	0.122	-0.045
21 Mar.	-0.052	-0.407	-0.530	-0.596	-0.740^{+}	-0.512	-0.581
5 Apr.	0.101	-0.318	-0.377	-0.405	-0.614	-0.368	-0.406
20 Apr.	-0.215	-0.462	-0.521	-0.648	-0.662	-0.560	-0.684
9 May	-0.119	-0.220	-0.198	-0.283	-0.323	-0.195	-0.284
20 May	-0.477	-0.745^{+}	-0.831*	-0.901*	-0.887*	-0.830*	-0.901*
Mean	-0.064	-0.328	-0.425	-0.521	-0.602	-0.319	-0.528

* See Table 6-5

6 - 24: Conductivity at 24 h predicted after 6 h , by using the equation of Y = 16.5 + 1.20 x

8 - 24: Conductivity at 24 h predicted after 8 h, by using the equation of Y = 29.3 + 0.90 x

6.1.2.5 Accelerated aging

The correlation between germination after accelerated aging and field emergence varied both among the aging periods and among the sowings (Table 6-7). The correlation coefficients for the first spring sowing and the first and second autumn sowings were higher than for the other sowings. 2 day AA gave the best correlations with field emergence over all sowings. The relationship was improved by surface sterilization (Table 6-7).

6.1.2.6 Controlled deterioration

Significant correlations were found between field emergence and germination after CD at all sowing dates (Table 6-8). The correlation coefficients following CD at low SMC (i.e. 16% , 18% and 20%) with the first spring and first, second and third autumn sowings were higher than that of the other sowings. The relationship was not as strong at higher SMC CD tests (Table 6-8). CD at 16% and 18% SMCs tended to be better indicators of field emergence than the higher SMCs, although at each SMC (16% to 24%) there were significant relationships(Table 6-8). Averaged over all the sowings, the correlation coefficient of field emergence with normal seedlings of CD were slightly higher than with that of radicle appearance (Table 6-8).

6.1.2.7 Germination rate

Germination rates (GR) at low temperatures (5 ^{o}C, 5/10 ^{o}C) showed positive and significant correlations with field emergence at 5 (5 ^{o}C) or 6 (5/10 ^{o}C) of the 8 sowings, but GR at high temperatures (10 ^{o}C, 15 ^{o}C, 20 ^{o}C) were weakly correlated to the field emergence (except for GR at 20 ^{o}C for 2 autumn sowings) (Table 6-9) .

Table 6-7 Correlation coefficient between germination after accelerated aging and field emergence in 6 Pawera seed lots at 8 sowing dates

Sowing dates	Correlation coefficient with					
	AA1	AA2	AA3	AASS1	AASS2	AASS3
23 Sep.	0.641	0.935**	0.824*	0.807^{+}	0.883*	0.944**
18 Oct.	0.382	0.688	0.430	0.563	0.712	0.759^{+}
13 Nov.	0.249	0.684	0.724	0.504	0.593	0.724
21 Mar.	0.720	0.896*	0.723	0.875*	0.931**	0.860*
5 Apr.	0.901*	0.894*	0.827*	0.927**	0.987**	0.796^{+}
20 Apr.	0.547	0.756^{+}	0.585	0.652	0.878*	0.788^{+}
9 May	0.377	0.547	0.413	0.662	0.699	0.478
20 May	0.543	0.683	0.395	0.596	0.783^{+}	0.732^{+}
Mean	0.545	0.760^{+}	0.615	0.698	0.846*	0.760^{+}

+, *, **: see Table 6-5,

AA1, AA2, AA3: 1, 2, 3 -day accelerated aging,

AASS1, AASS2, AASS3: seeds surface sterilized prior to 1, 2, 3 days AA.

Table 6-8 Correlation coefficient between germination after controlled deterioration and field emergence in 6 Pawera seed lots at 8 sowing dates

Sowing date	Charactor	Correlation coefficent with CD at different seed moisture contents				
		16%	18%	20%	22%	24%
23 Sep.	NS	0.956**	0.972**	0.895*	0.851*	0.903*
	RA	0.918**	0.957**	0.915*	0.869*	0.881*
18 Oct.	NS	0.735^{+}	0.769^{+}	0.698	0.829*	0.889*
	RA	0.771^{+}	0.700	0.712	0.814*	0.913*
13 Nov.	NS	0.827*	0.736^{+}	0.755^{+}	0.906*	0.535
	RA	0.941**	0.868*	0.748^{+}	0.912*	0.594
21 Mar.	NS	0.938**	0.948**	0.786^{+}	0.745^{+}	0.951**
	RA	0.861*	0.905*	0.825*	0.755^{+}	0.922**
5 Apr.	NS	0.935**	0.925**	0.786^{+}	0.572	0.799^{+}
	RA	0.771^{+}	0.883*	0.831*	0.608	0.687
20 Apr.	NS	0.880*	0.855*	0.824*	0.884*	0.828*
	RA	0.885*	0.810^{+}	0.840*	0.887*	0.791^{+}
9 May	NS	0.793^{+}	0.656	0.433	0.683	0.659
	RA	0.849*	0.746^{+}	0.475	0.657	0.734^{+}
20 May	NS	0.688	0.758^{+}	0.690	0.688	0.900*
	RA	0.638	0.612	0.713	0.685	0.850*
Mean	NS	0.844*	0.827*	0.733*	0.770^{+}	0.795^{+}
	RA	0.829*	0.810*	0.757^{+}	0.773^{+}	0.797^{+}

+, *, **: see Table 6-5

NS: Normal seedlings

RA: Radicle appearance

Table 6-9 Correlation coefficent between germination rate and field emergence in 4 Pawera seed lots at 8 sowings

Sowing date	Correlation coefficent with germination rate at					
	5 °C	5/10 °C	10 °C	15 °C	20 °C	SG
23 Sep.	0.271	0.538*	-0.056	-0.324	-0.222	-0.043
18 Oct.	0.519*	0.520*	-0.090	0.059	-0.356	0.085
13 Nov.	0.649**	0.452	0.077	-0.158	0.159	0.213
21 Mar.	0.490	0.568*	0.139	-0.008	-0.281	0.090
5 Apr.	0.280	0.352	-0.337	-0.260	-0.592*	-0.176
20 Apr.	0.517*	0.584*	-0.184	-0.237	-0.571*	0.193
9 May	0.721**	0.515*	0.134	0.366	-0.036	0.033
20 May	0.592*	0.608*	0.384	0.020	-0.230	0.012
Mean	0.505*	0.517*	0.008	-0.068	-0.266	0.051

*, **: See Table 6-5

6.1.2.8 Predicting field emergence by the best vigour tests

The most successful vigour methods in this experiment are summarized in Table 6-10. These data are based on the tests which were correlated to field emergence ($r^2 > 0.30$; i.e. $r > 0.548$) for at least 6 of the 8 sowings. CD16, CD18, and CD22 for both normal seedlings and radicle appearance, CD24 based on radicle appearance, and AASS2 gave the best results for indicating field emergence (Table 6-10). These tests were highly correlated ($r > 0.548$) to field emergence at all of the 8 sowings; these were followed by MD, AA2, AASS1, CD20%, CD24% (based on normal seedlings), at 7 of the sowings and then by GR5/10 and CON24, at 6 of the 8 sowings. The standard germination had the lowest correlation (Table 6-10).

The very high correlation coefficients (Table 6-10) indicate that field emergence at this experimental field environmental site was able to be predicted by laboratory vigour tests. The following regression formulae represent the relationship between field emergence and several vigour tests:

(1) for AA2

$Y = 36.1 + 0.458X$

(2) for AASS2

$Y = 30.6 + 0.555X$

(3) for CD16%

$Y = 38.9 + 0.455X$

(4) for CD18%

$Y = 51.4 + 0.386X$

where Y represents the field emergence%, and X represents germination% after the vigour test.

These relationships are also illustrated in Figure 6-1.

Table 6-10 Summary of the best vigour tests i.e. those were which highly correlated ($r^2 > 0.30$, i.e. $r > 0.548$) to field emergence at least at 6 of the 8 sowings

Test	No. of the 8 sowings	MR	Test	No. of the 8 sowings	MR
SG	3	0.506	CD16	8 (8)	0.933^{**} (0.829^{*})
MD	7	-0.657	CD18	8 (8)	0.911^{*} (0.810^{+})
CON24	6	-0.602	CD20	7 (7)	0.733^{+} (0.759^{+})
AA2	7	0.840^{*}	CD22	8 (8)	0.770^{+} (0.773^{+})
AASS1	7	0.698	CD24	7 (8)	0.808^{+} (0.797^{+})
AASS2	8	0.903^{*}	GR5/10	6	0.517^{*}
AASS3	7	0.760^{+}			

+, *, **: see Table 6-5
MR: Mean correlation coefficient over the 8 sowings
MD: Mechanicaly damaged seed
CON24: Conductivity after 24 hr soaking
AA2: 2-day AA
AASS1, AASS2, AASS3: Surface sterilised prior to 1, 2 and 3 days AA respectively.
CD16, CD18, CD20, CD22 CD24: CD with SMC of 16%, 18%, 20%, 22% and 24% respectively, based on the % of normal seedlings; the data in the brackets refer to radicle appearance.
GR 5/10: Germination rate at 5/10 ^{o}C.

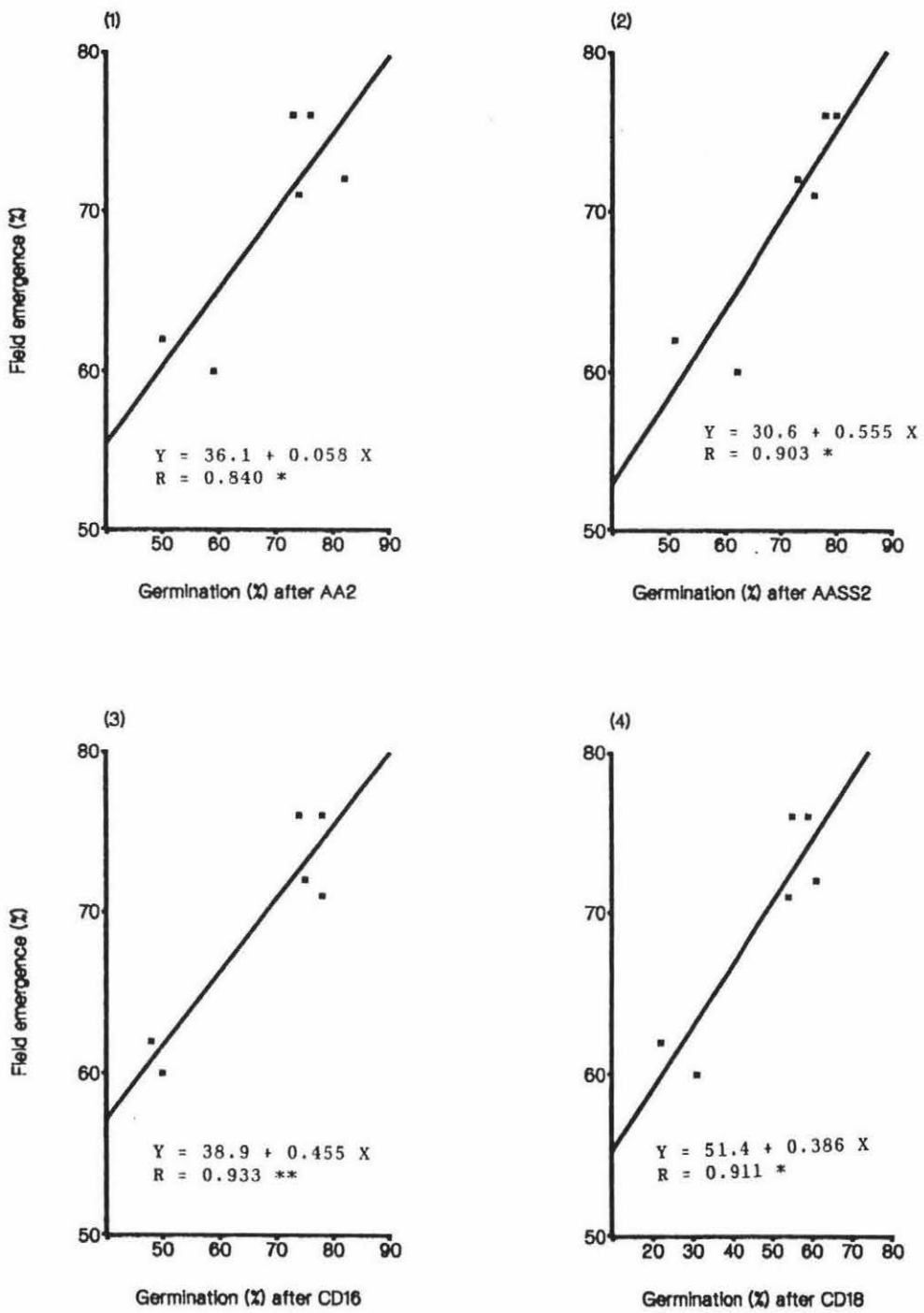

Figure 6-1 Relationship between AA2 (1), AASS2 (2), CD16 (3), and CD18 (4), and field emergence averaged across the 8 sowings.

6.2 Relationship between vigour and storability

The correlations between the main initial seed quality tests and germination after storage at different conditions are shown in Tables 6-13, 6-14, 6-15, and 6-16 respectively. Since similar correlation results were obtained from the AA tests for both with and without surface sterilized seed, and from CD either based on normal seedlings or radicle appearance, the results of AA without seed surface sterilization and CD based on normal seedlings only are presented in the Tables.

6.2.1 Storage at 20 oC-45% RH

Among the 15 initial quality tests listed, GR5, AA3 and CD20 were significantly ($P < 0.05$) related to germination after storage for at least 4 of the 5 storage periods (Table 6-11). The other tests were only weakly correlated (Table 6-11).

6.2.2 Storage at 20 oC-60% RH

Under 20 ^{o}C-60% RH storage conditions, GR5, GR5/10, CON24, CD16, CD18, and all AA tests were strongly correlated ($P < 0.05$) to the germination after storage for at least 4 of the 5 storage periods (Table 6-12). There were no other significant correlations with the exception of GR20 and CD24 at 1 and 9 and 11 months respectively (Table 6-12).

6.2.3 Storage at ambient conditions

At ambient conditions GR5, AA2, AA3, CD16, CD18 and CD20 were highly correlated with germination after storage, followed by GR5/10, CON24, CD22 and CD24. The other tests, including the SG were not correlated (Table 6-13).

For AA and CD, the correlation coefficients generally increased as the storage periods increased (Table 6-13).

6.2.4 Storage at 20 oC-75% RH and 20 oC-90% RH

About 50% of seeds had died following storage at 20 ^{o}C-90% RH for 1 month, and at 20 ^{o}C-75% RH for 5 months (Chapter 5). All methods, excepting SG, GR5, GR10,

GR15, GR20 and CD22 were significantly correlated ($P < 0.05$) to germination after storage at 90% RH for 1 month (Table 6-14). At 75% RH, GR5/10, CON24, and all AA and CD (except for CD22) were highly correlated with germination, followed by GR5 and GR10. Similarly to the other tests, initial SG, and GR at high temperatures (10, 15 and 20 ^{o}C) showed the poorest relationship with germination after storage. Correlation coefficients at 75% RH also generally increased as the storage period increased, particularly during the first 3 months (Table 6-14).

Table 6-11 Correlation coefficients between initial quality of 4 Pawera seed lots and germination after storage for different periods at 20 ^{o}C-45% RH

Quality test	Correlation coefficient with germination after different storage periods (months)					
	1	5	7	9	11	Mean
SG	0.228	0.427	0.537*	0.597*	0.335	0.425
SGR5	0.540*	0.406	0.734**	0.630**	0.530*	0.568*
GR5/10	0.426	0.318	0.596*	0.596*	0.340	0.455+
GR10	-0.101	-0.084	-0.131	-0.490	-0.307	-0.223
GR15	-0.310	-0.199	-0.250	-0.299	-0.141	-0.240
GR20	0.086	0.055	-0.138	-0.251	-0.126	-0.075
Con24	-0.221	-0.292	-0.393	-0.370	-0.380	-0.331
AA1	-0.097	0.283	0.194	-0.091	-0.030	0.052
AA2	0.397	0.583*	0.525*	0.330	0.545*	0.476+
AA3	0.551*	0.707**	0.612*	0.442	0.606*	0.584*
CD16	0.374	0.622*	0.612*	0.492	0.634**	0.547*
CD18	0.420	0.596*	0.478	0.400	0.485	0.476+
CD20	0.513*	0.569*	0.546*	0.511*	0.546*	0.537*
CD22	0.491	0.367	0.566*	0.584*	0.430	0.488+
CD24	0.446	0.197	0.696**	0.469	0.546*	0.471+

*, **: See Table 6-9

Table 6-12 Correlation coefficients between initial quality of 4 Pawera seed lots and germination after storage for different periods at 20 °C-60% RH

Quality test	Correlation coefficient with germination after different storage periods (months)					
	1	5	7	9	11	Mean
SG	0.063	-0.008	0.111	0.172	0.321	0.132
GR5	0.294	0.698**	0.541*	0.556*	0.559*	0.530*
R5/10	0.511*	0.746**	0.499*	0.528*	0.617*	0.582*
GR10	-0.200	0.042	0.001	-0.177	0.163	-0.034
GR15	0.077	-0.178	-0.004	-0.240	-0.044	-0.078
GR20	0.502*	-0.338	0.040	-0.568*	-0.248	-0.131
Con24	-0.514*	-0.617*	-0.120	-0.754***	-0.557*	-0.512*
AA1	0.630**	0.632**	0.272	0.630**	0.557*	0.544*
AA2	0.641**	0.729**	0.588*	0.674**	0.751***	0.677**
AA3	0.581*	0.708**	0.425	0.784***	0.643**	0.628**
CD16	0.650**	0.749***	0.599*	0.733**	0.674**	0.681**
CD18	0.642**	0.733**	0.557*	0.735**	0.670**	0.667**
CD20	0.471	0.676**	0.456	0.791***	0.652**	0.609*
CD22	0.134	0.246	0.195	0.276	0.442	0.259
CD24	0.447	0.449	0.225	0.523*	0.786***	0.486+

*, **: See Table 6-9 ***: at 0.1% probability level

Table 6-13 Correlation coefficient for initial quality of 4 Pawera seed lots and germination after storage for different periods at ambient conditions

Quality test	Correlation coefficient with germination after different storage periods (months)					
	1	5	7	9	11	Mean
SG	-0.046	0.247	0.129	0.288	0.196	0.163
GR5	0.540*	0.485	0.584*	0.651**	0.590*	0.570*
GR5/10	0.414	0.265	0.684**	0.696**	0.569*	0.526*
GR10	0.091	-0.266	-0.103	0.267	-0.097	-0.022
GR15	-0.230	0.055	-0.442	-0.117	-0.232	-0.193
GR20	-0.127	-0.326	-0.334	-0.272	-0.486	-0.309
Con24	-0.287	-0.316	-0.656**	-0.663**	-0.696**	-0.524*
AA1	0.421	0.164	0.375	0.639**	0.601*	0.440+
AA2	0.548*	0.548*	0.545*	0.782***	0.831***	0.651**
AA3	0.558*	0.570*	0.627**	0.664**	0.842***	0.652**
CD16	0.369	0.605**	0.614*	0.841***	0.891***	0.664**
CD18	0.546*	0.570*	0.701*	0.836***	0.904***	0.711**
CD20	0.521*	0.636**	0.732*	0.861***	0.914***	0.733**
CD22	0.066	0.524*	0.449	0.711**	0.556*	0.461+
CD24	0.405	0.545*	0.417	0.619*	0.615*	0.520*

*, **, ***: See Table 6-12

Table 6-14 Correlation coefficient between initial quality of 4 Pawera seed lots and germination after storage for different periods at 20 °C-75% RH and 20 °C-90% RH

Quality test	Correlation coefficient with germination after storage at					
	90% RH	75% RH				
	1 month	1 month	2-month	3-month	5-month	mean
SG	-0.039	0.153	0.285	0.039	0.284	0.190
GR5	0.382	0.408	0.432	0.437	0.447	0.431+
GR5/10	0.521*	0.069	0.663**	0.736**	0.535*	0.501*
GR10	0.070	-0.295	0.168	0.188	0.009	0.018
GR15	-0.068	0.142	-0.209	-0.054	0.159	0.010
GR20	-0.367	-0.209	-0.288	-0.522	-0.317	-0.334
Con24	-0.524*	-0.225	-0.654**	-0.733**	-0.510	-0.532*
AA1	0.708**	-0.002	0.705**	0.897***	0.687**	0.573*
AA2	0.727**	0.325	0.647**	0.806***	0.721**	0.625**
AA3	0.815***	0.443	0.670**	0.707**	0.808**	0.657**
CD16	0.581*	0.353	0.624**	0.775***	0.689**	0.610*
CD18	0.716**	0.332	0.666***	0.821***	0.685**	0.626*
CD20	0.593*	0.450	0.606*	0.704**	0.566*	0.582*
CD22	0.271	0.220	0.214	0.286	0.309	0.527
CD24	0.632**	0.387	0.521*	0.677**	0.621*	0.552*

*, **, ***: See Table 6-8

6.2.5 Predicting storability at ambient conditions by vigour testing

When seed was stored at ambient conditions from spring to autumn (i.e. 7 months of storage), or to the next spring (i.e. 11 months of storage), the germination of the low vigour lot (lot 3) fell by 14% and 24% respectively, but that of the high vigour lot (eg. lot 4) did not change significantly (Chapter 5). There was a good relationship [(r^2 > 0.30, i.e. r > 0.548)] between seed germination after storage and some initial vigour parameters in different seed lots (Table 6-15). These data suggest that a seed lot's storability under these ambient conditions can be predicted by some vigour tests, particularly AA3, CD18 and CD20. The relationships between CON24, AA3, CD18 and CD20 and seed storability for 7 and 11 months are illustrated in Figures 6-2, 6-3, 6-4 and 6-5.

Table 6-15 Summary of best vigour tests for predicting seed storability at ambient conditions

Storage period	Test	R	Regression equation
7 months	GR5	0.584*	
	GR0/10	0.684**	
	CON24	-0.656**	Y = 157 - 0.788 X
	AA3	0.627**	Y = 72.6 + 0.229 X
	CD16	0.614*	
	CD18	0.701**	Y = 67.5 + 0.294 X
	CD20	0.732**	Y = 71.8 + 0.289 X
11 months	GR5	0.590*	
	GR5/10	0.569*	
	CON24	-0.696**	Y = 205 - 1.28 X
	AA1	0.601*	
	AA2	0.831***	
	AA3	0.842***	Y = 63.1 + 0.476 X
	CD16	0.891***	
	CD18	0.904***	Y = 53.6 + 0.595 X
	CD20	0.914***	Y = 62.0 + 0.587 X

*, **, ***: See Table 6-8.

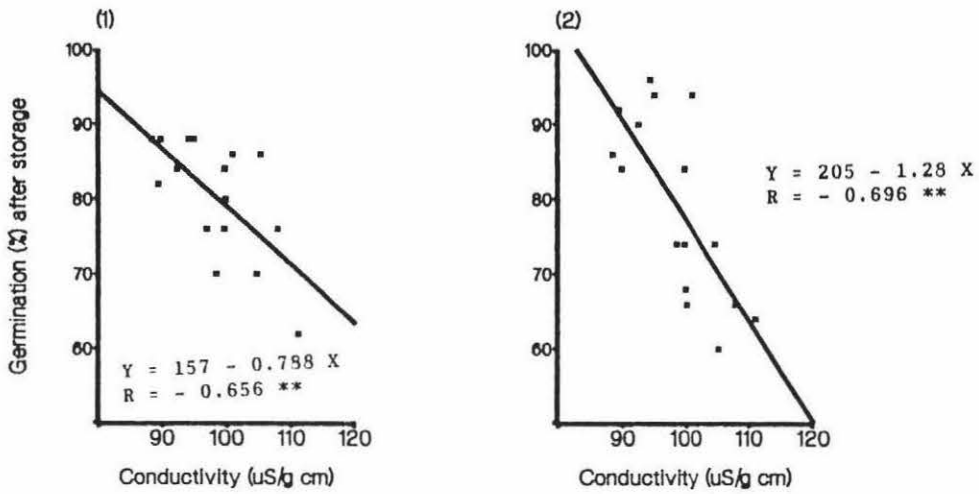

Figure 6-2 Relationship between conductivity and seed storability at ambient conditions for 7 months (1), and 11 months (2).

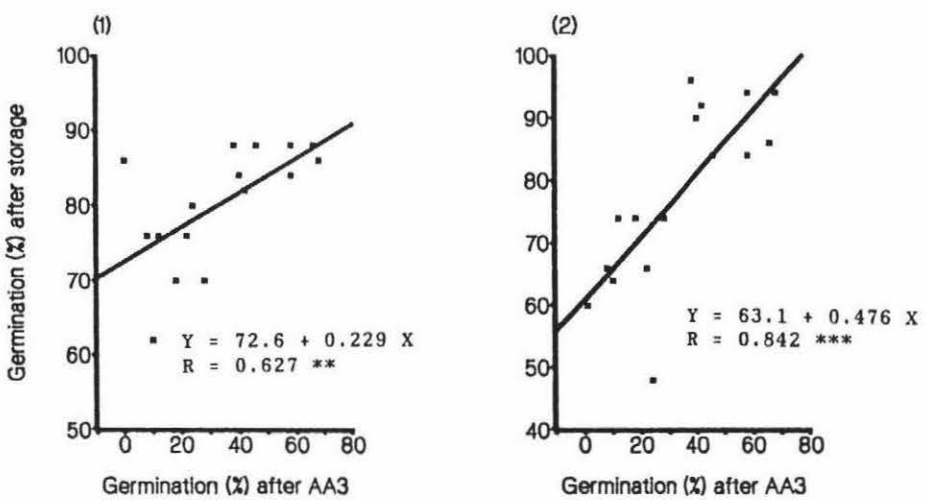

Figure 6-3 Relationship between AA3 and seed storability at ambient conditions for 7 months (1), and 11 months (2).

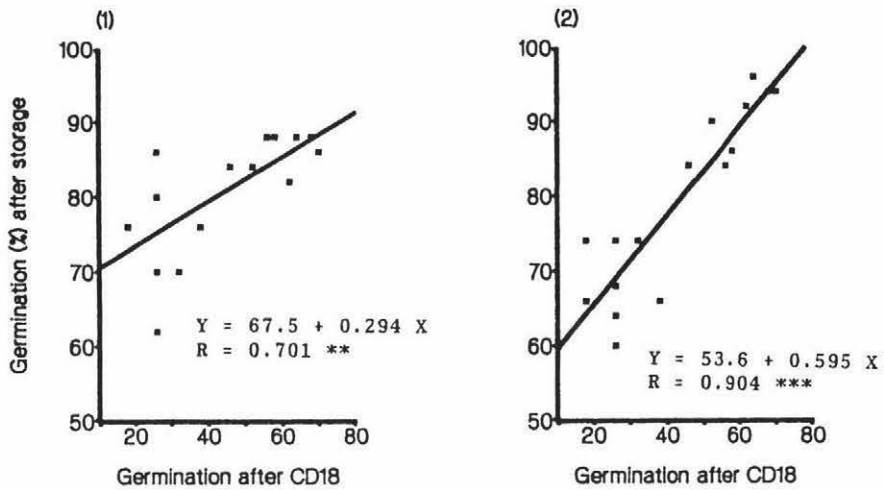

Figure 6-4 Relationship between CD18 and seed storability at ambient conditions for 7 months (1), and 11 months (2).

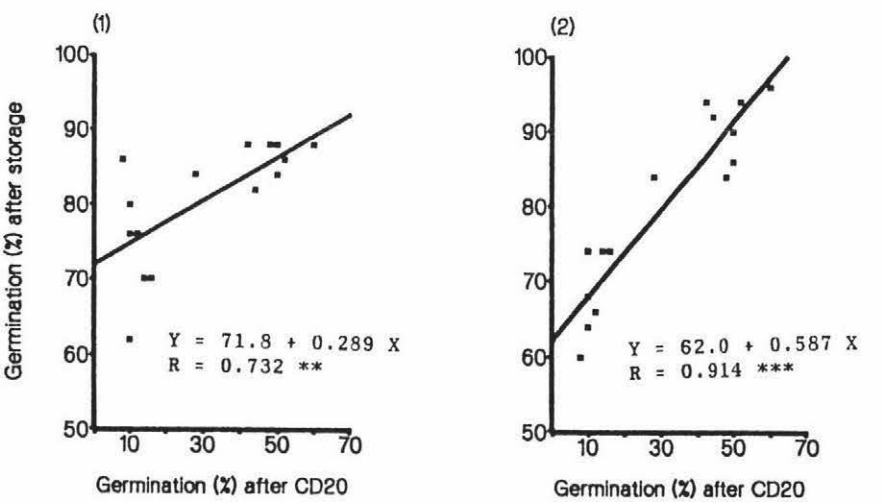

Figure 6-5 Relationship between CD20 and seed storability at ambient conditions for 7 months (1), and 11 months (2).

CHAPTER 7

DISCUSSION

7.1 Seed vigour and seed quality

7.1.1 Differences in vigour of seed lots

This study has confirmed that vigour differences exist within seed lots of red clover cv. Pawera, white clover cv. Huia and lucerne cv. Wairau, which did not differ significantly in laboratory standard germination, and were of the same chronological age (Chapter 4). The results are similar to those previously reported for large seeded legumes such as garden pea (Hampton and Scott, 1982), soybean (Delouche, 1973; TeKrony and Egli, 1977) and field bean (Hegarty, 1977); and for small seeded legumes such as Lotus corniculatus (McKersie et al 1981), lucerne (Larsen and Isely, 1967) and crimson clover (Helmer et al., 1962).

Results for red clover seed vigour agreed well with reports for other species which have shown that seed vigour may affect field emergence (Fiala, 1987; Perry, 1981; Marshall and Naylor, 1985; Nuswantoro, 1988), seed storability (Delouche and Baskin, 1973; Baskin and Vieira, 1980; Helmer et al., 1962; Nuswantoro, 1988;) and possibly plant stand (Hampton and Scott, 1982; Egli et at 1977). The difference in performance between low vigour (e.g. lot 3) and high vigour (e.g. lot 4) lots was considerable. For example, the difference was 15% in final field emergence, 33% in emergence rate over all the sowings, and 28% in seedling dry weight in autumn sowings (Section 6.1.1). Under ambient storage conditions, the differences in germination were 10% after 7 months storage and 24% after 11 months storage; under adverse conditions e.g. 75% RH, the difference was even bigger and occurred over a shorter storage period (Section 5.3.1). These results strongly suggest that vigour is an important quality factor influencing seed performance in red clover, and that more attention should be given to both research and the practical application of vigour test results for small seeded legumes.

7.1.2 Seed vigour and its relationship to other seed quality parameters

Many factors may cause variation in vigour between seed lots (ISTA, 1987). Some of them, such as seed weight (or size), mechanical integrity, seed moisture content and seed health status (e.g. storage fungi) can be detected through routine seed testing procedures. This type of quality information may therefore reveal some possible causes of the differences in vigour between seed lots. The 7 Pawera seed lots used were harvested in the same year, and the initial quality assessment indicated that there were no marked differences in seed moisture content levels and storage fungi, but there were significant differences in thousand seed weight, mechanically damaged seed and seed coat permeability (imbibition rate) (Section 4.2).

It is generally assumed that large seed has more food reserves, and thus may be more vigorous than small seed. Many reports have confirmed this positive correlation in small herbage legumes (e.g. Charlton, 1989; Haskins and Gorz, 1975; Henson and Tayman, 1961; Jenson et al., 1974; Stickler and Wasson, 1963), but some work has also demonstrated that seed weight is not related to seed vigour (e.g. McKersie et al. 1981). Of the 7 Pawera seed lots used, the lot with the highest seed weight (lot 1) was low in vigour, while the lot with the lowest seed weight (lot 2) was higher in vigour, and seed weight was only poorly related to field emergence. The lot with the highest seed weight also had the highest amount of mechanically damaged seed, but even excluding this lot did not much improve the relationship with field emergence. It is evident that it is the physiological status of the seed which determines seed vigour rather than the physical status, although the latter may influence the former (e.g. through mechanical damage). Such inconsistent relationships between seed vigour and specific seed lot size have also been noted by other authors (e.g. Charlton, 1989; Scott and Hampton, 1985). Seed lot TSW alone is not a reliable parameter for indicating seed lot vigour; in fact the weight (or size) within the lots may be a better indicator than that between the lots (Evans, 1973; Hampton, 1986, McKersie et al., 1981), since individual seeds have a common background.

Although lot 1 had a low field emergence and emergence rate under the same plant density, the seedlings were longer and had a greater dry weight than those of some of the other lots (Section 6.1.1). Results where seed weight has a greater influence on seedling performance than on seed emergence have also been reported in ryegrass

(Hampton, 1986), Trifolium subterranneum (Tayler, 1972) and Melilotus officinalis (Haskins and Gorz, 1975). The importance of plant population, and whether low plant numbers can be compensated for by greater per seedling performance requires further investigation in herbage legume species.

Mechanical damage of seed markedly reduced both seed viability and vigour in Pawera red clover in the laboratory and the field (Chapter 4, 6). These results were expected and confirm general observations on large seeded legumes (e.g. Mason, et al. 1982; Oliveira et al., 1984; Powell and Matthews, 1979). Mechanical damage can occur during seed harvesting and processing, but may not necessarily be severe enough to be visible externally. However, evidence of some physical damage is common in New Zealand red clover, particularly when seed has been harvested at unsuitable moisture contents and incorrect scarification techniques have been used (Hampton, personal communication). More subtle, but yet very important internal damage is unlikely to be detected through the laboratory germination test and external observation. The effects of seed production and processing practices on red clover seed quality require further investigation and consideration.

There was considerable variation in the rate of imbibition between red clover seed lots (Figure 4-3). The seed lots with a high rate of water uptake during the first few hours contained a high proportion of damaged seed and were low in both vigour and viability (e.g. lots 1 and 7). These results agree with those for other species (Faymi, 1951; Peiffer et al. , 1960; Powell and Matthews, 1979; Powell et al., 1986). However, imbibition rate is more likely to be an indicator of direct physical seed damage, rather than physiological seed damage, as lot 3 was low in vigour, yet imbibed only slowly over the first few hours (Section 4.2.6). Imbibition rate is therefore probably a better indicator of seed viability than of seed vigour.

7.2 Evaluation of vigour methods

The main consideration in this study was to assess the suitability of each of several important vigour techniques for indicating seed performance in red clover. The assessment was based on whether the test could provide a more close and consistent relationship with seed performance than the standard germination test, which is one of the most important requirements of a vigour test (Hampton and Coolbear, 1989; Perry,

in ISTA, 1987). In order to explore the possible potential application of any technique, a number of modifications were made, and the seed performance was monitored under a range of conditions both in the field and in storage.

The results showed that all the techniques were able to provide a better indication of seed planting and storage value than the laboratory germination test. However, the correlation coefficients varied between tests within each of the techniques, between the techniques and between the different environments. It is necessary to understand the variability and assumptions involved in each of the tests and the techniques.

7.2.1 Individual techniques

7.2.1.1 Conductivity

A soaking time of 24h is recommended for the conductivity test for large seeded legumes (ISTA, 1987), and this also seems the test time most suitable for red clover, as correlations with field emergence were consistently better than for other times. However, there were no major changes in lot ranking (good or poor vigour) between 8 and 24 h soakings, and conductivity results from 8 and 24 h were highly correlated with each other ($r=0.929$, $P < 0.001$) (Section 4.2.7.1). These observations suggest that the time of soaking in the conductivity test could possibly be reduced from 24 h to 8 h, and thus the test could be completed within a day. This supports the suggestions of Brouwer and Mulder (1982) in bean (4h soaking), and Loeffler et al. (1988) in soybean (6h soaking), but will require further confirmation in red clover.

The best correlation between conductivity and field emergence was obtained at the last autumn sowing, when the soil temperature was very low (8.9 ^{o}C) (Section 6.1.2.4). This result is in good agreement with the observation on large seeded legumes, that conductivity gives a more reliable prediction of performance under unfavorable field conditions (Beckendam, et al. 1987; Kraak et al, 1984; Perry, 1978). The present experiment further found that the conductivity test was also best related to seed storability under adverse storage conditions (Section 6.2).

Significant differences in conductivity existed between the seed lots; however, leaching from the 7 lots appeared to stop at the same time (about 24 h), indicating that the cell membranes of the seed lots had similar reorganizing rates during seed

dehydration . According to Coolbear's (1988a) interpretation, the fact that more solutes were available to leak out in some lots would seem to be due to other factors rather than membrane damage, possibly problems with the control of mobilization of seed food reserves; and/or a high incidence of damaged seed coats.

7.2.1.2 Accelerated aging (AA)

Germination after accelerated ageing (for 1, 2, 3 days, with and without surface sterilizing) was better correlated with field emergence than standard germination (Section 6.1.2.5). Four and 5 days of ageing was too severe on the seed lots and killed the seed. These results support those previously reported for the large seeded legume soybean (Fiala, 1987; Kulik and Yaklich, 1982; TeKrony, 1985 and Tomes et al. 1988).

Of the ageing treatments, 2 days provided the most significant correlations with field emergence, and at most sowing dates was more strongly related than 3 days, which is the standard recommendation (ISTA, 1987). However, 3 day's results were better correlated with storability than 2 day 's results (Section 6.2). Fungal growth after 3 days AA caused problems with identification of normal seedlings (Section 4.2.7.2), and surface sterilizing provided better correlations with field emergence than untreated seed after both 2 and 3 days AA.

Heydecker (1969) stated that fungicidal seed treatment can make a considerable difference to the vigour results obtained, and it may be necessary to test both treated and untreated samples. This experiment showed that 'Janola' surface sterilization reduced fungal growth on the seeds, but did not improve the germination percentage. In some lots the germination percentage was reduced, particularly for longer aging periods (2 to 4 days) (Section 4.2.7.2). It is possible that imbibition damage occurred during the seed surface sterilization process (i.e. the seeds were soaked in 10% Janola for 10 minutes, then rinsed under tap water for a further 10 minutes). Imbibition damage occurring in the outer layers of cells of the cotyledons during the first 2 to 30 minutes of rapid water uptake has been recorded in pea (Powell and Matthews, 1981), and has been interpreted as the result of physical disruption of cell membranes causing cell death (Powell and Matthews, 1979). Such damage has been found to cause the failure of the cells on the surface of the cotyledons to stain in a TZ test, to increase conductivity and to reduce the germination and field emergence in large seeded

legumes such as pea and soybean (Powell, 1988). This study suggests that imbibition damage also occurred in seed lots of red clover during surface sterilization, and this may explain why surface sterilization AA results were better correlated with field emergence than untreated seed, particularly if imbibition damage occurred in the field. This requires further investigation. If this interpretation is correct, surface sterilization did not improve the prediction of storability (Section 6.2) because imbibition damage did not occur during seed storage.

The correlation of AA with field emergence at some sowings was higher than at others (Section 6.1.2.5). Such a phenomenon has also been found by several other authors (e.g. Johnson and Wax, 1978; Samimy et al., 1987). In this experiment the testing stress provided by AA was likely to be more closer to the environment at some sowings than at others. For example, the soil temperature of the sowings which were best correlated with the AA tests (i.e. the first spring sowing, and first and second autumn sowings) were very similar, around 14 ^{o}C; the poorer relationships occurred at sowings where the soil temperature was either higher or lower (Section 6.1).

7.2.1.3 Controlled deterioration

The CD results at each of the seed moisture contents used (16 to 24%) were all able to give a better indication of seed performance than the standard germination test and were significantly correlated to seed performances in most cases. However, 16% and 18% SMC's were generally better than higher SMC's for predicting field emergence and 16%, 18% and 20% SMC for forecasting lot storability. This may be due to the fact that SMC's from 16 to 20% distinguished seed lot vigour more clearly than did higher SMCs. At these lower seed moisture contents, lots showed a wide range of germination following deterioration and the treatments were not so severe that they killed the seed (Section 4.2.7.3).

The relationship between the CD test and field emergence for the last two autumn sowings, when soil temperatures were very low (Chapter 6), was poorer than for other sowings (Table 6-8). In Italian ryegrass, Naylor and Syversen (1988) reported that the CD test could not predict seedling emergence from soil in cool or hot temperature regimes, but could do so at moderate temperature. This suggests that the CD test may be unreliable as a predictor of field emergence under extremely adverse conditions. However, even though the correlations at the last two autumn sowings were not as

good as for other sowings, they were still greater than the standard germination test (Table 6-8).

Overall, the CD test using normal seedling counts showed a better indication of field emergence than when germination was based on radicle appearance (Tables 6-8, 6-10). This was expected since radicle appearance may overestimate seed performance because abnormal seedlings are not detected.

7.2.1.4 Speed of germination

The first count of the standard germination test has been used to estimate field emergence for lucerne (Larsen, 1964) and soybean (Burris et al., 1969; TeKrony and Egli, 1977), but, no advantages were demonstrated in this study. Although the 4-day and 7-day counts did more sensitively separate the seed lots than the standard germination, lot rankings were quite different to those for other vigour tests (Chapter 4) and the relationship with emergence was poorer than for standard germination (Section 6.1.2.1). This could possibly have been due to variability of the results. Perry (1978) also noted that the intermediate germination counts were more variable and lots within a test were frequently less well differentiated.

The germination rate (GR) and field emergence rate calculated using the Maguire (1962) method showed that temperature which ranged from 5 to 20 ^{o}C in the laboratory and averaged 14 to 18 ^{o}C in the field did not affect final germination percentage or emergence. These observations confirm the notion of Hampton et al. (1987) that red clover is less temperature dependent and can be sown in the late autumn or early spring without significantly reducing establishment.. This experiment confirmed that only at very low temperatures i.e. 5 and 5/10 ^{o}C did differences in germination rate between seed lots become apparent. Therefore, this characteristic of the species is possibly the main reason why the GR at high temperature, and first and intermediate counts, did not show very promising results as vigour tests.

7.2.2 Promising techniques

The four techniques i.e. conductivity, AA, CD and germination speed used in this study are generally considered as having the important characteristics of being objective, relatively rapid, simple, economically practical and do have good predictive

value for seed performance (AOSA, 1983; ISTA, 1987). The evidence from the present study has further confirmed that these tests can also predict planting and storage values in red clover. However, predictability varied among the techniques. For predicting field emergence averaged across all sowings, the best result was obtained from the CD test, followed by AA, and conductivity. Although the GR was conducted on only 4 seed lots the correlation coefficient did not show this test as a promising vigour technique. For predicting storability, the best result was given by both AA and CD under controlled storage conditions and by CD under ambient conditions, followed by conductivity and then GR. These differences could be due to one or more of the following:

(1) differences in the principle or basis used between the tests
(2) problems of lower reproducibility in some tests
(3) the species effect and/or
(4) the environmental effect

Both AA and CD tests are based on the assumption that high vigour seeds will tolerate adverse stresses of high temperature and high moisture treatment and thus retain their capability to germinate. However, the CD test gives better control of the seed moisture content than the AA test (ISTA, 1987). Many factors have been reported which may cause variation in the moisture content during the AA test and thus lead to the test being poorer in reproducibility e.g. the initial seed moisture content (McDonald, 1977), seed size (Tomes et al. 1988), the position of seed within the container (Tao, 1978b; 1979) and possibly the testa integrity and permeability (Hampton and Coolbear, 1989). The present study has shown that longer aging periods (e.g. after 3 days) of AA caused mould growth on the seeds and made the seedling evaluation difficult. In addition, different seed coat permeability and integrity also existed among the seed lots (Chapter 4). These are possibly the reasons why the AA tests did not perform as well as the CD test. However, the factors which affect the reproducibility of the 'Jar' AA test method in small seed legume vigour testing needs to be further investigated.

The conductivity test has been efficiently used for testing seed vigour in peas (ISTA, 1978) and beans (Matthews and Bradnock, 1967), but there have been a few results which have shown that the test as an indicator of field emergence is not as good as the SG test, e.g. in Cicer arietinum (Ram, et al., 1989). In this study, although the test was a better indicator of seed performance than the SG, it was not as good as the AA and CD tests. It has been reported that seed coat permeability can affect the precision of the

test results (Powell et al., 1986a, b), and big variations in seed coat permeability are common in small seed legumes. Whether the conductivity test is really a promising vigour technique in small seeded legumes needs further exploration .

Although the speed of germination is one of the oldest vigour techniques, it has not been accepted as widely as the AA , CD and conductivity tests. The test has the limitation that the result is difficult to link with field performance value. In this study, the correlation coefficients of GR with seed performance both in the field and in storability were the poorest of the four vigour techniques, and this suggests that the test is not suitable for this species.

The present study has demonstrated that the relationship between laboratory germination, vigour tests and seed performance, both in the field and during storage, are strongly affected by the environment. This observation is supported by previous reports (e.g. Beckendam et al., 1987; Jonson and Wax, 1978; Perry, 1978; Saming, et al., 1987; Naylor and Syversen 1988). Hampton and Coolbear (1989) state that "It appears increasingly unlikely that any one test , whether germination, physiological or biochemical, will be appropriate for even a single species under all conditions. Thus there is a need to consider more carefully development work on promising tests within a local context". The AA and CD tests showed the best accuracy for predicting seed performance under the present experimental conditions, suggesting that these two tests possibly could be used for indicating field emergence in New Zealand and for predicting seed storability under temperate storage conditions in red clover. However, the regression equations established in this test need to be further confirmed or modified through more study, by using more seed lots and more field sites.

The reduction in seedling dry weight in low vigour seed lots (e.g. lot 3) in autumn sowings was greater than in the spring, which support the previous notion that low vigour seeds were more sensitive to an adverse environment than high vigour seeds (Roberts, 1986), and that the effects of vigour level may persist to influence plant yield (ISTA, 1987; Roberts, 1986).

It could be argued that establishment of vigour differences between Pawera seed lots, and the indication that significant differences in seedling performance exist with autumn sowings is of little practical significance, because farmers usually use sowing rates far higher than are actually needed. However, Thom et al. (1985) noted that the

tendency for high seed rates to be used in practice probably reflects farmers lack of confidence in the ability of species to establish under their particular farm circumstances. Lancashire (1985) reported that around 80% of farmers sow pasture seeds in autumn, and Hampton et al. (1987) and this experiment showed the negative influence of low autumn temperatures on germination rate. If this is further complicated by poor seed vigour, the ability to establish, produce dry matter and survive, particularly in a competitive multispecies sward becomes extremely important. Further work is required to confirm this, not only for Grasslands Pawera red clover, but also for other red clover cultivars and herbage legume species.

7.3 Some aspects of the sequence of changes during seed deterioration

7.3.1 General aspects of seed deterioration

Storage of Pawera red clover seeds under open and controlled storage conditions for 11 months, resulted in seed deterioration. This deterioration was associated with reduced germination rate (first count), viability (via. germination %), and field emergence; and with increasing conductivity, abnormal seedlings and dead seed content (Chapter 5). These results were expected and supported the general principles of seed deterioration summarized by previous researchers (e.g., Delouche and Baskin, 1973; Justice and Bass, 1978; Kozlowski; 1972; McDonald and Nelson, 1986).

In general, as the storage period increased, a significant loss of vigour occurred in each of the seed lots as measured by the conductivity test. This effect occurred earlier than loss of germination percentage during storage (Figure 5-3, 5-4, 5-7, 5-8, and 5-9), which confirms the general suggestion that loss of vigour precedes viability loss (AOSA, 1983; ISTA, 1987) and supports the assumption of Delouche and Baskin (1973) that cell membrane damage may be one of the earliest events occurring during seed deterioration. The experiment failed to demonstrate that reduced speed of germination (via. four-day count) occurred earlier than the final reduction of germination during the sequence of changes in seed deterioration (Figures 5-3, 5-4, 5-5 and 5-6). The variation of results between the replicates as indicated by the LSD value for the first count was much higher than for the SG test (Section 5.3.2). The decrease in germination percentage was initially closely associated with an increased number of abnormal seedlings, and then was gradually followed by increasing dead seed content (Figures 5-3, 5-4; Tables 5-3, 5-5). The main categories of abnormal seedlings were

short roots or stunted growth, -- typical symptoms of aged seed. These occurred under most storage conditions i.e. 45% RH, 60% RH, 70% RH and ambient conditions, and more decayed seedlings caused by the storage fungi were recorded at high RHs. An attempt has been made to examine histological and physiological differences between normal and various abnormal crimson clover seedlings (Ching, 1958). It was found that abnormal seedlings showed injury to essential tissues e.g., meristemic and cortical, and that their respiratory rate was lower, and respiratory quotient was higher, than that of normal seedlings (Ching, 1958). It was suggested that extensive basic research on the biochemical differences between normal and abnormal seedlings or in sound, deteriorating, and dead seed lots would be desirable to define the causes of seed deterioration (Ching et al., 1959).

In 20 ^{o}C-75% RH controlled storage conditions, the viability decline of red clover seed followed a normal distribution pattern (Figure 5-4a). However, in the low vigour lot (lot 3) viability started declining earlier than in the higher vigour lot. The survival curves for each of the 4 lots tended to be parallel with each other, particularly during the phase when viability declined quickly (from 2 to 5 months). Such seed survival patterns both within and between seed lots are typical of seed survival curves described by Roberts (1986).

Evans (1957) stated that drier storage conditions tend to produce a higher hard seed content in red clover. In this study, under relatively dry storage conditions i.e. 20 ^{o}C-45% RH, although there were no significant changes in hard seed content at the final count of the SG test (Table 5-4), germination rate (via 4-day count) was decreased and was much lower than in seed stored at high RH e.g. 60% RH and ambient conditions during the first 3 months of storage. After this time the germination rate gradually increased (Figure 5-5). The possibility is that seed coat permeability was lowered as the seed was quickly desiccated, then raised again as the storage period increased, thus resulting in changes in seed imbibition rate, and therefore germination rate.

7.3.2 Factors affecting seed deterioration

The present study showed that as the RH of the storage environment increased, the higher was the SMC reached, and the quicker seed quality fell. Similar results were also obtained from previous work on this crop (e.g., MacKay and Flood ,1969;

Evans, 1957). Evans (1957) reported that the moisture content of red clover seed is the most important single factor governing its longevity. Seed with high SMC (e.g. under storage at 75% and 90% RH) was associated with rapid increases in storage fungi and reduced seed quality (Section 5.1, 5.2, and 5.3.1). Therefore, in order to keep high seed viability, maintaining low seed moisture is one of the most important factors to be taken into account in seed store management.

Evans (1957) reported that in a humid climate such as is found in Britain it is unsafe to hold valuable clover seeds for more than one year in ordinary stores, even when seed are reasonably well dried and have a high initial viability. Evans (1957) suggested that at ordinary ambient temperatures, the moisture content should be reduced to below 10% if quality red clover seed is required to be carried forward for sowing purposes after more than one year. The present experiment has partly confirmed this suggestion. For example, when seed was stored at ambient conditions, the average SMC over the storage period (11 months) was below the critical value of 10% i.e 8.8%. High viability and high vigour seed (lots 2 and 4) did maintain its high viability over the storage period. However, in seed lots with high viability but low vigour (lots 1 and 3) viability was significantly reduced after 7 months. The quality tests conducted after 7 months storage revealed that all 7 lots had markedly reduced vigour (via. conductivity test) and field emergence (Section 5.5). As the storage period increased, the rate of this loss of viability increased until after 11 months, a 24% loss in viability had occurred in the low vigour seed lot. Several previous researchers have studied the factors involved in the loss of seed viability in red clover seed during storage. However, it seems only Helmer (1962) as cited in Helmer et al. (1962) was concerned with responses of different seed lots to storage conditions. He demonstrated that under stress storage conditions, seed lots initially low in vigour lost germination very rapidly, while lots high in vigour were relatively unaffected. This experiment also further documented that significant reductions in viability of low vigour lots occurred even under reasonably good storage conditions, e.g., 45% RH-20 ^{o}C for 9 months and 60% RH-20 ^{o}C for 7 months. As the storage conditions became adverse, the loss of viability by the low vigour lot was generally quicker than the that of the high vigour lots (Section 5.3.1). The observations from the present storage experiment have the following two practical lessons for farmers and seed companies. Firstly, under ambient storage conditions, it is wise to store even initially high vigour seed lots only for a short term (e.g. 6-12 months). Secondly, it is necessary to test seed quality before sowing, and not to use test results which are more than 6 months old. In New Zealand,

it is recommended by the Seed Testing Station that the seed testing result is only valid for 6 months. However, in practice, many people do not follow this advice, and in most cases sow seed in the autumn, after using test results obtained up to 12 months earlier. Storage of low vigour lots and the use of outdated germination data may help explain some of the recent establishment problems that have occurred with Grasslands Pawera red clover, particularly in Northland (D. J. Scott, personal communication).

CHAPTER 8

CONCLUSION

Based on the results and discussions from this study, the following conclusions can be drawn:

1. Significant vigour differences existed between seed lots of red clover cv. Pawera, white clover cv. Huia and lucerne cv. Wairau, even though they were similar in standard germination capacity and were of the same chronological age.

2. The differences in vigour between red clover seed lots resulted in differences in field emergence, emergence rate and seedling dry weight (under adverse conditions) over 8 sowings; and differences in seed viability after storage under a range of simulated temperate conditions. The low vigour lots (e.g. lot 3) had less ability to tolerate adverse conditions both in the field and in storage than the high vigour lots (e.g. lots 2 and 4).

3. With a few exceptions, a number of vigour tests modified from each of four vigour techniques were all able to provide more sensitive and accurate results for indicating seed planting and storage performance than the standard germination test. The best tests obtained from each of the individual techniques were as follows:

(1) CD:
- -- At either 16 or 18% seed moisture content (for field emergence)
- -- At either 18 or 20% seed moisture content (for storability).

(2) AA:
- -- 2-day AA of surface sterilized seed (for field emergence).
- -- 3-day AA (for storability).

(3) Conductivity:
- -- 24 h soaking (for both field emergence and storability).

(4) Speed of germination:

-- GR at 5 ^{o}C and 5/10 ^{o}C (for both field emergence and storability).

Among the different techniques, the best result for predicting seed field performance was obtained from the CD test, followed by the AA and conductivity; for estimating seed storability, the best results were given by both CD (for all storage conditions) and AA (for controlled storage conditions) and CD (for ambient storage conditions), followed by conductivity and then GR.

4. The relationship between laboratory vigour tests and field emergence / storability were influenced by the field / storage environments, the extent of influence varying among the techniques. The test results strongly support the recent view by Hampton and Coolbear (1989) that there is a need to consider more carefully development work on promising tests within a local context. The CD and AA tests were significantly and consistently correlated (R^2 > 80%) to seed performance under a range of conditions. This therefore suggests that the regression equations established in this study has the potential to be further developed and modified for predicting potential seed field emergence and storability under temperate storage conditions. However, more studies in a greater range of environments are still required.

5. Some physical properties of seed can affect seed viability and vigour.

(1) Mechanical damage markedly reduced seed germination and vigour through directly injuring the seed's essential tissues and / or consequently causing imbibition damage during the process of germination and / or emergence. Therefore, in order to improve red clover seed quality it is very important to reduce the incidence of mechanically damaged seed during seed harvesting and processing.

(2) The imbibition rates (IR) were better correlated with viability than vigour in red clover, although the seeds which were swollen at the end of 4 h were low in viability and vigour; and seeds swollen during 4 to 6 h were high in viability and vigour.

(3) Seed weight (or size) was a less reliable or inaccurate parameter to indicate seed viability and vigour in red clover, since many factors may indirectly affect the test results.

6. The loss of viability at high RH was closely associated with the increased incidence of storage fungi infection. The main storage fungi were Penicillium sp at 90% RH , and Aspergillus sp at 75% RH. The loss of vigour as measured by the conductivity test, appeared earlier than the loss of viability during storage.

7. Under NZ ambient open storage conditions, it is unsafe to store low vigour seed lots, even with high initial viability, for more than a year. In general, seed vigour and field emergence were significantly reduced after six months' storage.

8. Under good storage conditions (e.g. 20 ^{o}C-45% RH and 20 ^{o}C-60% RH), the high vigour lots (e.g. lots 2 and 4) maintained their high initial viability over the storage periods (11 months), but the low vigour lots (e.g. lot 3) significantly lost viability during storage (after 9 months at 45% RH and 7 months at 60% RH).

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APPENDICES

Appendix 1 Seed moisture content (%) of 4 Pawera seed lots stored for 11 months at different relative humidities

Storage condition	Lot	Storage period (months) 1	2	3	5	7	9	11
20 °C-45% RH	1	6.4	6.0	6.0	6.6	--	6.6	
	2	6.5	5.8	6.2	6.8	--	7.3	
	3	6.8	6.1	6.1	6.9	--	7.1	
	4	6.7	6.0	6.3	6.7	--	6.9	
20 °C-60% RH	1	9.7	9.3	9.8	9.3	--	9.8	
	2	9.7	9.1	9.9	9.5	--	9.9	
	3	9.9	9.8	10.2	9.4	--	9.5	
	4	9.5	9.5	9.8	9.4	--	9.7	
ambient	1	9.4	9.7	9.0	7.8	7.1	8.3	9.7
	2	9.1	9.5	9.2	8.1	6.9	8.3	9.8
	3	9.1	9.9	9.3	8.4	7.2	8.7	10.0
	4	8.9	9.4	9.1	7.9	7.4	8.0	10.1
20 °C-75% RH	1	13.7	14.4	14.4	14.3	--	13.7	
	2	14.2	14.1	14.4	14.4	--	14.0	
	3	14.5	14.1	14.8	14.9	--	13.7	
	4	13.9	14.0	14.3	14.9	--	14.1	
20 °C-90% RH	1	27.7	30.7					
	2	27.0	30.4					
	3	27.8	30.9					
	4	27.2	30.5					

SMC prior to storage was 11.9, 11.8, 12.4, 10.9% for seed lots 1-4 respectively.

Appendix 2 Storage fungi content of 4 Pawera seed lots stored for 11 months at different relative humidities

Storage condition	Lot	Storage period (months) 1	2	3	5	7	9	11
20 °C-45% RH	1	1b	--	--	0a	11a	0a	0a
	2	6ab	--	--	2a	1a	1a	2a
	3	9a	--	--	3a	0a	2a	0a
	4	5ab	--	--	0a	1a	0a	1a
20 °-60% RH	1	0b	--	--	0a	1a	1a	0a
	2	0b	--	--	4a	1a	1a	1a
	3	6a	--	--	4a	0a	0a	1a
	4	2ab	--	--	2a	0a	0a	0a
ambient	1	4a	--	--	6a	2b	3a	1a
	2	6a	--	--	0a	11a	0a	1a
	3	6a	--	--	5a	1b	2a	1a
	4	1a	--	--	4a	0b	2a	1a
20 °C-75% RH	1	3a	29a	65a	75b	98a	100a	
	2	3a	43a	62a	90a	92ab	100a	
	3	5a	37a	52ab	88a	88b	100a	
	4	3a	30a	37b	79ab	90b	100a	
20 °C-90% RH	1	3a	100a					
	2	6a	100a					
	3	2a	100a					
	4	10a	100a					

* For each condition, means within any column followed by the same letter do not differ significantly at P=0.05