EFFECTS OF SEED VIGOUR ON SEED PRODUCTION AND QUALITY IN ZEA MAYS L. CV. ILLINI GOLD

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1993

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A thesis presented in partial fulfilment
of the requirements for the

Degree of Master of Agricultural Science

in Seed Technology, at Massey University
Palmerston North
New Zealand

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1993

ABSTRACT

MASHAURI, I.M (1993). EFFECTS OF SEED VIGOUR ON SEED PRODUCTION AND QUALITY IN *ZEA MAYS* L. CV. ILLINI GOLD

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Some of the consequences of seed deterioration can be a reduction in field emergence and stand establishment, retarded plant growth and reduced seed yield. From the available literature it is not clear whether the reported lower seed yields are solely a consequence of lower population density resulting from low vigour seed and hence poor emergence, or are a result of poor individual performance of plants grown from low vigour seed.

Two high vigour seed lots of shrunken-2 super sweet corn (*Zea mays* L.) cv. Illini Gold were artificially aged to obtain differential vigour levels (high and low vigour seed lots). These seed lots were then used to study the effects of seed vigour on field emergence and emergence rate, vegetative and reproductive growth and development, and seed yield and quality. The quality of unaged and aged seed lots was judged by the use of standard germination, health and vigour tests. This last category included seedling growth and evaluation tests, eg seedling growth test; stress tests, eg cold germination, soil cold test, complex stress vigour test (CSVT) and soak germination test; and biochemical tests, eg electroconductivity, respiration and tetrazolium tests.

The two high vigour seed lots differed significantly ($P < 0.05$) in thousand seed weight and mechanical damage levels, but not in their germination and vigour performance. However, both these seed lots were heavily infected by *Fusarium subglutinans*. The internally-borne *Fusarium subglutinans* survived the high temperature employed during the artificial ageing treatment, and was able to cause severe damage in the deteriorated seed lots. Aged seed lots demonstrated a decrease in germination and vigour as illustrated by a slower rate of seedling growth and accumulation of dry weight, an increase in electroconductivity leakage, a decrease in respiratory oxygen uptake and an increase in respiratory quotients. Interestingly, however, the performance of these seed lots in the soil cold test, cold germination test, and low temperature respiration test, did not differ significantly from that of the unaged seed lots, presumably because the activity of the seed-borne *Fusarium subglutinans* was reduced by low temperature (10°C or less). This cultivar

has some cold tolerance and exposing seed lots to low temperature did not cause the extent of physiological disorder expected, possibly because of adaptive protection mechanisms that allowed the seeds to undertake some cellular repair.

Field emergence and emergence rates were poor but did not differ significantly between high and low vigour seed lots over three spring sowings at the same site, probably because the environment exerted little stress, but more probably because the effects of both seed-borne and soil-borne *Fusarium* spp on chemically untreated seeds masked any other seed quality differences. As a consequence, no seed quality test was significantly related to field emergence, with the exception of the CSVT which was significantly ($P < 0.05$) correlated with field emergence for the November sowing. A greater ($P < 0.05$) loss of plants from low vigour seed lots due to post emergence damping-off and seedling blight caused by seed-borne and soil-borne pathogens was recorded in the October and December sowings, with the result that plant population was significantly reduced when compared to that of high vigour lots. While the reason for this increased loss was not explained conclusively, it is possible that seedlings produced from low vigour seeds were less able to withstand the fungal attack. However, in plants which survived there were no differences in heterotrophic plant growth as demonstrated by similarities in leaf, stem and plant dry weight at the 3rd leaf stage for all seed lots within any one sowing date. Significant sowing date effects were recorded, however, during autotrophic growth (5th leaf stage), the performance of high vigour seed lots (measured as leaf, stem and plant dry weight) was significantly ($P < 0.05$) superior to that of low vigour seed lots. However, these differences decreased as the plants grew and were no longer present at the 7th leaf stage, because plants from low vigour seed lots had produced thicker tillers.

From silking on, no significant differences among populations were recorded as grain filling rate and days to maximum grain dry weight did not differ. However, for the two sowings (October and December) when plant population differed significantly, plants grown from the low vigour seed lots significantly ($P < 0.05$) outyielded those grown from high vigour seed lots because the former compensated for lost plants by producing more tillers and hence reproductive parts.

The seed vigour status of the parent did not affect the seed vigour of the progeny as demonstrated by similarities in seed quality, vigour and field performance among seed lots of freshly harvested seeds. However, seed quality differed with sowing date, with seed harvested from the November and December sowings having a significantly ($P < 0.05$) lower quality than that from the October sowing. These results should be treated with caution,

because seed quality from all harvests was poor, due to heavy infection of seeds by *Fusarium* species and also damage incurred during drying. The performance of the original seed lots after storage for 14 months showed that the high vigour seed lots had a significantly ($P < 0.05$) higher germinability, vigour and field performance in comparison with their pre-storage performance because the occurrence and activity of *Fusarium subglutinans* which was rampant in unstored seed lots, decrease considerably following storage. Although seed-borne pathogens also decreased in low vigour seed lots after storage, the deterioration originally induced by the accelerated ageing had increased. For stored seed lots, a significant relationship existed between field emergence and both the standard germination and cold germination tests.

The results from both the laboratory tests and field trials were confounded by *Fusarium subglutinans* and other fungi. This work should be repeated in the absence of particularly seed-borne pathogens before any definite conclusions can be drawn.

ACKNOWLEDGMENTS

My studies and the preparation of this thesis depended on the support of many individuals and organisations. I gratefully thank my Chief Supervisor, Professor John G. Hampton of the Seed Technology Centre, Massey University, for his excellent supervision, understanding, and warm encouragement throughout my study, especially for his enthusiastic help, constructive criticism, endless patience and his concern for my personal welfare throughout. Without him, this work would not have been possible. I owe a great debt to him.

I also wish to express my sincere thanks to Mrs Karen A. Hill and Mr Allan K. Hardacre, my Co-supervisors, for their friendship, help and valuable suggestions.

Appreciation and thanks are also extended to:

Professor Murray J. Hill, Director of the Seed Technology Centre, Massey University, for his guidance, support, parental care, his efforts to obtain scholarship funding and concern for my personal welfare, without all of which this work would have not been possible. I can't thank him enough.

Flavia Kabeere, PhD student, Seed Technology Centre, for her expert help in the seed health testing which played a vital role in completion of this thesis.

Dr Peter Coolbear, Seed Technology Centre, Massey University, for his support, persistent efforts to obtain scholarship funding, discussion on the physiological topics and his searching questions which stimulated my lateral thinking.

All the staff of the Seed Technology Centre, Massey University, for their valuable help in so many ways, particularly Mr Craig McGill and Mr Robert Southward for their excellent technical help.

Drs S. Ganeshanandam and Charles R.O. Lawoko, Statistics Department, Massey University, for many helpful suggestions regarding statistical analysis and computing. Mrs Kathryn Hamilton for her humour and skillful typing of this manuscript, and Mr Graeme Donaldson for his help in the graphics.

Financial support for this research programme was received from the New Zealand Ministry of Foreign Affairs and Trade and the Helen E. Akers Scholarship. Professors Murray J. Hill

and John G. Hampton strongly supported my efforts to obtain extra funding, without which my family's welfare would have been jeopardised. My employer, the General Manager, Tanzania Seed Company Limited (TZ) for granting me leave of absence for the duration of study. Mrs Halima I. Kamote for her encouragement and constant support and care in very many ways.

The students of the Seed Technology Centre, Massey University, for their valuable help, especially Cunibert Mtolera, Bambang Budhianto and Daniel Hampton.

Lastly, a huge vote of thanks is due to my family for support, resilience, sacrifice and understanding.

Finally, I thank the LORD for showering me HIS graces of good health, luck and good predisposition.

Isaka M. Mashauri

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CHAPTER 1

INTRODUCTION

This study investigated the influence of seed vigour differences in high and low viour seed lots of sweet corn (*Zea mays* L.) on field emergence, vegetative growth and seed production. The relationship between time of sowing and seed lot vigour status, and the combined effects of these two factors on crop performance were determined. This chapter commences with a general introduction, highlighting the significance and the overall aim of the entire study with an overview of the background literature. It then presents specific problems pertaining to seed production of Shrunken-2 (sh-2) super sweetcorn (*Zea mays* L.), followed with an outline of the specific objectives of the present study. The chapter concludes by presenting a synthesis and organisation of this thesis.

1.1 GENERAL INTRODUCTION

Agronomically, one of the greatest hazards in agricultural production is sowing seeds which do not have the capacity to produce an abundant yield of the required cultivar. Planting seeds which possess those physiological traits for rapid, uniform emergence and optimum number of maturing plants (high germinability and vigour) is one of the surest ways of improving agricultural production.

Decrease in seed planting quality has been attributed to genotypic factors and/or to deterioration during seed development, harvesting, processing and storage (section 2.4) leading to viability and vigour loss of the seeds. This may be due to degradation of cellular systems and functions, including membranes, nucleic acids and cellular organelles (section 2.5). Some of the consequences of seed deterioration are a reduction in field emergence and stand establishment, retarded plant growth and reduced grain yield (Edje and Burris, 1970; Heydecker, 1977; ISTA, 1987). The effects on stand establishment as influenced by emergence is one of the critical factors affecting grain production in cereals (Roberts, 1972; Pollock and Ross, 1972; Heydecker, 1977). These effects can be either through total emergence, rate of emergence or uniformity of emergence.

Total emergence determines plant density, and there is a strong relationship between plant density and grain yield (Pollock and Ross, 1972; Egli and Te Krony, 1979; Perry, 1980). Because of environmental constraints, eg inadequate or excess moisture, pH, temperatures, soil-borne pathogens, soil crusting on silt soils, poorly prepared seed beds or deeper planting (section 2.4), unsatisfactory emergence and establishment may occur (Powell, 1988). The impact of these adverse conditions can be severe and economically very

damaging when low vigour seeds are planted. Apart from decline in the overall yield as a consequence of poor stand resulting from poor emergence (Hampton and Scott, 1982), emergence failure may occur, necessitating resowing, and this could mean a delay in harvest, resulting in a fall in market prices, and lower profits from the harvest (Matthews and Powell, 1986). As there is variability in vigour of individual seed (high, medium and low vigour and dead seeds) in a seed lot, variability in emergence rate is expected, and the late emerging seedlings are at a competitive disadvantage and may result in weaker plants of doubtful survival until maturity (Tseng and Lin, 1962; Webster, 1966). The surviving medium or low vigour seedlings will inevitably cause variability in uniformity during plant growth and development (eg Perry, 1980; Adegbuyi and Burris, 1989; Egli *et al.*, 1990; Te Krony and Egli, 1991). This can potentially affect dry matter accumulation by plants and thus potentially influence seed production.

Production of high quality seeds depends on achieving full maturity (Knittle and Burris, 1976; Bennett *et al.*, 1988). However, due to variability in emergence, growth and maturity rates, the harvested seeds may inevitably vary in size and/or quality. The smaller seeds are likely to be immature seeds of lower dry weight, or mature seeds of unbalanced chemical composition, have suffered stress during development, causing low vigour in a seed lot (Wilson and Trawatha, 1991). Although sophisticated machinery (eg specific-gravity separators, aspirators, etc) capable of sorting seeds by density or size are available, small, immature, low vigour seed lots cannot be upgraded. Much research work has also been pursued in a quest of assessing the effects of seed vigour on seed production. Some of these research findings (eg Funk *et al.*, 1962; Camargo and Vaughan, 1973; Burris, 1975) indicate that vigour effects may be carried over to influence grain yield. However, whether the reduced yield is due to seed vigour effects *per se*, or is simply because of reduced plant population is questionable. There is growing evidence showing that when plant population is not a factor, there are negative or no effects of seed vigour on grain yield (eg Johnson and Wax, 1981; Barla-Szabo, Dolinka and Berzy, 1989; Adegbuyi and Burris, 1989; Te Krony *et al.*, 1989). Factors such as genotypic diversity, variability in vigour levels of the seed lots used, environmental interactions, and experimental techniques employed might be responsible for these conflicting seed vigour results. This last factor (vigour experimental techniques) is crucial as appropriate and reliable methods for determining seed vigour (sections 2.3 and 2.7.5), which will effectively predict field emergence and thus help to improve crop stand and yield are still required.

From the available literature, it is not clear whether the reported lower yields are due to other seed vigour effects, or merely a consequence of lower population density resulting

from low vigour and hence poor emergence. A study carefully designed to identify the influence of seed vigour *per se* on seed production is important.

1.2 SEED PRODUCTION PROBLEMS OF SUPER SWEETCORN (*Zea mays* L.)

Fresh market sweet corn hybrids are popular in many countries, eg USA, China, Japan, New Zealand, Netherlands, etc. In New Zealand, fresh market sweet corn earns approximately 6 million dollars annually (NZ Agriculture Statistics, 1991). Shrunken (sh_2) super sweet corn genotypes in particular, have dominated the market because of their extra sweetness and extended post harvest kernel quality. This provides longer periods for transport, process, sale and consumption of sweet corn of superior quality (Juvic *et al.*, 1993). The biochemistry and physiology behind high sugar and low starch concentrations in developing sweet corn kernels and the associated attributes are described in section 2.2

However, sh-2 genes are not without some drawbacks. Although they can enhance eating quality, they pose seed production problems. Achieving optimum and uniform maturing plant populations of sh-2 sweet corn is an ever challenging problem to growers and researchers, a problem which can become severe, even devastating because sh-2 seed germination is often low due to poor quality (Juvic *et al.*, 1993). The cause of problems related to stand establishment and vigour with sh-2 genotype are numerous, and have been documented. As the kernels remain with high moisture and sugar content at harvest, delayed harvesting may occur, making the seeds liable to weather and/or pathogen damage. If harvested at high seed moisture content, mechanical drying is necessary. This may cause mechanical and/or drying damage and seed quality reductions. Reduced starch levels in sh-2 kernels may not provide sufficient carbohydrate reserves for optimal emergence and vigorous seedling growth rates (Wann, 1980). The dramatically reduced starch content of sh-2 kernels results in severe collapsed endosperm, cracks in the pericarp, and air pockets between the pericarp and aleurone layer (Styer and Cantliffe, 1983). Styer and Cantliffe (1984) suggested that poor performance of seeds of sweet corn may be due to dysfunction of the scutellum or embryonic axis in relation to utilisation of carbohydrates. But Harris and De Mason (1989) disputed this and attributed poor seedling performance to a delay or reduction of aleurone-controlled mobilisation of reserves needed for embryo growth. High sugar content of kernels elevate osmotic potential which in turn may cause membrane and pericarp damage as a result of too rapid an influx of water during imbibition (Simon, 1978). This may be associated with increased leaching of sugars such as sucrose, glucose and fructose (Caplan, 1984) from the seeds, and hence increase conductivity (Wann, 1986).

Solute leakage not only reduces metabolic energy available for embryo growth during germination, but also stimulates growth of pathogenic micro-organisms which may decrease emergence and seedling vigour significantly (eg Styer and Cantliffe, 1984; Headrick and Pataky, 1989; Headrick *et al*., 1990).

Due to the complexity of factors influencing seed vigour, and the wide variation in field planting conditions, it is important to investigate the effects of seed vigour on seed production and quality using both high and low vigour seed lots under different environmental conditions, and to see how long such effects persist in the crop cycle.

1.3 OBJECTIVES OF THE STUDY

Considering all these aspects, this study was designed to investigate the effects of seed vigour on:

1. Field emergence and emergence rate
2. Crop vegetative and reproductive development
3. Seed yield and quality.

1.4 SYNTHESIS AND ORGANISATION OF THIS THESIS

This thesis comprises 7 chapters. Following this introductory chapter is a review of the literature (Chapter 2) pertaining to seed vigour and its measurement. Factors affecting seed vigour such as genetic effects, environmental conditions during seed development, harvesting and processing techniques and storage conditions are discussed in some depth, with some coverage on the physiological basis of the factors limiting seed quality and especially vigour. Chapter 3 describes the general materials and methods used in this study. Specific experimental procedures are presented alongside the appropriate data under the relevant chapters. Chapter 4 describes extensive work on seed quality and vigour assessments in two experiments. Experiment One employed a series of seed quality and vigour assays aimed to give an estimate of potential performance of seed lots under sundry environmental conditions. In Experiment Two, a wide range of techniques were used, and were designed either to confirm and/or investigate further the results in Experiment One so as to give clearer and more detailed information of seed planting value before planting in the field. In Chapter 5, the effects of seed vigour on field emergence, vegetative and

reproductive development, maturity, seed yield and seed yield components, harvesting and drying are evaluated. The potential seed quality, vigour status and field performance of fresh harvested and original stored seed lots are examined in Chapter 6. In the last chapter (Chapter 7), a short discussion, key conclusions and scope for future work are given.

CHAPTER 2

REVIEW OF LITERATURE

2.1 INTRODUCTION

A seed is a highly sophisticated, self-contained living system. As successful performance of the seed is related to its quality, it is important that this quality be preserved. In order to maximise its chances of producing a successful plant, it needs to be treated with care during production, harvesting, processing and storage. This review first introduces the crop seed used in this course of study, and because of their importance in agriculture, accumulation of seed food reserves have been highlighted. Seed vigour, the focus of this study, is then addressed briefly, highlighting the importance of seed vigour in successful agricultural seed production, techniques for seed vigour assessment and the underlying principles and limitations of these tests. The factors affecting seed vigour are discussed in some detail, opening with a short discussion on the complex soil environment where the planted seeds must either emerge to produce a new generation, or perish. The influence of genetic factors during the various steps of seed production is considered first, followed by the seed production field where the seeds form, develop and mature, particularly the cultural and environmental conditions during seed development, eg mineral nutrients, temperatures, water potential, pathogens, etc. Untimely and improper seed harvesting, threshing, drying and cleaning are discussed next. Lastly the seed storage environment is considered, especially the ambient air temperature and relative humidity (hence moisture content) of the seed store.

2.2 *Zea mays* L.

Maize, or corn is a tall, vigorous growing annual plant, with flowers occurring on separate male and female inflorescences. The male flowers are borne on a terminal inflorescence called a tassel. The female inflorescences, are located half way down the plant, and only one of the flowers in each spikelet is fertile. The paired spikelets are unstalked and are located on a spadix known as the cob. It is this monoecious nature of the plant that allows for its regulated or controlled crossing. The cob develops as a swollen tip of a branch with extremely short internodes. Overlapping modified leaves develop from these compressed nodes and form the husk which sheaths the cob. The species is characterised by monocotyledonous endospermic caryopses, each consisting of an endosperm and an embryonic axis (Johann, 1935; Berrie, 1977; Justice and Bass, 1978). At maturity, the endosperm is packed solidly with dead reserve materials that will be hydrolysed to provide the growing embryo with nutrients until it establishes itself as an independent seedling. The only remaining live cells are the aleurone layer and embryonic

axis. The aleurone layer cells contain some hydrolytic enzymes capable of starting the processes of food reserve breakdown upon subsequent germination. The embryo will differentiate into the radicle and plumule, and also contains some plant growth regulators responsible for controlling the processes involved in germination and the mobilisation of food reserves (Black, 1972; Diekma and Jones, 1986; Hammerton and Ho, 1986).

There are many types of maize which vary both genotypically and phenotypically, but most fall into five main categories.

1. Dent maize: A type of maize which has kernels with a central core of soft starch which shrinks more in drying than the surrounding hard endosperm to leave a shallow depression at the top of the kernel.

2. Flint maize: This type of maize has kernels with hard endosperm but with a small amount of soft starch near the centre.

3. Floury maize: A type of maize which has kernels of mostly soft endosperm with a thin layer of hard endosperm around the surface.

4. Popcorn: A type of maize which has kernels of mostly hard endosperm. The kernels of popcorn pop or explode when heated.

5. Sweetcorn: A type of maize which has kernels with a relatively high sugar content.

The last category of maize, the sweetcorn, differs from the other types of maize in that the sucrose-starch conversion is inhibited, and as a result the kernels remain with a high sugar content (Ferguson *et al.*, 1978).

In normal maize, starchy formation in the endosperm of a developing seed involves several reactions catalysed by enzymes such as sucrose synthase, UDP-G pyrophosphorylase, ADP-G pyrophosphorylase and starch synthase, among others (Bewley and Black, 1978; Preiss, 1978; Salisbury and Ross, 1978; Goodwin and Mercer, 1985). The primary substrate for the starch synthesis is ADP-glucose, although Uridine 5-diphosphate (UDP)-glucose can also be used *in vitro* (Akatsuka and Nelson, 1966; Lavintman *et al.*, 1974). ADP-glucose is formed from ATP and glucose-1-phosphate in a reaction catalysed

by ADP-glucose pyrophosphorylase. This is an allosteric enzyme activated by ATP and by some glycolytic intermediates that might regulate starch synthesis (Tsai and Nelson, 1966; Dickinson and Preiss, 1969; Ozbun *et al.*, 1973). ADP-glucose can also be synthesised directly from sucrose and ADP in steps mediated by sucrose synthase enzyme. It can also be formed indirectly from UDP-glucose (Defekete and Cardini, 1964; Su and Preiss, 1978). UDP-glucose is converted to glucose-1-phosphate by UDP-glucose pyrophosphorylase, and glucose-1-phosphate can then be used by ADP-glucose pyrophosphorylase to produce ADP-glucose (Tsai *et al.*, 1970). As the glucose incorporated into starch during starch formation is from the ADP-G (Bewley and Black, 1978; Salisbury and Ross, 1978; Goodwin and Mercer, 1985), many workers have suggested that ADP-G pyrophosphorylase is the most important enzyme in starch synthesis of a developing endosperm in maize (Tsai and Nelson, 1966; Dickinson and Preiss, 1969; Preiss, 1978; Bewley and Black, 1978; Goodwin and Mercer, 1985).

The work of Dickinson and Preiss (1969) showed that the 3-P-glycerate, which is produced by photosynthesising pericarp tissue in a developing seed, activated the ADP-G pyrophosphorylase during starch synthesis. They suggested that activation of this enzyme by 3-P-glycerate has a role in regulation of starch synthesis. This activation had previously been observed with ADP-G pyrophosphorylase from other photosynthetic tissue (Ghosh and Preiss, 1966) and in non-photosynthetic tissue (Sanwal and Preiss, 1967). Presumably 3-P-glycerate has physiological importance in modulation of starch synthesis in these tissues (Dickinson and Preiss, 1969; Ghosh and Preiss, 1967; Sanwal and Preiss, 1967; Sanwal *et al.*, 1968).

Another interesting view regarding the interaction between ADP-G pyrophosphorylase and 3-P-glycerate in starch synthesis of a developing seed, is that of cessation of starch synthesis at mass maturity. There can be little doubt as to the importance of ADP-G pyrophosphorylase and 3-P-glycerate in starch synthesis; however, it is known that the linear increase of food reserve accumulation indicated in stage II of seed development (section 2.4.3.1), is ultimately not source limited; rather, it is sink controlled (Bewley and Black, 1978). This view was demonstrated by Jenner and Rathjen (1975) who showed that at mass maturity, where maximum dry weight is attained (Brooking, 1990), there was still plenty of sucrose available to the developing seed. Coolbear (1990) suggested that the key factor in cessation of starch synthesis may be the 3-P-glycerate which is produced by the synthesising pericarp of a developing seed. 3-P-glycerate is required to activate the key enzyme in starch synthesis, the ADP-G pyrophosphorylase

(Dickinson and Preiss, 1969; Coolbear, 1990). The time at which the pericarp loses its photosynthetic activity is when a cessation of starch synthesis is expected (Coolbear, 1990).

Incorporation of mutants in maize breeding programmes dramatically alters the characteristics of the resultant hybrids. In maize, there are many morphological identifiable mutant genes affecting virtually every level of the plant's life cycle (Neuffer *et al.*, 1968; Coe and Neuffer, 1977). Endosperm mutants, which in maize have been given names such as defective endosperm (de), shrunken endosperm (sh), brittle (bt), defective kernel (dek) among others (Laughnan, 1953; Neuffer and Sheridan, 1980; Soave and Salimini, 1984; Clark and Sheridan, 1986), exert their effects in the quantity and quality of the starch in the endosperm. Kernels appear shrunken, wrinkled, brittle, frequently collapsed or giving indented crowns (Neuffer *et al.*, 1968; Soave and Salamini, 1984). (For review see Scandalios, 1982; Soave and Salamini, 1984).

In sweetcorn, the incorporation of the high sugar endosperm mutant genes (eg sh_2, bt_2) alters the carbohydrate synthesis pathway in the endosperm, particularly inhibiting formation of water insoluble polysaccharides and thus raising the sugar levels (Gonzales *et al.*, 1976; Ferguson *et al.*, 1978). Many workers have suggested that the site of inhibition of starch formation by these mutants is the enzymatic system responsible for starch synthesis, particularly ADP-G pyrophosphorylase (Tsai and Nelson, 1966; Vidra and Loerch, 1968). Dickinson and Preiss (1969) presented data showing that sh_2 or bt_2 genes arrested 92 to 95% of the endosperm ADP-G pyrophosphorylase activity. They also observed less activation of ADP-G pyrophosphorylase by 3-P-glycerate in mutants than in normal maize. Chourey and Nelson (1976) reported that sh_2 mutant genes control the major form of sucrose synthase activity. Hannah *et al.* (1980) demonstrated that all ADP-G pyrophosphorylase detectable in the endosperm was under the control of sh_2 and bt_2 mutant loci, and Tsai and Nelson (1966) showed that the maize mutant, sh_2 and bt_2 contained no detectable ADP-G pyrophosphorylase enzyme. The accumulation of starch in these mutants was estimated to be only 25% of the starch found in non-mutant (normal) endosperm (Greech, 1965; Tsai and Nelson, 1966). This was supported by Bewley and Black (1978) who reported that the sh_2 mutant of maize had only 10-12% of ADP-G pyrophosphorylase activity and synthesised only 25-30% of the starch of normal maize, resulting in small kernels with smaller endosperms (Wann, 1980). In addition to disruption of starch synthesis, mutants slow both the rate of change of the endosperm carbohydrate fractions and moisture loss as seed maturity advances (Soberalske and Andrew, 1978), and thus interfere with seed maturation (Churchill and Andrew, 1983) (Section 2.4.3.1).

2.3 SEED VIGOUR AND FIELD EMERGENCE

For many agricultural and horticultural crops, specified plant populations are recommended for maximising yield and/or quality (Hampton and Coolbear, 1990), and the first prerequisite in achieving this goal is the use of seeds which possess those physiological traits for maximum yield and/or quality of the required cultivar. Unfortunately, however, because of the interplay of many deteriorative factors affecting the seeds, from the time of inception at flowering of the parent plants until they germinate, emerge and establish in a complex soil environment, availability of such high quality seeds may become a limiting factor. Assaying of seed quality before planting is thus vital and is the objective of much effort in many countries, leading to development of various techniques for detection and estimation of potential field performance of seeds.

2.3.1 Standard germination and field emergence

The main method for determining the planting value of seeds, and hence their value to producers, is seed testing for various aspects of seed quality. Seed viability is considered the major component and most widely accepted parameter for assessing seed quality, and germination testing has become the universal method for determining seed viability (AOSA, 1983; ISTA, 1987). The test reports the percentage of normal seedlings, abnormal seedlings (which are probably produced from those seeds close to death - Roberts, 1986), and dead or ungerminable seeds in the seed lots, and germination results less than an acceptable standard (eg 90% for cereals - Hampton and Coolbear, 1990) usually indicate deterioration.

There is often a good correlation between germination test results and field emergence under favourable conditions (eg Thompson, 1979; Roberts, 1984; Bekendam *et al.*, 1987). The Bekendam *et al.* (1987) study for example, showed that aged and unaged seed lots of maize had similar performance in the standard germination test, and that during favourable field conditions, the germination capacity showed the highest correlation with field emergence. However, under unfavourable conditions, the standard germination test often over-estimates the actual field performance of the seeds (AOSA, 1983; Hampton and Coolbear, 1990). For example, Bekendam *et al.* (1987) demonstrated that although the standard germination results of both aged and unaged seeds were similar, aged seeds performed poorly in the cold test, and the conductivity test, and emerged poorly in the field, and cold test and conductivity test results showed higher correlation with field emergence than the standard germination test (cf Perry, 1978).

This observation can be attributed to two factors: the standard germination test is not sensitive enough to distinguish small but important differences in the ageing or deterioration processes, and that in the germination test the soil physical properties (temperatures, water potential and strength) and the biotic factors (insect pests and micro-organisms - section 2.7.1) are either excluded or optimised, while in the field the physical and biotic factors are rarely completely optimal, and can reduce the level of field emergence and establishment to below that predicted by the standard germination test. The standard germination test, thus assesses the maximum percentage of seed sown which can potentially germinate, emerge and establish, but the actual field emergence essentially depends on the prevailing environmental conditions. There will be differences in field emergence between high germinating seed lots of the same cultivar due to differences in seed vigour (Hampton and Scott, 1982; ISTA, 1987).

2.3.2 Seed Vigour

Seed vigour has been defined as those properties of a seed lot which determine its potential to rapidly produce a uniform and healthy plant stand under a wide range of field conditions (AOSA, 1983). Seed vigour may be viewed as the potential of a seed lot to germinate, emerge rapidly or store well under less than optimal environmental conditions (Hampton and Coolbear, 1990). Maximum seed quality occurs at mass maturity, when maximum seed dry weight is attained, after which vigour and viability can deteriorate both pre- and post-harvest. Loss of vigour may result from a matrix of inter-related deteriorative processes involved with seed ageing, which may be viewed as an inevitable consequence of initial membrane deterioration (section 2.5). As deterioration increases, both field emergence and storability decrease (Delouche and Baskin, 1973), and the seed lot will exhibit ageing symptoms including a slow rate of germination and emergence, poor seedling growth, decreased tolerance to sub-optimal conditions, low oxygen uptake during the early stage of germination and enhanced solute leakage (section 2.7). However, there are distinct, though interacting, factors such as genetic constitution, environmental conditions during seed development, tolerance/susceptibility to mechanical or heat damage during harvesting, drying and cleaning, storage conditions and pathogens which are fundamental factors considered to influence vigour potential through their effects on membrane integrity (section 2.4).

2.3.3 Vigour assessments and their relationship with field emergence

The performance differences in field emergence between seed lots which the standard germination test indicates are of similar quality (section 2.3.1), obviously require a more sensitive differential of potential seed performance to be able to predict field emergence and establishment with more accuracy. Although this can in theory be achieved by predictive simulation models frequently used by agricultural engineers and agronomists (Wood, 1992), this method is over complicated and unreliable as such simulation models require accurate information on the environmental conditions in the field (that are often variable, and hence, unpredictable) and on the response of seed lots to the environmental variables. Seed vigour testing has become very important because it can often provide a better estimation of field emergence under stress conditions than the results of standard germination tests (Te Krony and Egli, 1977; Johanson and Wax, 1978; AOSA, 1983; ISTA, 1987; Hampton and Coolbear, 1990).

A variety of seed vigour tests have been developed, and AOSA (1983) categorised them as (i) seedling growth and evaluation tests (eg seedling growth, speed of germination, etc), (ii) stress tests (eg cold test, accelerated ageing, etc), (iii) biochemical tests (eg conductivity test, respiration, etc). The principles and suggested procedures for these tests have been published by AOSA (1983) and ISTA (1987) and those often used for evaluation of maize seed vigour are discussed in detail in section 2.7. Although these tests have been shown to provide results which are more closely correlated with seed performance in the field under some conditions than the germination test, they suffer reproducibility problems (for review, see Hampton and Coolbear, 1990), and in contrast to the standard germination test, there is no standard vigour test or combination of tests widely used to predict field emergence at present. This particular problem is discussed in section 2.7.5. Hampton and Coolbear (1990) reviewed the progress of vigour testing during the past 25 years. Their conclusion on the development of a vigour test to give an indication of the potential performance of a seed lot was that without an understanding of the variables and assumptions involved in some of the tests, with all the complexities involved, and the vast number of possible interactions of seeds with different post harvest and planting environmental conditions, it is most unlikely that any one test, whether germinative, physiological or biochemical will be appropriate for even a single species under all conditions. However, if the internationally applicability of these tests (standardisation problem) is left out, many of these tests have been shown to be successful under local conditions.

2.4 FACTORS INFLUENCING SEED VIGOUR

The planted seeds are the end product of several steps. These include growing in the seed production field, harvesting, processing and storage (Justice and Bass, 1978). The conditions and practices the seeds encounter in these processes have their ultimate test when the seeds are planted in the complex environment of the soil.

Soil moisture levels range from insufficient for seed germination and development, to water saturated soil which can cause soaking injury. Water logged soils may also cause oxygen deficiency that can inhibit germination and seedling elongation. Soil temperatures range from below zero to above 30°C, and those < 15°C (depending on genotype) can cause chilling injury. A combination of decreased soil temperature and soil moisture content and an increasing planting depth, can be critical to successful seedling emergence (Perry, 1976; Chopra and Chaudhary, 1981). This can reduce emergence rate, making the seeds more prone to soil pathogen attack, causing a decrease in total emergence. The pH in agricultural soils normally varies from 3 to 8, while the levels of organic matter, amount of various ions and soil texture also differ greatly, and this too may affect germination (Cal and Obendorf, 1972; Etherington, 1978; Harman, 1983). Maximum germination and seedling growth rate for most kinds of seeds occur in pH ranging from 4.0 to 7.0 (Justice and Reece, 1954; ISTA, 1985). Hydrogen-ion concentration 4 < 7 may thus affect germination and emergence. Soil strength (compaction) opposes penetration and growth of the root system and also shoot growth prior to emergence. This opposing force increases with decreasing pore diameter in the soil, levels of organic matter and water potential, ie as the soil dries (Currie, 1984). Without adequate vigour, the elongating root experiences resistance or fails to penetrate compacted soils. The greatest barrier to shoot elongation is a surface crust, formed when unstable soil, often with a too fine tilth, slakes on wetting then hardens on drying (Arndt, 1965a, b; Currie, 1984). Throughout this range of soil conditions there are numerous insects and about 1,500 types of micro-organisms and virus that can survive and adversely affect the germinating seed (Agarwal and Sinclair, 1987).

The uncertainty of environmental conditions demands that the seed producers must plant seeds of high vigour because the subsequent performance of seeds under adverse conditions usually hinges on the ability of the seeds to survive the wide range of environmental conditions. However, there are many factors which are considered to influence vigour. These can be categorised as genetic effects, environmental conditions during seed development, harvesting and processing techniques, pathogens and deterioration during storage (Ching and Kronstad, 1972; Burris, 1977; Bulisan and Warner,

1980; ISTA, 1987; Bdliya and Burris, 1988; Mashauri *et al.* 1992). These factors can all be linked to seed ageing, due to the damage inflicted to the structural integrity of cell membranes during seed development, drying, handling and storage.

2.4.1 Genetic effects

Genotype can have a significant effect on seed quality, and there is considerable evidence of genotype differences in seed quality characteristics or in characteristics which indirectly influence seed quality. The minimum potential seed quality with regard to germination, emergence, vigour and tolerance to sub-optimal conditions is genotypically controlled, with environmental conditions determining how well this potential is fulfilled (Burris, 1975; Dickson, 1980; Bdliya and Burris, 1988).

Rapid, high and uniform emergence and vigorous seedling development are good indicators of high vigour value in a seed lot (Perry, 1980). However, there are many reports of genotype differences in seed quality characteristics responsible for successful and rapid establishment in maize crops. For example, Burris (1977) showed that some inbred lines had a highly significantly greater germination, shoot and root dry weights compared with other inbred lines, and Barla-Szabo *et al.* (1990) demonstrated that shoots of some inbred lines were significantly longer than others. Complex stressing vigour test (CSVT) results indicated that high vigour inbred lines, as determined by the seedling growth test, developed stronger, faster and produced healthier seedlings after complex stressing vigour testing. Other workers (eg Odiemah, 1991) have also shown genotype differences in maize germination and seedling vigour.

Seed vigour is a manifestation of the ability to survive a wide range of environmental stresses during germination and the subsequent growth and development of the plant (ISTA, 1987). Low temperature stress, for example, has been recorded in both low temperature germination and low temperature growth in maize (Eagles and Hardacre, 1979a; Eagles, 1982). However, these two factors are not necessarily correlated, and the ability to germinate at low temperatures is often independent of the ability to grow at low temperatures (Dickson, 1980). Anderson (1970) suggested that germination and seedling growth, although closely related, are regulated by two different mechanisms. The same is also likely to be true of the ability to germinate and the ability to grow under other stress conditions such as salinity or drought (Sulisbury, 1992).

Low temperature stress of 5-15°C (depending on genotype) can cause poor germination as a result of physiological disorders (Cal and Obendorf, 1972) - refer section 2.7.3. The Miedama *et al.* (1982) study for instance showed that seedling elongation rate, measured as time to emergence of seeds sown at a depth of 4 cm, was recorded as 23 days at 10°C, 8 days at 15°C, 4 days at 21°C and 2 days at 32°C. Menkir and Larter (1987) showed a reduction of seedling dry mass in 12 inbred lines at lower temperatures. On average shoot dry weights were 6.57 g (18°C), 1.12 g (14°C) and 0.18 g (10°C). Many workers (eg Eagles and Hardacre, 1979a,b; Eagles and Brooking, 1981), however, have demonstrated genotype differences in germination, germination rate and seedling vigour at low temperature in maize. The genetic control of this low temperature stress characteristic has been investigated. The work of Eagles and Hardacre (1979a) indicated that maternal effects appeared to be of considerable importance for germination percentage at low temperature in maize, while the result of Eagles (1982) suggested that genotype of the embryo and endosperm was of much greater importance than the genotype of the maternal parent in determining differences in time to emergence and seedling growth at low temperatures.

Exposing young maize seedlings to temperatures in a chilling range can lead to various types of physiological dysfunction, manifested in abnormal development of the plant (Miedama, 1984). In extreme low temperatures, frost may occur. Frost temperatures may kill the foliage and stalk of the immature plant or may inflict damage to the seeds themselves (Rossman, 1949a) (see section 2.4.2 for more detailed discussion). However, Salisbury (1992) presented data indicating that genotypic differences in seedling chilling tolerance exist in maize. Other workers (eg Eagles *et al.*, 1983) showed genotypic variation in seedling frost tolerance in maize, while Rossman (1949a) reported a significant difference in tolerance/susceptibility of seed to frosting temperatures among inbred lines.

Mechanical damage, imbibition damage (section 2.4.3.4), and weathering damage (section 2.4.2) can all affect seed quality in many crop species. There are, however, genotype differences for many of these characteristics. For example, Sirivastavas *et al.* (1974) reported maize cultivar differences in the load necessary to rupture the kernel pericarp, and Wortman and Rinke (1951) observed that some maize hybrids have larger and heavier kernels while others have a thicker covering over the germ and are therefore more resistant to damage. Horlings *et al.* (1991) showed that soybean lines, with large seed size and a permeable seed coat, were more highly susceptible to weathering damage, than lines with small seed size. The work of Powell *et al.* (1986) demonstrated that white cultivars of beans were more susceptible to imbibition damage and poor field emergence. White

cultivars have higher rates of water uptake, presumably because of faulty testas which are easily damaged during threshing. Although impairment of membrane integrity (measured as electrolyte leakage), can be influenced by either weathering, imbibition damage (Powell, 1986) or mechanical damage (Bekendam *et al.*, 1987; Bruggink *et al.*, 1991a), membrane integrity *per se* is genotype dependent (Joo *et al.*, 1980; Tao, 1980a).

Viability and vigour levels of stored seeds decline as a result of spoilage by storage fungi or natural ageing (section 2.4.4) with vigour loss preceding viability loss (Justice and Bass, 1978). It is generally accepted that seeds of high initial quality usually tolerate adverse conditions better than seeds of initially low quality (Byrd and Delouche, 1971; Roberts and Black, 1989). A small difference in germination in different seed lots of the same cultivar can sometimes cause a big difference in seed storage life (Ellis *et al.*, 1988). However, there are significant differences in seed longevity among cultivars in maize. Odiemah (1991) observed significant differences in vigour and storability (estimated by the accelerated aging technique) among maize hybrids. Attempts have been made to link better storage ability of cultivars with chemical quality or other characteristics of the seed. In maize for instance, correlation between higher polyamine content and superior storage characteristics has been reported (Lonzono *et al.*, 1989). These authors demonstrated that storage characteristics were determined by dominant nuclear genes, without any maternal effects.

2.4.2 Environmental conditions during seed development

Environmental conditions during seed development may affect the degree to which the seed's potential can be reached. Levels of soil fertility and moisture supply influences sink size and weight, and there is evidence that seed weight is associated with germination and vigour (Heydecker, 1972; Austin, 1972; Ueyama, 1975; Odiemah, 1985), but there is also a large body of evidence suggesting the opposite (eg Smith and Camper, 1975; Delouche, 1980; TeKrony and Egli, 1991).

It has been demonstrated that nitrogen fertiliser application increases protein content in cereal seed crops (Chang and Robertson, 1968; McNeal *et al.*, 1971; Lopezi and Grabe, 1973; Bulisan and Warner, 1980), and that seeds with high levels of protein perform better than low protein content seeds of the same cultivar. High positive correlations have been reported between high protein content and seedling vigour (Lowe *et al.*, 1972; Lowe and Ries, 1973; Ries and Everson, 1973). Interaction of nitrogen and phosphorus nutrition supplies to the mother plant have been reported to increase vigour (measured as leachate

conductivity) in peas (Hadavizadeh and George, 1988). Deficiencies in these elements may thus affect membrane permeability (and hence, vigour) through effects on the protein and phospholipid components of membranes.

It is known that plants have evolved a capacity to adjust seed production to the resource available, and thus the response of plants to chronic water (drought) stress is a reduction of quantity and sink size (Hall *et al.*, 1981; Schussler and Westgate, 1991). This is because of asynchrony in floral development, egg sac abortion and zygote abortion (Moss and Downey, 1971; Herrero and Johnson, 1981; Westgate and Boyer, 1986). This last problem may be caused by substrate starvation because water deficiency affects photosynthesis and reduces carbohydrate reserves at pollination (Westgate and Boyer, 1986). On the other hand, an acute deficiency resulting from temporary but severe deficiencies can have disastrous effects because once seeds are set, many crops have little capacity to adjust seed number to the available assimilates and the result is low weight, shrivelled and small size seeds (Delouche, 1980). This last effect (seed size), generally shows that within a seed lot, larger seeds have better performance than small seeds. However, between seed lots, although the effect of seed size on potential performance has been extensively studied, most of the results are conflicting (TeKrony and Egli, 1991). This may be due to the fact that seed vigour is often not related to seed size, and other factors such as mechanical damage levels, ageing and incidence of micro-organisms (see other sections) may well differ between individual cultivars, or seed lots, and conceal any seed size effects.

Maturing seeds can be damaged on the mother plant by insects, birds and rodents. These may affect the subsequent performance of the seeds. Additionally, pathogens may invade the seeds through the damaged areas, or can be internally transported to the seed tissue as a result of systemic infection (Neergaard, 1977). Once infection has occurred, be it from internal or external sources, the pathogen can cause disease (eg *Fusarium* spp may cause a severe ear and/or kernel rot, Shurtleff, 1980). The infected seeds in the field may survive and be a source of primary inoculum in the next generation (Christensen and Kaufman, 1969, 1974). The topic of pathogens is addressed in section 2.6.

Ambient temperature regimes during seed development and maturation may affect the potential performance of the seeds. Browning and George (1981) reported that high temperatures and sunny weather during ripening and maturation can induce low vigour in peas, and Green *et al.* (1965) showed that soybean seeds that experienced hot and dry weather during maturation exhibited reduced germination and field emergence. In maize,

increased temperature reduced the duration of the linear phase of kernel growth (Badu-Apraku *et al.*, 1983) - the sequence of seed development is described in section 2.4.3.1. This in turn may reduce vigour as a result of unbalanced chemical composition of reserves. Alternatively, extended periods of extreme temperatures may affect the potential assimilate production, assimilate supply and assimilate translocation to the kernel (Tollenaar and Daynard, 1978; Tollenaar and Bruulsema, 1988). Thermal effects may impair the endosperm cell division process, causing a severe reduction in number and/or size of endosperm cells or starch granule formation (Radley, 1978; Jones *et al.*, 1985) that may subsequently reduce seed weight and hence vigour level. This is because kernel sink capacity or potential to accumulate dry weight is determined by the number and size of endosperm cells formed during the growth phase (Stage I) of seed development (Bingham, 1969; Jenner, 1979), which in turn may determine the size and number of sites of starch deposition during the food reserve accumulation phase (Stage II) of seed development (Jones *et al.*, 1985).

Early frosts may occur in the seed production field before harvest, and this may kill the leaves and stalks, stopping the translocation of food materials to the developing seed so that the kernels fail to accumulate the necessary food reserves for subsequent seed germination and seedling growth. The seeds themselves may be damaged (Rossman, 1949a). This damage can be progressive, causing a reduction in seed vigour, increase in abnormal seedlings and finally, a complete death of the seeds. This type of damage might have been caused by intercellular ice formation, accompanied by dehydration and eventually an irreversible physiological change of the protoplasm (Rossman, 1949a; Aboul-Ela, 1951). Aternatively, frost may cause a localised killing of the embryonic axis (Aboul-Ela, 1951). Rossman (1949a) for example, demonstrated a failure of some frozen seeds to develop either a plumule or a radicle. He suggested that localised intracellular ice formation might have occurred in the embryo. The ice crystals formed may lacerate the plasma membrane and produce instant loss of semipermeability and death of the cells (Levitt, 1980). Frost temperatures may also affect syntheses of storage protein (zein, glutelins, globulins) during seed development (Aboul-Ela, 1951).

However, many workers (eg Rossman, 1949a,b; Aboul-Ela, 1951) have reported that moisture content of the seed is a major factor in freezing injury. Rossman (1949a) showed that frozen seeds of high moisture content become spongy and soft and thus are susceptible to rapid infection, and on their subsequent drying, they showed darkened embryos. At low SMC, the water bound more closely to the protoplasm and was thus less subject to crystallisation. The protoplasm also becomes viscous and elastic, therefore resisting dehyration and reducing the amount of cell shrinkage during freezing. A seed moisture

content of 25% is regarded as being safe from frost injury in maize (Aboul-Ela, 1951). However, genotype (section 2.4.1), temperature, duration of exposure and seed maturity are also important factors affecting frost damage in maize (Rossman, 1949a,b; Delouche, 1980).

Rossman (1949b) working on sweetcorn, dent maize and pop corn seeds, observed that sweetcorn inbreds were more tolerant to frost damage than dent maize inbreds, while pop corn inbreds were most susceptible to frost damage, presumably because of differences in sugar level, which tend to be higher in sweetcorn than in dent maize or pop corn (section 2.2). Sugars have a protective effect during temperature and water stress (freezing, desiccation and heat) and have been reported to protect sensitive membranes against freezing damage. This protective effect increases with increasing sugar concentration (Santarius, 1973). The mechanism involved in protecting cell membranes from frost damage is much the same as that which occurs during seed maturation and desiccation (Santarius, 1973), described in detail in section 2.4.3.2.4.

Climatic conditions during the post-maturation and pre-harvest periods also have a great influence on seed quality. Stresses set up by cycles of wetting and drying causing alternative swelling and contracting of seed tissues (weathering damage) may occur after maturation (Moore, 1965; Delouche, 1980). This may cause physiological (eg membrane, respiratory) damage (Coolbear, 1990). In some cases, especially in cereals with reduced sensitivity or low levels of ABA, imbibition (wetting) may initiate the gearing up of the seed's metabolic machinery for growth. Hydrolytic enzymes (eg α-amylase, protease) will be released in response to gibberelic acid (GA) and/or calcium ions stimulus and start the process of mobilisation of food reserves while the seeds are still on the mother plant (Black, 1972; Bewley and Black, 1978; Hermerton and Ho, 1986). This may initiate germination on the ear itself (sprouting damage) (Gordon *et al.*, 1977; Matthews and Powell, 1986).

2.4.3 Harvesting and processing techniques

Improper techniques and inaccurate time and methodology for harvesting, drying, threshing and cleaning can substantially lower the quality of seeds before or after storage.

Harvest timing is one of the most important factors affecting the maximum seed potential performance in maize, because harvesting too early, too late, too dry or too wet may be detrimental to the seeds. Harvesting too early may terminate in small, immature and shrivelled seeds of poor vigour due to inadequate food reserves, while harvesting too late may cause losses of seed quality due to sprouting, weathering damage, insects, birds and

mould attack (section 2.4.2). While harvesting too dry makes the seeds liable to cracking damage or breakage, harvesting too wet and with improper harvesting techniques may result in bruising damage associated with internal disruption, all of which result in doubtful planting value in the field. In addition to this, seeds with high moisture content are more susceptible to drying damage (section 2.4.3.2.5).

Harvesting at the right stage and with the properly adjusted equipment or drying with accurate methods and properly adjusted temperatures is essential for minimising mechanical injury, drying damage, field deterioration, damage by birds, rodents or moulds. Monitoring changes taking place during seed development are thus of particular importance as they can be used to assess seed maturity and indicate the correct time for harvesting. Furthermore, seed development knowledge is very useful during the subsequent seed drying after harvest, as the SMC determines the subsequent method of drying, type of dryer, and setting or adjustment of safe air flow rate and temperature.

2.4.3.1 Seed development

The basic structure and rudimentary tissues of the mature seed are laid out in a consistent and orderly sequence of developmental events. This complex process is believed to involve the temporal tuning and control of numerous genes during the coordinated development of the zygote in the mature seed (Sheridan and Neuffer, 1981; Scandalios, 1982; Clark and Sheridan, 1986). The seed development process involves various physiological, biochemical and morphological changes (Bewley and Black, 1978; Bryant, 1985). Some of the processes and mechanisms underlying seed development are not completely known or understood and the available information is either unconfirmed or conflicting (Bewley and Black, 1978; Karmode *et al.*, 1989; Olsen *et al.* 1992). However, for the farmer, and seedman in particular, development of the seed at the whole seed level is much more important than details of histo-differentiation and molecular biology of developing seed (Coolbear, 1990). The basic process can be followed by monitoring seed moisture content (SMC), and fresh and dry weight changes in the developing seeds. The sequence of seed development in most crops can be grouped into three stages (Bryant, 1985; Coolbear, 1990):

1. Stage I. The growth stage:

   This stage is characterised by rapid cell division in the almost fully developed embryo. Both fresh and dry weight increase rapidly, and SMC is very high and approximately constant. At the end of this stage the seed is just viable but of low vigour because of limited food reserves at this stage.

2. Stage II. The food reserve accumulation stage:

This stage is characterised by cell expansion. There is a continued, often linear, increase in dry weight, lower or no increase in fresh weight and the SMC begins to fall. At the end of this stage, the seed reaches mass maturity. The inner cells of the endosperm are generally dead tissue, packed solidly with starch such that any surviving cytoplasm is very distorted and does not survive the subsequent desiccation (Olsen *et al.*, 1992). In the aleurone layer cells, processes related to desiccation protection similar to that of the embryo must be assumed to take place (Bartels *et al.*, 1988), enabling the cells to survive seed desiccation damage (section 2.4.3.4). In maize, the seed is isolated from the maternal plant tissue by the formation of a black cell layer (Daynard and Duncan, 1969).

3. Stage III. The ripening stage:

During this stage dry weight stays constant and fresh weight and SMC fall rapidly as the seed tissue dehydrates to a suitable moisture for harvesting (harvest ripeness). The dehydration is initially an active process, but it can be affected by ambient RH (Bryant, 1985) (section 2.4.3.2).

As can be seen from the sequence of seed development, maize water loss is biphasic. Before mass maturity moisture declines linearly as dry matter is deposited in the seed, and after mass maturity moisture loss proceeds at a falling rate as water is lost to the atmosphere (Brooking, 1990). In contrast, seed development studies in sweetcorn (Wilson and Trawatha, 1991) demonstrated that moisture loss declined linearly with maturity. They suggested that drying characteristics of sh_2 kernels are possibly different from that of starchy maize, and that the concentrated sucrose solution in the sweetcorn endosperm resisted drying. Additionally, the common definition of mass maturity - "attainment of peak dry matter" (Brooking, 1990), may not be entirely relevant in sweetcorn because the sh_2 genes incorporated in sweetcorn (section 2.2) are developmental mutants and dry matter accumulation into the endosperm is brought to a premature halt by a failure in starch synthesis (Wilson and Trawatha, 1991).

It should be noted that the ripening stage (stage III) of seed development is not simply a passive process of tissue desiccation, it has an important consequence (Karmode *et al.*, 1989). Mashauri (1990) (unpublished data) showed that freshly harvested maize seeds (55% SMC, after mass maturity) recorded 2% germinability. Drying of seeds to 21.5% SMC increased the germination to 96%. For most seeds desiccation or maturation drying is the terminal phase of development. This leads to a state of metabolic quiescence which

interpolates between development and germination. Subsequent hydration of mature seeds leads to their germination. Therefore, drying, whether natural (Karmode and Bewley, 1985) or imposed (Herter and Burris, 1989a) acts to terminate development processes and initiate those metabolic processes necessary to prepare the seeds for germination and subsequent growth (Evans *et al.*, 1975; Dasgupta and Bewley, 1982; Karmode and Bewley, 1985; Karmode *et al.*, 1989). Alternatively, desiccation may operate through decreasing ABA (the germination inhibitor) concentration or sensitivity, increasing GA (the germination promoter) concentration, or may affect the genome directly (Karmode and Bewley, 1985; Karmode *et al.*, 1989). Evans *et al.* (1975) reported that improper drying in the ripening stage of the seed may make the aleurone cells insensitive to GA produced by the embryo, and this may result in vigour differences as a result of breakdown of events during the mobilisation of food reserves.

2.4.3.2 Seed drying

As we have seen from the ripening phase (stage III) of seed development (section 2.4.3.1), seed moisture content falls rapidly to a suitable moisture for harvesting-ripening maturity. At this stage, the seeds will eventually equilibrate with the RH of the ambient atmosphere. If RH is low, no further drying is required, but if RH is high, or if the growing season is too short to allow for natural drying (as is the case for maize in some temperate regions), or if it is harvested during wet weather, the seeds are moist and must be dried as quickly as possible to a seed moisture content in equilibrium with 70% RH or lower so as to retain germinability and prevent the action of microbes (Neergaard, 1977).

2.4.3.2.1 Principles of Seed Drying

Seed moisture (water) is of two distinct types; chemically and physically bound water. Chemically bound water is part of the chemical composition of the seed, and its removal would cause structural alteration and thus reduce the seeds economic value. It is therefore undesirable. Physically bound water can be either adsorbed or absorbed water. Adsorbed water is a layer several molecules thick held on the surface of material by attractional forces between molecules of the material and the water, while absorbed water is held in the spaces in and around the individual grains of a crop seed in liquid or vapour form. There is no boundary between the two types. Physically bound water is the one which is removed during the drying process (Hill, 1990).

Drying takes place where there is a net movement of water out of the seed into the surrounding air. In order for this to occur, the RH of the ambient atmosphere must be reduced to a level that establishes a water vapour pressure gradient from the seed surface

to the ambient air. This causes evaporation from the surface of the seed until equilibrium is reached. The rate at which seed will lose water to the atmosphere is determined by how fast moisture migrates from the interior to the seed surface and by the rate at which this surface moisture is moved into the surrounding air (MAF, 1985; Hill, 1990).

2.4.3.2.2 Methods of Seed Drying

Drying can be achieved in a number of ways:

Natural drying:

The use of the sun's rays and drying by natural ventilation are the most widespread methods of drying crops, particularly in the tropics. The seeds can be laid out directly in the sun's rays on a flat surface or placed on a simple platform or in a krib, raised above the ground. The method is cheap and is often used for maize in African countries. With this method, however, several disadvantages are apparent: in sunlight, the crop temperature can build to a high level (up to 70°C) after only a few hours of exposure. This can cause uneven drying of the seeds with consequent cracking damage. Also moisture from the earth condenses on the bottom layer of the seeds resulting in a low drying rate, while in the case of the ventilation or krib method, because of the low drying rate, moulds, insects and birds become a considerable risk. Additionally, serious problems arise in humid areas where RH tends to be high and this can reduce seed drying rate considerably, which in turn may make the seeds liable to fungal attack or heating up (Hill, 1990).

Artificial drying

As the quantity of seeds produced is increased, natural drying methods become inadequate and artificial drying must be considered. Many methods and systems exist, but all should conform to certain requirements, eg they should be able to dry the seeds at a predictable rate and the seed's germinative energy must be retained.

Artificial drying systems may be classified into low, medium and high temperature drying. Low temperature drying is the simplest artificial drying arrangement where ambient air may be blown through the seed by means of a fan. Such drying is relatively cheap, and as unheated air is being used, there is obviously no risk of overheating, and the danger of overdrying is small. Once again, the method can only be effective when ambient air is dry (Hill, 1990).

Where natural drying and low temperature drying methods are not satisfactory due to climatic considerations, some simple medium temperature drying systems have been

developed for situations where moisture extraction rates are required. In this system, warm air is blown through a bed of seeds of controlled depth. With this method, care is needed to prevent overdrying and overheating. A more sophisticated arrangement is the use of high temperature continuous driers, which reduce the drying time by subjecting the seeds to high temperatures for a short period of time. This method can cause both physical and physiological damage (Seyedin *et al.*, 1984; Herter and Burris, 1989a,b) and thus requires skilled staff to ensure maximum performance without overheating or overdrying.

Commercially, 45°C is considered to be a safe maximum drying temperature for cereals, but the specific "safe" drying temperature varies markedly with species, cultivar, seed moisture, air flow rate, etc (Justice and Bass, 1978; Seyedin *et al.*, 1984). However, use of such elevated temperatures has been correlated with the development of subcellular abnormalities, lowered viability and vigour as substantiated later in section 2.4.3.5 and 2.4.3.6.

2.4.3.2.3 The complexity of seed drying

The drying of seeds (artificial drying in particular) is considered to be one of the least understood aspects of seed production and one which can cause severe loss of seed quality if not carried out properly (Burris and Navratil, 1980). The study of artificial drying is complex because the system is dynamic (Roberts, 1981): the seeds begin in a moist condition and gradually become drier, they also begin cool and gradually become warmer because, although a constant inlet-air temperature may be used in the drier, the seeds themselves follow a complicated warming curve which is influenced by the fact that the warming due to the exchange of heat between the air and the seed is partly counteracted by the cooling effect due to the latent heat of evaporation, which is initially pronounced but becomes less so as the seed dries. In addition to this, the temperature and RH of the ambient air changes as it passes through the drier, following a pattern which depends on the design of the drier. In particular the pattern varies markedly depending on the direction of air flow; that is, whether it is cross-flow, concurrent-flow or counter-flow (Nellist, 1980).

Because of this complexity, it is not surprising that in the devising of recommendations for artificial drying, reliance has been placed on empirical rules that have been arrived at from crude practical observations (Roberts, 1981). Such rules involve the concept of "safe" drying temperatures, either of the air or the seed. It is disconcerting, however, that different countries, organisations and individual workers have arrived at different "safe" drying temperatures even within the same species (Nellist, 1978). Given the variability of drier specifications and technology and genotypic diversity of seeds which

mediate the level of drying damage, eg heat tolerance, seed size, pericarp thickness and permeability, endosperm types, etc, it is unrealistic to expect uniformity of "safe" drying temperatures even within the same species.

2.4.3.2.4 The physiology of seed survival during desiccation

When the seed desiccates during the ripening phase, the cell organelles/membranes undergo dramatic changes as the seed moisture content falls. For successful seed germination and seedling establishment, disorganisation of the organelle/membrane system during maturation and desiccation must be undergone in an orderly manner so that its reorganisation after imbibition becomes possible in the shortest possible time, and upon rehydration, cell membranes must become fully reorganised before the cells become fully hydrated otherwise vigour differences may occur (Bryant, 1985).

Substantial literature has accumulated in recent years which suggests that water is important not only as a solvent for biochemical reactions, but as a stabiliser of structure (Tanford, 1980). Hydrophilic and hydrophobic interactions impart structure to macromolecules and organelles within cells. Membrane structure in particular depends on these complex interactions, and is often regarded as a primary site of desiccation damage (Crevecouer *et al.*, 1976). In various desiccation tolerant organisms, several compounds such as glycogen, glycerol and trehalose were found to be synthesised and accumulated during preparation for the drying phase (Crowe *et al.*, 1987). Crowe and Crowe (1982) reported that in anhydrobiotic organisms, tolerance to dehydration is associated with stabilisation of lipid membranes by the disaccharides, trehalose. They suggested that trehalose acts as a spacer, preventing contact between the collapsing membrane during drying. This spacer inhibits lipid fusions that result in membrane lipid changes. Similar stabilisation reactions have been suggested to operate in the desiccation process of drying seed and pollen (Hoekstra *et al.*, 1989; Koster and Leopold, 1988).

In seeds of higher plants, raffinose-family oligosaccharides are believed to be a key factor in protecting membranes in drying seeds (Koster and Leopold, 1988; Hoesktra *et al.*, 1989). These sugars inhibit crystallisation of sucrose, allowing the formation of H-bonding glass that stablises membrane structure in the dry seed (Koster and Leopold, 1988). Recent desiccation tolerance studies in maize (Chen and Burris, 1990, 1991) demonstrated that the ratio of sucrose to raffinose has been implicated as a factor in development of drying tolerance in maize. These results suggest that the high sugar crops (eg sweetcorn) are fraught with desiccation tolerance problems. Wilson and Trawatha (1991) suggested that

the sh_2 lesion may interfere with raffinose synthesis directly, or might alter the raffinose/sucrose ratio by flooding the system with sucrose.

Seeds can be intolerant to desiccation, particularly to rapid desiccation rates or high temperature desiccation, during the early stage of their development. Karmode and Bewley (1985) reported that seeds may undergo transition from a desiccation intolerant to desiccation tolerant state at a particular time in the course of seed development. This change occurs at different stages of development for different species and cultivars (Herter and Burris, 1989a; Chen and Burris, 1990). If seeds are dried before reaching the desiccation tolerance of maturity, they will not germinate (Adams *et al.*, 1983; Senaratna and McKersie, 1983) because extensive lipolysis occurs when desiccation intolerant tissues are dried (Senaratna *et al.*, 1985). Additionally, when desiccation tolerance is lost during dehydration, reducing monosaccharides (eg glucose) accumulate in the seed (Koster and Leopold, 1988). These reducing sugars participate in the maillard reactions, a complex series of non-enzymic reactions that can lead to hundreds of end products, depending on reaction conditions (Nursten and O'Reilly, 1986). The first steps of reaction can occur at low moisture level (Saltmarch *et al.*, 1981) such as those experienced by drying seeds (Koster and Leopold, 1988). Occurrence of maillard reactions has been reported to cause protein and nucleic damage (Fujimak *et al.*, 1986), thus threatening the viability of the seeds.

Normal (eg dent) maize and sweetcorn seeds are often harvested on the ear at seed moisture as high as 40% and 55%, respectively (Herter and Burris, 1989a; Wilson and Trawatha, 1991). Therefore ears must be dried mechanically with heated air before shelling and safe storage. At this stage, normal maize seeds are intolerant to high temperature desiccation or excessively low drying temperature, but they can tolerate moderate temperature desiccation (Herter and Burris, 1989a). With progressive moisture loss in the field, or pre-conditioning by exposing to 35°C for 1-2 days before subjecting them to high temperature drying, maize seeds become more tolerant to high temperature drying (Herter and Burris, 1985; 1989b) (section 2.4.3.2.6). In sweetcorn, seeds are intolerant to high drying rates caused by high temperatures. In the field they dry very slowly (Wilson and Trawatha, 1991). Even pre-conditioning at 35°C which induces tolerance (protection) to subsequent elevated temperatures (Lindquist, 1986; Vandeventer and Lock, 1992), produced no or little increase in drying tolerance (Schleppi and Burris, 1989). Wilson and Trawatha (1991) observed that desiccation tolerance was gained very slowly in sh_2 sweetcorn and that low seed vigour resulted as a consequence of desiccation damage. They suggested that slow drying might prevent such injury.

2.4.3.2.5 Drying damage

It is known that the principles of control of loss of viability and vigour during drying extend to high SMC and high drying rates caused by high temperatures (Herter and Burris, 1989a,b). High drying rates caused by high temperatures in drying wet seed are particularly injurious (Hill and Johnstone, 1985). During drying, the moisture is initially removed from the outer layers, resulting in a moisture gradient from the centre to the periphery. If this gradient is too great, as a result of high drying rate, internal stress will cause cracking damage (Thompson and Foster, 1963; Escasinas, 1986) with a resulting loss of vigour (Escasinas, 1986). Alternatively, "case hardening" can occur, where the outer layers of the seed dry out and become impermeable to water even though the inner part of the seed remains wet (Hill and Johnstone, 1985). The SMC will later tend to equalise during storage, creating quality problems (Arvier, 1983). Additionally, during the initial stage of seed drying, the absorbed (free surface) water is removed quickly, and thus rapid evaporation removes heat from the seeds fast enough to keep the actual seed temperature depressed. As the seed moisture drops, the supply of water for evaporation is reduced, and the actual seed temperature will increase (Hampton, 1992). This is in support of Washko (1941) who observed that at drying temperatures of 52°C, seed harvested at 44% SMC was heat tolerant whereas that harvested at 32% SMC showed drying injury, as measured by the standard germination test. In contrast, the work of Navratil and Burris (1984) showed that, seeds harvested at 45% SMC and dried at 50°C had good germination but poor vigour as indicated by the cold test and seedling dry weight test, while seeds harvested at 25% SMC and dried at 50°C showed better performance in terms of germination, seedling dry weight and cold tests. Standard germination and cold test values were 0% where ears of different cultivars were harvested at 43-47% SMC and dried at 50°C (Herter and Burris, 1989a). Seyeden *et al.*, (1984) reported that seeds harvested over a range of 49-52% SMC and dried at 50°C leached significantly more sugars than did those dried at 35°C, while those harvested over a range of 18-25% SMC leached very little sugars regardless of drying temperatures.

Washko (1941) addressed the question of when injury occurs during the drying process. For seeds harvested at an average seed moisture of 46% and dried at 49°C to 16% SMC, injury (as indicated by the standard germination test) became evident after 36 h of drying. Navratil and Burris (1984) argued that most drying injury occurs early in the drying process at high seed moisture content. Seedling dry weight declined substantially even after only a few hours of drying at 50°C indicating that low levels of injury occurred but these could not be detected by standard germination and cold tests (Herter and Burris, 1989c). They suggested that drying injury to the seeds is not a single shot process, but occurs in small increments that accumulate and finally result in abnormal seedlings or death of the

seeds. These discrepancies may be due to the exact mechanical configuration of the drier used, genotypic variation in the factors mediating drying injury such as heat tolerance etc, or most importantly, on the way injury was subsequently evaluated.

Navratil and Burris (1984) found in two years of investigation that maize samples from seed parent A632 were more drying susceptible at seed moistures of 30 to 35% than at moisture from 35 to 45% or from 15 to 20%. In contrast, seed parent B73 and MO17 gained drying tolerance as drying progressed in the field. These results suggest that in some instances, maize seed may be more drying susceptible over the moisture range at which most maize seed is harvested. In the preliminary work of Burris and Navratil (1980), results indicated that drying injury may occur in a specific moisture range.

A comparison of drying rates among different inbred lines showed that the heat tolerant genotypes dried at the fastest rate in both the laboratory and the field (Navratil and Burris, 1984). Faster drying rates have been associated with thinner pericarps (Purdy and Crane, 1967) or endosperm types - low level of hydrophilic compounds which are genetically controlled (Nass and Crane, 1970). Probably drying tolerant cultivars are able to dissipate moisture at a greater rate than heat intolerant genotypes, and this failure to dissipate moisture by heat intolerant cultivars would result in prolonged drying, and thus prolonged exposure to high temperatures at high seed moisture content (Navratil and Burris, 1984), and this in turn may result in drying damage as suggested by Herter and Burris (1989a,b).

An interesting observation presented by some workers (Loeffler and Burris, 1982; Herter and Burris, 1989c) was that during the drying process different parts of the seed dry at a different pace. Pabis and Hall (1962) reported that during mechanical drying of ears at 46°C, cobs dried much more rapidly than the seed, and that the endosperm dried more rapidly than the embryo, and Loeffler and Burris (1982) showed that embryo moisture was much greater than seed moisture after 12 hrs drying at 50°C. This view was substantiated later by Herter and Burris (1989c) who demonstrated that for seed harvested at 47% SMC, embryo moisture was 57%, and after drying for 40 hrs at 35°C, whole seed moisture was 30% while that of embryo was still as high as 47%. Struve (1958) as cited by Herter and Burris (1989c) indicated that through the seed moisture range of 30 to 40%, embryo moisture remained 15% higher than whole seed. Below 30% SMC the embryo moisture dropped rapidly and reached the whole moisture at 15%. Herter and Burris (1989c) reported similar findings. They suggested that embryos dry slowly in the early drying phase because of their position on the ear and that the trend is typical of the drying condition in the field. Herter and Burris (1989b) who harvested ears with 48% SMC and dried at 50°C

for up to 15 hrs, reported that the start of seed deterioration during high temperature drying seems to coincide with the start of embryo drying. However, whether or not the embryo drying process actually caused the injury, or whether the initial safe 50°C drying was also a minimum exposure period required for deterioration to start, needs further research. Washko (1941) performed a similar study and he concluded that injury occurs during the later phase of drying when evaporative cooling is reduced and the embryo temperature increased (cf. Hampton, 1992).

2.4.3.2.6 The physiology of high temperature desiccation

Artificial drying of maize may affect various physiological factors that could either directly or indirectly reduce viability and seed vigour. Navratil and Burris (1984) and Herter and Burris (1989b) demonstrated that radicles are more susceptible to high temperature (50°C) drying than are plumules, and Cal and Obendorf (1972) observed that radicles were frequently aborted after imbibitional chilling treatment. This suggests that high temperature drying might have damaged the radicle primordia. Results from embryo culture (Seyedin *et al.*, 1984) showed that root, shoot dry weights and percent germination were significantly reduced in seedlings grown from embryos of seeds harvested at 47% SMC and dried at 50°C, compared with seedlings grown from embryos of seeds dried at 35°C. The work of Madden and Burris (1992) on embryo culture clearly showed a delay in initiation of elongation of isolated embryos from high temperature (45°C) drying compared with that of embryos from seeds dried at 35°C. These results demonstrate that a significant portion of the injury is localised in the embryo *per se*, and that seedling dry weights, which are used for growth analysis during this phase of development in maize (Stewart *et al.*, 1990), characterise the injury as a limitation in early growth potential.

Respiratory measurements during imbibition using excised embryos (Madden and Burris, 1992) demonstrated that embryos from seeds dried at high temperatures (45°C) were not taking up oxygen or producing ATPs at the same rate as those dried at low temperature (35°C), suggesting that high temperature drying effectively reduces the activity of mitochondria or its respiratory enzyme complex. Histo-chemical light microscopy studies of maize embryos (Seyedin *et al.*, 1984) showed that 50°C temperature drying at 49-52% SMC, resulted in a substantial reduction in the number and size of starch grains in the embryonic axis compared with those dried at 35°C, with a corresponding significant sugar leakage, while those dried at 18-25% SMC leached very few sugars regardless of drying temperatures. This starch hydrolysis in the embryonic axis was suggested to lead to the increased sugar leakage upon hydration.

A significantly increased level of electrolyte leachates have been recorded at high temperature drying than at low temperature (Seyedin *et al.*, 1984) suggesting membrane damage inflicted by high temperatures. Biochemical and microscopic investigation on heat stress also have been done with other plant tissues. Daniel *et al.* (1969) reported disorganisation of tonoplast, plasmalemma and chloroplast membranes at the thermal death point and concluded that disintegration of cell membranes is the primary target of heat injury. Gausman *et al.* (1952) reported that seed dried at 53.9°C or 43.2°C showed significant differences in the vitamin pantothenic acid, Riboflavin and pyridoxin levels, and Roberts (1981) concluded in a literature survey that major subcellular systems, including the genome, could be damaged during ageing or drying.

With progressive drying in the field, seed gains heat tolerance to the subsequent high temperature drying, and thus avoids the deleterious effects of high temperature on seed quality. Simulation of field drying by initially drying (preconditioning) at low temperature (35°C) renders high moisture seed, in most cases, tolerant to subsequent drying at high temperature (eg 50°C) (Herter and Burris, 1989c). These authors suggested that preconditioning appears to accelerate a maturation process that normally occurs in the field. Both exposure time and moisture loss at the moderate temperatures (20-35°C) seem to be factors of the mechanism that produce drying tolerance at the subsequent high temperatures (Herter and Burris, 1989c). There is a growing body of current literature which shows that heat shock (HS) at a permissive temperature allows the corresponding cells to tolerate or survive an otherwise lethal temperature (eg Lin *et al.*, 1984; Abernethy *et al.*, 1989; Helm and Abernethy, 1990). A brief sublethal heat shock induces the production of a number of so-called heat shock proteins (HSPs) (Cooper and Ho, 1983; Van de venter and Lock, 1992). These HSPs are widely accepted as being involved in thermotolerance (Lindquist, 1986). Helm and Abernethy (1990) suggested that HSPs may function in seed developmental processes (cf Herter and Burris, 1989c), protection from desiccation stresses during embryo maturation, and enhance stress tolerance during imbibition (see also section 2.5.4).

From the preceding discussion, it is clear that drying is a very effective mechanism for seeds to gain heat tolerance. However, there are other possible mechanisms that render the seed tolerant to drying; evidence is presented in the literature that protein denaturation is involved in heat stress of plants (Levitt, 1980) and that proteins become more heat tolerant at low moisture contents. Elevated temperatures may change the folding of newly synthesised proteins and render them more stable (Levitt, 1980). The work of Yutani *et al.* (1977) demonstrated an increased thermostability, where only a few suitable amino acids were changed. Crowe and Crowe (1982) suggested that lipid membrane stablisation may

occur in drying tolerant systems, which may involve the production of simple disaccharides or trisaccharides which prevent lipid fusion during drying (see section 2.4.3.2.4 for a more detailed discussion).

2.4.3.3 Processing damage

Both static and dynamic forces apply to maize kernels as they pass through harvesting, shelling and cleaning machinery. Depending on the seeds viscoelastic material properties (eg tensile strength) and the magnitude of the impacting or compression forces, internal and/or external damage may occur (Choudhury and Buchele, 1978; Moreira *et al.*, 1981; Mashauri *et al.*, 1992). Internal damage may be either stress cracks or disorganisation of endosperm and/or embryo, while external damage can range from spots of pericarp missing through severe pericarp damage, open cracks, endosperm or embryo disorientation to chipped, crushed or broken kernels (Choudhury and Buchele, 1976; Verma, 1978; Barasteire *et al.*, 1982; Pierce and Hanna, 1985; Mashauri, 1991).

These kinds of damage can severely affect the quality of the seeds before or after storage (Waelti and Buchele, 1969; Moore, 1972; Pana, 1977). Waelti and Buchele (1969) observed that kernel injury affected both short and long term maize storage life, and that damaged seeds deteriorated two or three times faster than undamaged seeds, and Nikilov and Kirilov (1983) showed that while damaged seeds performed under favourable (20°C) conditions they performed poorly when the conditions deteriorated (10°C).

The type of damage, position and magnitude of the damage are important factors for seed quality. Mashauri *et al.* (1992) observed that bruised seeds with internal disruption had a more severe reduction in germination and vigour than cracked seeds. Bruising causes collapse of tissue and release of hydrolytic enzymes which may subsequently do extensive damage to the seeds (O'Brien *et al.*, 1984) and reduce germination and vigour as a result. The effect of cracking damage on seed performance, however, depends on the position and magnitude of the damage (Coolbear and Hill, 1988). Bartsch (1979) found that seeds impacted near the radicle had the largest reduction in tetrazolium index, and Escasinas (1986) observed a reduction in germination after storage for seeds with cracks situated either alongside of or extending into the middle of the germ area, than for seeds with cracks outside the embryos.

Damaged seeds (eg disorganisation of endosperm and/or embryo, etc) may develop problems in the mobilisation of food reserves or growth metabolism, and are often more liable to injury during the early stage of imbibition (imbibition damage), resulting in poor germinability (Simon and Mills, 1983). This last problem is because several cells within the seeds, especially those near the site of external damage, cannot cope with too rapid an influx of water, and eventually burst or undergo autohydrolysis, thus losing their contents to the surrounding medium (Matthews and Powell, 1986) at higher rates than undamaged seeds (Bruggink *et al.*, 1991a). Careful processing and handling techniques that do not damage the seed coat or pericarp, decreased the amount of electrolyte leakage and injury (Perry and Harrison, 1970; Powell and Matthews, 1978), and have been shown to improve seed performance (Dickson *et al.*, 1973).

While small internal injuries may not immediately cause loss of viability, they can reduce vigour potential and storage life, with production of high amounts and different types of seedling abnormalities (Moore, 1972; Justice and Bass, 1978; Mashauri, 1991). Additionally, any physical or mechanical damage to the seed, no matter how minute, readily allows invasion of the underlying tissue by pests and diseases (Agarwal and Sinclair, 1987) that can affect viability and/or vigour (refer section 2.6).

2.4.4 Deterioration of seeds during storage

Seeds placed into storage will undergo natural aging (Berjak and Villiers, 1970; Roberts, 1972, 1983). Vital systems and functions of the cell membrane, organelles, nuclear materials and biochemical mechanisms that control the physiological processes involved in the resumption of growth, are progressively impaired until they become incapable of supporting the resumption of growth (AOSA, 1983). This physiology of seed deterioration will be briefly discussed in section 2.5. Alternatively, micro-organisms (storage fungi in particular) can infest the seeds during storage, and are also responsible for spoilage of seed quality (see Christensen and Kaufmann, 1974 for review). Temperature and RH (hence, SMC) both influence the rate at which the seed will deteriorate and also control the activity of micro-organisms. The higher the SMC and temperatures, the higher the deterioration rate, and if the seeds are physically or mechanically damaged, or if the storage pathogens (fungi, insects, mites) invade, seed quality loss may occur substantially and very quickly (Neergaard, 1977; Roberts, 1983; Agarwal and Sinclair, 1987; Mashauri, 1991).

Low SMC and temperature prolong seed storage life. Recent studies (Williams and Leopold, 1989; Bruni and Leopold, 1991, 1992a, 1992b) have shown that the cytoplasm of

several seed species including maize, is in a glassy state at low SMC and temperature storage. A glass is a liquid of high viscosity such that it stops or slows down all chemical reactions requiring molecular diffusion, and so prevents damaging interactions between cell components and thus assure quiescence and stability over time (Franks, 1985; Burke, 1986; Bruni and Leopold, 1992b). Although dry and cold storage conditions can increase storage life of seeds, the optimum SMC and temperature are still a subject of an ongoing debate (Ellis *et al.*, 1990, 1991; Vertucci and Ross, 1990, 1991). However, too low a SMC and temperatures can also be detrimental to the seeds. Too low a moisture content would cause structural alteration as the intercellular bound water could be removed (Aksenow *et al.*, 1977). Bruni and Leopold (1992b) suggested that too low a SMC may cause crystallisation in the sugar matrix, which in turn may perturb membranes, bringing an irreversible damage (section 2.4.3.2.4). The poor storage performance observed at extremely low SMC (Ellis *et al.*, 1990; Vertucci and Ross, 1990) could therefore be related to this event. At low temperature (eg 5°C and below), although insects become inactive, some micro-organisms such as some group members of *Aspergillus*, *Penicillium* and *Fusarium* species, can continue to degrade the seeds (Christensen and Kaufmann, 1974; Neergaard, 1977; Mashauri, 1991).

2.5 THE PHYSIOLOGY OF SEED DETERIORATION

Loss of viability and vigour may result from deterioration processes involved during seed ageing, both pre- and post-harvest (see other sections), and seed ageing is now considered as a major cause of reduced vigour in many species (Delouche, 1980; Powell *et al.*, 1984).

Aged seeds exhibit various forms of physiological change which are a measure of how far ageing has progressed. These are reflected as reduced rates of emergence and growth and decreased tolerance to suboptimal conditions (Roberts, 1972; 1983). These symptoms may or may not appear at one time in a single sample of seeds and some are peculiar to seeds of certain species of plants or one set of ageing conditions. For example, reduction in seedling growth, which either precedes or accompanies loss of germinability, does not necessarily occur in every case of seed deterioration and Anderson (1970) suggested that germinability and seedling growth, although closely related, are regulated by different mechanisms which seem to operate independently during deterioration. Often, the time which marks the first detectable loss of vigour or viability does not coincide with

the actual beginning of biochemical changes associated with deterioration (eg Harrington, 1973; Powell and Matthews, 1977).

At the cellular level, there are several biochemical changes that can cause or contribute to seed deterioration. These may include; changes in membrane composition and integrity, enzyme changes, respiratory changes, damage to protein and RNA synthesis, genetic damage, hormonal changes, and accumulation of toxic metabolites (Abdul-Baki, 1980; Blowers *et al.*, 1980; Woodstock *et al.*, 1984; Dourado and Roberts, 1984; Francis and Coolbear, 1987; Priestly, 1986).

Often it is very difficult to differentiate between primary cause and secondary effects from these events because one or more of these degradative changes may or may not be casually related to the deterioration process, whereas other damage may occur as a secondary response to some primary damage event or may occur as independent but coincident processes related to the primary cause of deterioration (see the following discussion). There is no reason to assume that any critical type of physiological damage in any species or under given set of ageing conditions may be very important in another species or set of ageing conditions. Probably it is best to regard all these mechanisms as a component of inter-related events of which the relative importance may depend on the exact conditions of the seeds both pre- and post-harvest (Priestly, 1986; Coolbear, 1990). These changes are discussed in the following sections.

2.5.1 Changes in membrane composition and integrity

Cellular membranes are based on a lipid bilayer structure composed of polar heads of phospholipids and apolar tails of fatty acid chains. Membranes not only constitute the cytoplasm boundary, but also are integral features of the structures of most cell organelles (Salisbury and Ross, 1978). The structural integrity of cell membranes (which depends on the stability of phospholipids and protein molecules; Bewley, 1986) acts as a selectively permeable barrier controlling the general diffusion of materials into and out of the cells, and is also important for many biochemical reactions essential for the physiology of living cells, and hence, the seed's potential performance (AOSA, 1983). Membrane damage is likely to be the most important cause of the loss of vigour and viability during ageing (Powell, 1988; Basavarajappa *et al.*, 1991). Membranes are fundamental to the life and organisation of each cell. Once they begin to break down, the cell can no longer compartmentalise its very diverse range of metabolic activities.

Many workers (eg Cherry, 1983; St Angelo and Orry, 1983; Priestly, 1986; Thompson *et al.*, 1987; Benson, 1990) have suggested that membrane damage of moist seeds may result from the activity of hydrolytic enzymes such as proteases, phospholipases etc (section 2.5.2) or from lipid peroxidation in dry seeds. As free radicals (eg hydroxyl (OH) or superoxide (O_2^-) form and accumulate during ageing as a result of autooxidation of polyunsaturated (double or triple bonded) fatty acids or enzyme (eg lipoxygenase) activity (Harrington, 1973; Wilson and McDonald, 1986; Benson, 1990), they can further react with oxygen to form new free radical species such as peroxides which can then react with another unsaturated fatty acids resulting in the production of lipid radicals and hydroperoxides, thus initiating a chain reaction ultimately leading to a whole range of toxic breakdown products which in turn may have substantial consequences on membrane integrity, and also on the mechanism of DNA, RNA and protein synthesis (Blowers *et al.*, 1980; Pukacka and Kuiper, 1988; Basavarajappa *et al.*, 1991).

However, free radical chain reactions may be halted by reactions with another free radical, natural antioxidants (eg α-tocopherols), quenching with water or activity of scavenger enzymes such as superoxide dismutases, catalases, or hydrogen superoxidases (eg Priestely *et al.*, 1985; Porter and Wagner, 1986; Puntarulo and Boveris, 1990; Basavarajappa *et al.*, 1991).

Damage to membranes may result in loss of semi-permeability characteristics resulting in metabolites leaking out from the cell and then from the seed into the imbibition medium. This facet will be addressed in detail in section 2.7.3. Alternatively, a decreased level of phospholipids may be evident, and many workers (eg Powell and Matthews, 1981; Senaratna *et al.*, 1988; Basavarajappa *et al.*, 1991) have indicated that loss of membrane phospholipids is associated with seed deterioration. Ultrastructural changes of cell organelles such as withdrawal of plasmalemma from the cell wall (Opik, 1972; Villiers, 1972), distension of the outer mitochondrial membrane (Hallam *et al.*, 1973) or coalescence of lipid bodies (Anderson *et al.*, 1970; Villiers, 1972) are another manifestation of cell membrane damage during ageing. However, whether these changes are one of the primary causes of seed deterioration or are just symptoms of the problem, is still unclear.

2.5.2 Changes in enzyme activity

Enzymes play a major role in the performance of seeds. Apart from being involved in the complex metabolic (catabolic and anabolic) reactions of the germinating seeds, the survival of the seeds from the rigors of germination depends mainly on the efficiency of

enzyme controlled mechanisms such as repair enzymes and the detoxification system. While the former synthesise phospholipids and proteins to repair damaged membranes or the mechanisms for synthesis of DNAs, RNAs and proteins which are the first important events taking place during early germination, the latter remove free radicals and other toxic metabolites.

In a deteriorated seed, enzyme activities are often considerably decreased (eg Anderson, 1970; Priestly, 1986). Loss of activity of repair or detoxification enzymes during ageing can compromise seed viability or vigour. The work of Stewart and Bewley (1980) for instance, demonstrated that non-viable seeds showed no detectable activity of superoxide dismutase (SOD) during the early stages of germination. Recent reports on this aspect (eg Puntarulo and Boveris, 1990) showed that SOD activity after 2 hours of imbibition indicated a marked decrease in activity which correlated with the losses of vigour during ageing, and after 30 hrs of imbibition the SOD activity in deteriorated axes was only 1/5 of that in fresh axes. A reduced activity of other enzymes such as DNA polermase (Vasquez Ramos *et al.*, 1988), amylase (Kole and Gupta, 1982), peptidase (Nowak and Mierzwinska, 1978), dehydrogenase (Basvarajappa *et al.*, 1991), DNA ligase (Vazquez *et al.*, 1991), peroxidase (Basavarajappa *et al.*, 1991), etc all have been shown to be associated with loss of seed quality performance.

However, this does not mean that the decreased viability or vigour always results from general damage to the entire spectrum of enzymes; rather, specific critical enzymes are influenced (Vazquez Ramos *et al.*, 1988). These authors demonstrated that while the activity of mitochondrial enzyme (malate dehydrogenase) and DNA enzyme (polymerase) were reduced by 75% and 50%, respectively, cytoplasmic enzymes (alcohol dehydrogenase and catalase) suffered little or no reduction in activity after accelerated ageing. This corroborates with earlier work of McLeod (1952) who reported that peroxidase and dehydrogenase activity decreased with loss of viability during natural ageing, while others such as amylase, phosphatase, proteinase were relatively stable. There is a suggestion that many hydrolase enzymes involved in metabolism are less affected by deterioration, while those involved in anabolic pathways are more vulnerable to damage by ageing, and may be this is an inherent property of these enzymes *in vivo* (Vazquez Ramos *et al.*, 1988).

Some workers (Perl, Luria and Gelmond, 1978), however, have reported an increased activity of one hydrolytic enzyme (protease), while others eg amylase, ribonuclease, glutamic-pyruvic-transaminase, glutamic acid decarboxylase decreased. Increased activity of protease was postulated to be the cause of decreases in all other types of enzyme

activity during accelerating ageing in sorghum seeds. In contrast to this, recent work in maize (Basavarajappa *et al.*, 1991) showed a continuous increased activity of hydrolase enzymes such as protease, amylase and phospholipase A, but a decreased activity of metabolic regulator enzymes (acid phosphatase and phosphomoesterase) and respiratory enzymes (peroxidase and dehydrogenase) during accelerated ageing. This may highlight the idea pointed out by Priestly (1986) and Coolbear (1990) that physiological damage may not be the same for different species or sets of ageing conditions. On the other hand, if the seeds are contaminated by storage fungi, some reported increase in hydrolytic activity in seeds may be due to hydrolyase enzymes produced by these fungi. No matter what the source is, an increased activity of hydrolase enzymes may erode the structural integrity of membranes, which in turn may cause or contribute to loss of control over enzyme activity which is membrane bound.

2.5.3 Respiration changes

Given the many changes in enzymatic activity and membrane damage previously described, reports for reduced viability or vigour as a result of reduced or disrupted respiratory metabolism during ageing are not surprising. This topic is discussed in detail in section 2.7.4.

2.5.4 Damage to protein and RNA synthesis

Protein synthesis is one of the earliest events in axes of germinating seeds, a period which includes important physiological events essential for cellular repair processes and production of some enzymes vital for controlling the necessary metabolic activities for optimum germination.

In a deteriorated seed, lower capacity or decreased rate for *in vivo* protein and RNA synthesis have been reported (eg Anderson and Baker, 1983), presumably because all these biosynthetic systems require ATP which has been shown to be at lower levels in aged seeds (section 2.7.4). This decreased protein synthesis is often associated with a general decrease in seed vigour or viability (Abdul-Baki, 1980; Smith and Bray, 1984). Recent studies of this aspect (eg Abernethy *et al.*, 1989; Helm *et al.*, 1989; Helm and Abernethy, 1990) suggest that loss of vigour might be related to lack of synthesis of specific sets of proteins, the so called heat shock proteins, which are produced as a protective response to stresses (eg heat, cold, anoxia, ethanol, etc (Lindquist, 1986)) - refer section 2.4.3.2.6. These proteins are potentially carrying out processes critical to successful seed germination (Helm *et al.*,

1989; Helm and Abernethty, 1990). This specific decreased response to stimulus (stresses) suggests that the low vigour embryo's ability to sense stress is impaired, and may be a manifestation of the numerous initial effects of loss of seed vigour. This reduction in stress protective response may make the seeds more vulnerable to stress-induced damage (Helm *et al.*, 1989).

Usually protein synthesis is organised on a membrane surface, and thus membrane damage may cause or contribute to loss of activity which in turn may have a severe consequence on the repair process. Impairment to the components of protein synthesis such as damage to the conserved mRNAs or impaired synthesis of new mRNAs may affect protein synthesis. Thompson *et al.* (1992) observed a marked reduction in mRNA content, and those seeds surviving the ageing process were more highly susceptible to degradation during the subsequent germination than the mRNAs of unaged seed (cf Smith and Bray, 1982), while the net accumulation of new mRNAs were also more greatly delayed in aged compared to unaged seeds. These workers suggested that transcriptional machinery and/or the genome itself was damaged by the ageing process.

Damage to the ribosome can cause a decreased rate of protein synthesis. Reduction in integrity of rRNA has been demonstrated in aged embryos (Roberts, 1972). Basavarajappa *et al.* (1991) suggested that these biochemical lesions could arise as a result of failure of the translation or transcription ability of stored or newly synthesised mRNA, or be due to an extensive and rapid degradation of RNA by nuclease or an impairment of the nuclear template. Loss of enzymatic activity (section 2.5.2) may also decrease protein and RNA synthesis. Deficiencies in hormone control in aged seeds may also affect synthesis of enzyme proteins as some of the germinative hydrolases have been shown to be under hormonal modulation (section 2.5.6).

2.5.5 Genetic damage

Ageing can cause an increase in mutation rate, and a corresponding increase in undesirable mutation may lead to metabolic malfunctions. A good correlation between levels of aberrant cell division (a good index of genetic damage) and loss of viability has been reported (Roberts *et al.*, 1967). These workers suggessted that while an increase in numbers of aberrant cells over a critical level may result in loss of seed viability, seeds with few aberrant cells may cause irregularities in the subsequent seedlings, that in some cases may be heritable (Dourado and Roberts, 1984). DNA rupture or breakdown in seeds and decrease in DNA metabolism as seeds imbibe are among the several metabolic defects

found in low vigour or non-viable seeds (Cheah and Osborne, 1978; Vazquez Ramos *et al.*, 1988). Although total DNA does not change much during ageing (Harrington, 1973), it has been shown to fragment markedly and in extreme cases, condensation of chromatin may occur, causing death of the seeds (Coolbear, 1990).

A decreased transcription as a result of damage to DNA is common in deteriorated seeds (Sen *et al.*, 1975; Bray and Dasgupta, 1976; Sen and Osborne, 1977). Although DNA repair to restore the genome with proper information transmission capacity is expected to occur after imbibition (Osborne *et al.*, 1984), declined activity of the key DNA repair enzymes such as polymerase or ligase (section 2.5.2) during early germination may produce a severe uncoupling of events (eg mRNA, protein synthesis, chemical reactions, hormone activity, etc) necessary for successful germination (Vazquez *et al.*, 1991). Alternatively, DNA repair may occur late. This was demonstrated by Vazquez Ramos *et al.* (1988) who observed a recovery of both high molecular weight DNA and DNA polymerase activity just near the completion of germination, but viability was not recovered presumably because other factors necessary for resumption of early germination might be involved in loss of viability. Once again, this may emphasise the idea presented earlier that defining a specific cause of seed deterioration can be very difficult. Vazquez *et al.* (1991) suggested that not only could defective enzymes of DNA metabolism bring germination to a stop, but also that a dephasing of events could produce a longer germination timing (maybe this is the basis of loss of seed vigour) or germination failure (loss of seed viability) resulting from defective transfer of functional information from the nucleus into the cytoplasm.

2.5.6 Changes in hormone levels or sensitivity

Plant growth regulators (hormones) such as gibberellins (GA), abscisic acid (ABA), auxins (IAA), ethylene, cytokinins (kinitins), etc play a fundamental role in the seed development, maturation, dormancy and germination. In the germination process for example, GA_3 or GA_3 and kinitin stimulate the aleurone cells to produce hydrolytic enzymes (eg α-amylase, protease) necessary for the mobilisation of endosperm food reserves, while auxins control growth and stimulate differentiation of vascular tissue (transport tissue eg phloem) to facilitate movement and distribution of the reserves. Ethylene stimulates cell division, and hence growth and may be one of the many factors responsible for resistance to ageing. For successful germination, the process of mobilisation of food reserves must be well coordinated with the metabolic changes taking place, otherwise vigour differences may occur.

In deteriorated seeds, change in endogenous hormone levels or sensitivity is expected to occur (Coves *et al.* 1965; Sreeramulu, 1983; Coolbear, 1990). The work of Sreeramulu (1983), showed evidence of reduced endogenous auxins in aged seeds. Since the site of some hormones such as ABA, or IAA is a plasmalemma membrane (Abdul-Baki, 1980), disruption of membranes during ageing probably could be the cause of changes in hormone levels and/or sensitivity. Although well organised experiments to establish the role of hormones in seed ageing are rare in the literature at present, success of hormone treatment studies for the purpose of improving performance of deterioated seeds (eg gibberellic acid (Miezwinska, 1977), ethylene (Takayanagi and Harrington, 1971; Petruzzelli and Taranto, 1985) or protecting seeds against ageing by pre-application of hormones before storage (eg gibberellins (Petruzzelli and Taranto, 1985), kinetin in acetone (Khan *et al.*, 1976) may serve as evidence for loss of hormone levels and/or sensitivity. Contrasting results, however, have been recorded (Aspinall and Pagel, 1971; Harrison, 1977), suggesting that one deteriorative component (in this case hormonal changes) may or may not be important in every case of seed deterioration.

2.5.7 Accumulation of toxic metabolites

In prolonged storage, seeds may accumulate toxic metabolites arising from biochemical reactions (Floris, 1970). As already seen from the foregoing discussion, uncoupling of respiration and subsequent anaerobic respiration of glucose or lipid peroxidation may result in ethanolic, aldehyde and peroxide compounds. Other toxic materials are short chain fatty acids and phenolic compounds that comes as a result of lipid peroxidation as secondary products (Priestly, 1986; Coolbear, 1990). These in turn may do extensive damage to the seeds, and especially to those with lamed repair and detoxification systems. Dey and Sircar (1968) reported that loss of viability during storage was associated with supra-optimal levels of indole acetic acid, and Sreeramula (1983) suggested that an increase in phenolics such as vanillic, ferulic and hydroxybenzoic acids could be the cause of loss of seed viability. However, whether these toxic compounds are more important than the biochemical reactions (damage) which produced them in the first place is still debatable.

2.6 PATHOGENS: *Fusarium*

There can be no doubt as to the importance of pathogens on seed production and quality (for review see Neergaard, 1977; Agarwal and Sinclair, 1987). As it is practically impossible to review the whole range of the maize disease spectrum here, this brief review

focuses on *Fusarium* species, currently a vexing problem in New Zealand, and therefore also most likely to exert effects in this study.

Fusarium spp. are well known plant pathogens, causing Fusarium diseases such as cortical rots or vascular wilts, among the most important of the cosmopolitan diseases. The fungi are present in soil, air and organic material and some species survive well with little oxygen or water and with unusual mixtures of gases and chemicals, and flourish under extremes of sundry environmental stresses. The diseases are a leading threat to most of the principle agricultural, horticultural, pastoral and forestry products of the world, and are very difficult to arrest once established. Victims range from cereals (eg maize, wheat, rice, etc), legumes (eg beans, peas, etc) through to oil (eg oil palms), fibre (eg cotton), ornamentals (eg carnation, chrysanthemum), pasture species (eg ryegrass, clover) to forestry plantings. Field outbreaks of *Fusarium* diseases have been reported in USA, USSR, Australia, Europe, North America, China, Africa and elsewhere (Cook, 1981; Cassin, 1981; Burgess *et al.*, 1981; Maric, 1981; Kommendahl and Windels, 1981; McGee, 1988). The problem is further confounded by the fact that Fusaria produce mycotoxins. These are toxic secondary metabolites produced by fungi which, apart from being in some cases phytotoxic to plants and seed tissues, are detrimental to man and his animals (Agarwal and Sinclair, 1987; Abbas *et al.*, 1989).

In maize, seedling blight, root, stalk, ear, and kernel rots can occur as a result of infection with one or a complex of *Fusarium* species. The degree of damage may be negligible or devastating, depending on environmental locality and conditions, and most important, the genotype of the crop (eg Warren, 1978; Gulya *et al.*, 1979). The frequently reported Fusaria (eg *Fusarium moniliforme*, *Fusarium graminearum* and *Fusarium subglutinans*) infect maize and cause damage when the plant is under stress from biotic, climatic or edaphic causes.

2.6.1 Mechanisms of seed transmission and pathogenicity

Harvested seed before storage is always associated with a certain amount of debris, broken kernels and other inert materials that may harbour propagules of *Fusarium* (Neergaard, 1977; Agarwal and Sinclair, 1987). The processing operation and transport into storage, apart from inflicting damage to the seeds, may facilitate contamination of the seeds with *Fusarium* fungi (Neergaard, 1977). Many species of insects and mites which also may carry *Fusarium* spores, may accompany the seeds and these too may inflict further physical injury and/or disseminate the fungal propagules to the seeds. The stored seeds may thus

be contaminated with *Fusarium* spp. either internal to the seed tissue as a result of systemic infection in the field, internal to the seed tissue due to contamination of the silk or physical injury during development, or externally located fungal bodies (Neergaard, 1977; Kulik and Schoen, 1982).

If the storage is relatively short term, *Fusarium* may dominate the micro-environment of the seed (Agarwal and Sinclair, 1987; Russell *et al.*, 1982; Mashauri, 1991) but with progressive increases in storage time and temperature, *Fusarium* propagules will lose vigour and viability (Mashauri, 1991). However, survival of some *Fusarium* spp at 4°C for up to 6 years also has been reported.

The soil in the seed production field may be contaminated with *Fusarium* spp. oospores, sporangia, chlamydospores and conidia are dormant microorganism structures of *Fusarium* pathogens frequently found in the soil (Hendrix and Campbell, 1973). Under the correct conditions of temperature, water availability, pH and nutrient supply, these, together with *Fusarium* fungal structures associated with the surface of the seeds, will germinate and any seed in proximity could become infected (Agarwal and Sinclair, 1987). Physically and mechanically damaged seeds are thus more prone to soilborne or seedborne *Fusarium* infection (Neergaard, 1977).

The seeds set to germinate in the moist soil may be internally and/or externally infected by *Fusarium* fungi (Agarwal and Sinclair, 1987). During germination and early establishment, these microflora will remain dominant, and virulent *Fusarium* spp can cause death of the seed or a severe pre- and/or post-emergence damping-off (Shurtleff, 1980). This can result in a lower yield as a consequence of poor stand in the field.

However, the actual infection of a germinating seed by soilborne or seedborne *Fusarium* is influenced by a number of factors, some of which also control the seed germination. Agarwal and Sinclair (1987) suggested that moisture levels and atmospheric temperature are important factors in controlling spore germination in the soil, the infection process and the subsequent disease development. Burgess *et al.* (1981) for example, demonstrated that *Fusarium* stalk rot is prevalent in drier than in wet environments, while Shurtleff (1980) showed that pre- and post-emergence diseases of maize caused by soilborne or seedborne *Fusarium* fungi are prevalent in poorly drained, excessively compacted or in cold (less than 10-13°C) and wet soils. Leach (1947) suggested that microorganisms have a relatively lower optimum temperature than that of seed germination and seedling growth, that in turn would allow soil and seedborne pathogens to grow,

multiply and attack the seeds or seedlings. This view gained the support of Christensen and Wilcoxson (1966) who reported that seedling blight of maize caused by *Gibberalla zeae* and other *Fusarium* spp is frequently prevalent during periods of cool weather when plants grow slowly. Additionally, Isley (1950) showed that the important factors are probably the extra length of time which seeds must lie in the soil in imbibed condition, and possibly low temperature metabolism behaviours of seed which renders it peculiarly susceptible to fungal infection.

In contrast, Covey (1959) found that some isolates of *Fusarium graminearum* produced a greater reduction of seedling stands at 25°C than at 15 or 20°C. Factors such as host genotype and resistance, presence of insects, levels of inoculum, as well as its type, aggressiveness and location in the rhizosphere relative to the seed are also important (Neergaard, 1977; Agarwal and Sinclair, 1987). Additionally, during seed germination there is leakage of carbohydrates and amino acids from the seed and these can stimulate spore germination in the soil or on the seed surface and thus aid the infection process (Short and Lacy, 1976; Agarwal and Sinclair, 1987). Low vigour seeds which often leach more electrolyte than high vigour seeds are thus more susceptible to fungal attack (Harman, 1983). This last problem, presumably because of high sugar content, has been reported to be more severe in sweetcorn than in normal maize (Berger and Wolf, 1974; Cantliffe *et al.*, 1975).

In the soil, the seedlings are usually infected during or immediately after germination (Voorhees, 1934; Lawrence *et al.*, 1981; Agarwal and Sinclair, 1987). *Fusarium moniliforme* for example, may ingress the cotyledonary plate region when the stem bud breaks through the pericarp, or when the plumule breaks through the apex of the coleoptile (Agarwal and Sinclair, 1987). Direct penetration of emerging adventitious roots and of primary radicles when they break through the coleorhiza also has been reported (Voohees, 1934). Additionally, since the soil particles are abrasive during the germination process, the protruding radicle and extending plumule are prone to physical injury. In keeping with their opportunistic invasive nature, soil and seedborne *Fusarium* may invade such wounds (Neergaard, 1977). Infection by either seedborne or soilborne *Fusarium* may result in poor field emergence (Kruger, 1989). However, the degree to which emergence is affected depends on the extent of seed infected before harvest or levels of mechanical damage (Harman, 1983; Kruger, 1989). Kruger (1989) reported that no seed will germinate from cobs which are covered partly by mycelium, and that if one third of the cob is covered with mycelium, the adjacent one third is also badly damaged.

In the seed production field, the plant is also prone to infection by *Fusarium* fungi. There are a number of acceptable mechanisms of infection and although each can be considered separately they are all probably involved in the natural infection (Hudson, 1986). The work of Lawrence *et al.* (1981) indicated that *Fusarium moniliforme* grew rapidly through the root system and crown but made little additional growth until anthesis, when the rest of the plant including the cobs and kernels was colonised. Kingsland and Wernham (1962) suggested that fungal invasion may occur through vascular tissues leading from the main stalk to the rudimentary ears. Invasion of vascular parenchyma can occur because airborne fungal spores of *Fusarium* spp frequently lodge between the leaf sheath and the leaf stalk, an area which provides ideal conditions for the spore to germinate (Kingsland and Wernham, 1962) and penetrate into the stalk (Foley, 1962; Kucharek and Kommedahl, 1966). Additionally, Griffiths and Lim (1966) suggested that pectolytic enzymes produced by the *Fusarium* fungi are most important during the early stage of infection, resulting in the occlusion of the xylem vessel element, with pectic substances as an early sign of infection. Koehler (1936, 1942) indicated that the pathogen entered in the region of silks, and then spread to the bracts and pedicels through the vascular cylinder, and finally spread into the shank. Internal kernel infection did not become established until the ears were approaching maturity. Alternatively, any sort of injury during plant growth and development can facilitate additional infection of the plant tissues from external sources. Apart from insect-induced damage, plants and seeds are also prone to damage by birds, heavy rains and hail, and animals (Kucharek and Kommedahl, 1966; Neergaard, 1977; Agarwal and Sinclair, 1987). Once infection has occurred, be it from external or internal sources, *Fusarium* may stunt seedling growth, induce wilting or even cause plant death (Neergaard, 1977; Agarwal and Sinclair, 1987).

The mechanisms by which *Fusarium* fungal pathogens move through the plant tissues varies with the particular pathogen involved, and many can assume more than one form (Neergaard, 1977; Agarwal and Sinclair, 1987). Most *Fusarium* species move through the plant tissue in mycelial form, for example *Fusarium moniliforme* in maize (Foley, 1962; Lawrence *et al.*, 1981). Others also occur in mycelial form, but move specifically through the plant's vascular system, for example *Fusarium oxysporum* in peas (Neergaard, 1977) and *Fusarium moniliforme* in sweetcorn (Lawrence *et al.*, 1981). In these cases, if infections are high, the pathogens can cause wilting and death by occluding the transpirational stream. Some *Fusarium* can assume a mycoplasmal form (Shurtleff, 1980) and move through the plant in that state, for example *Fusarium moniliforme* in maize (Lawrence *et al.*, 1981).

No matter what the mode(s) of infection or transmission is, the developing seed is open to systemic infection by *Fusarium* (Neergaard, 1977). At this stage, some species of *Fusarium* can cause severe ear and kernel rots (Shurtleff, 1980; Commedahl and Windels, 1987; McGee, 1988). Seeds infected in the seed production field may survive and be the source of primary inoculation in the next generation. Such pathogens must be specialised in their ability to survive the various stages of plant and seed development and the subsequent storage in order to be transmitted successfully to the next generation (Agarwal and Sinclair, 1987).

2.7 THE PRINCIPLES OF THE EVALUATION OF SEED VIGOUR IN MAIZE

In deteriorating seed, several changes occur at the cellular level. Vital systems and functions of cell membrane, organelles, nuclear materials and biochemical mechanisms that control the physiological processes involved in the resumption of growth (eg protein synthesis, respiration, etc) are progressively impaired until they become incapable of supporting the resumption of growth (AOSA, 1983) (refer section 2.5 for a more detailed discussion). This is manifested as a narrowing of the environmental range over which the seed will germinate, reduced rates of germination and emergence, poor seedling growth, production of abnormal seedlings and ultimately a total loss of viability (Berjak and Villiers, 1970, 1972a,b,c; Roberts, 1972, 1983; AOSA, 1983).

Seed vigour is a multi-dimensional property of the seed. At the germination level, it involves the rate and totality of germination, rupture force of seedlings in relation to natural robustness, and tolerance of seeds and seedlings to sub-optimal conditions (AOSA, 1983). Vigour measurements such as seedling growth testing (ISTA, 1987; Mashauri, 1991), the cold test with soil (Hoppe, 1955; Perry, 1984) or without soil (Loeffler *et al.*, 1985; Mashauri, 1991), complex stressing vigour testing (Barla-Szabo and Dolinka, 1988), the accelerated ageing test (Fiala, 1987), etc have been frequently used to assess these aspects, and have often been shown to correlate better with field emergence than the standard germination test (Grabe, 1965; Perry, 1981; Fiala, 1987; Barla-Szabo and Dolinka, 1988).

At the biochemical level, biosynthesis of energy and metabolic compounds (eg nucleic acids, proteins, carbohydrates, lipids etc) and the coordination of the seed's metabolic activities associated with germination performance such as cellular activities, membrane composition and integrity or mobilisation of food reserve (eg respiration and production of ATPs) are related to the seed's germination and growth, and hence vigour

value (Ching, 1973a,b; AOSA, 1983). Vigour assessments based on these aspects include measurement of membrane integrity (determined by conductivity testing), and integrity of respiratory apparatus metabolisms measured as oxygen uptake rates and ATP content etc (Ching, 1973a,b; Styer *et al.*, 1980; Abdul-Baki, 1980; AOSA, 1983; Priestly, 1986). These too, have been shown to correlate with field emergence (Tao, 1980a,b; Lunn and Madsen, 1981). The following are some of the principles of the evaluation of seed vigour in maize.

2.7.1 Principle based on tolerance/susceptibility to sub-optimal field conditions

Standard germination test results often over-estimate the actual field performance because the test is not sensitive enough to distinguish small but important performance differences resulting from the ageing or deterioration processes, and also uses optimum conditions, whereas field conditions are rarely completely favourable (AOSA, 1983; ISTA, 1987). The severity of stress that seed experiences in the field depends on many factors, including temperature, availability of oxygen, water and soil pathogens (Herner, 1986).

In early spring, the weather is often wet and cold. Maize seeds which are physically/mechanically damaged are unable to germinate under wet soil, presumably because of soaking injury (too rapid water uptake or lack of oxygen) (Svien and Isely, 1955) and may undergo decaying due to soil-borne micro-organisms (Isely, 1950). Optimal temperature for germination of maize ranges from 32-35°C (Mayer and Poljakoff-Mayber, 1982). However, soil temperature in many maize producing areas is often much lower than this at the time of planting, and may cause poor germination because physiological disorder (chilling injury) occurs at 5-15°C, depending on genotype (Cal and Obendorf, 1972).

A number of studies on effects of low temperature imbibition or wet soil have been reported. They include inhibition of respiratory metabolism (Woodstock and Pollock, 1965; Crawford, 1977; Leopold and Musgrave, 1979) or membrane damage (Bramlage *et al.*, 1978). Oxygen uptake can be markedly reduced at lower temperatures (Woodstock and Pollock, 1965) presumably by affecting mitochondrial activity (Lyons and Raison, 1970; Duke *et al.*, 1977) resulting in decreased concentration of adenosine triphosphate (ATP) and other nucleotides (Stewart and Guinn, 1969, 1971). RNA, protein and lipid-soluble phosphate also decrease (Guinn, 1971) and this may affect the potential emergence and/or seedling vigour. Seeds planted in cold, wet soils may undergo anaerobic respiration and the production of ethanol and acetaldehyde (Raison, 1980; Martin, 1986, 1987; Martin *et al.*, 1988) both of which are toxic metabolites, and their presence may disrupt membranes (Crawford, 1977) causing higher solute leakage, depending on vigour level, which in turn may cause poor

performance. Leakage of solutes from the seed and its effect on seed performance will be discussed in detail in section 2.7.3.

Chilling injury in the field is also related to an increased susceptibility to soil and seed-borne pathogens (Isely, 1950; Schulz and Bateman, 1968; Short and Lacy, 1976). This is an important problem in the soil environment, especially for physically or mechanically damaged seeds. Pathogens can ingress through the seed's damaged areas and invade the inner tissue; the leakage of solutes mentioned earlier, which tends to be more severe in damaged seeds, may then stimulate the further activity of soil micro-organisms. Additionally, soil micro-organisms have a relatively lower optimum temperature for growth than that necessary for seed germination, and thus they are capable of growing, multiplying and attacking the seed at lower temperatures (Leach, 1947). Low vigour seeds planted under these adverse conditions may culminate in poor germination, reduced seedling emergence, decreased seedling vigour and ultimately loss of the subsequent seed quality and/or yield, while the counterpart high vigour seeds are better able to express maximum potential growth (Zheng, 1991). Vigour assessment techniques such as the soil cold test (section 3.4.1.6), which simulates field conditions in the laboratory, evaluates the ability of the seeds to perform under such adverse field conditions.

As with the soil cold test, the complex stressing vigour test (section 3.4.1.5) also uses water and temperature stresses adjusted to correspond to unfavourable weather conditions at sowing. The stress caused by two days of soaking used in the first phase promotes the initiation of germinative metabolisms at the initial optimum temperature (25°C). Soaking causes permanent oxygen deficiency, thus the soaked seeds respire anaerobically, and the initiated biochemical processes slow down and eventually stop. Moreover, the soaked seeds leak solutes essential to the seed including organic compounds and inorganic ions, the extent depending on the degree of membrane integrity (vigour level). The subsequent low temperature (5°C) applied on the second two days of soaking may cause further damage to the physiology of seeds suffering from a chronic oxygen deficiency, particularly for the cell division and synthesis processes that are most sensitive at this stage (Barla-Szabo and Dolinka, 1988).

However, the response of the seeds to these adverse conditions is influenced by genotype (cold tolerance/susceptibility) (Burris, 1977; Van de Venter and Grobbelaar, 1985). Mashauri (1991), working with a cold resistant cultivar of maize, observed a substantial germination increase from cold stressed (10°C for 7 days; 25°C for 6 days) seeds compared to seeds sown at 25°C for 7 days only. An interesting aspect of physiological changes

taking place in the course of chilling imbibition was observed by Gu *et al.* (1981) and Zheng *et al.*, (1981) who reported a temporary increase of some physiological and biochemical processes (eg respiration rates, enzymatic activity) in the initial stage of imbibition. For example, Gu *et al.* (1981) demonstrated a temporary dramatic increase in oxygen uptake of cold stressed seeds in the initial stage of chilling imbibition, and Zheng *et al.* (1981) showed that cold stressed seeds (3-5°C) exhibited a temporary increase in activity of dehydrogenase, peroxidase and Na^+ - K^+ - ATPase. This adaptive protection reaction occurred in the early stage of chilling imbibition, followed by a decrease and then a complete inhibition as the stress (damage) time increased. The duration of these dramatic changes varied with species and cultivars (Zheng, 1991). The period of adaptive protection reaction of dehydrogenase (measured by the tetrazolium test) for example was longer in cultivars with moderate cold tolerance than in cold intolerant cultivars (Zheng *et al.*, 1981).

2.7.2 Principle based on emergence, seedling growth and accumulation of dry matter

The uncertainty of environmental conditions requires that high vigour seeds be planted. Rapid and uniform emergence and vigorous seedling growth are a good indication of high vigour value in a seed lot (Perry, 1980). However, as we have seen previously in section 2.4, deterioration of the seeds may begin during seed development itself and the subsequent rate of deterioration is influenced by genotype, pre-storage history conditions (eg mechanical damage, maturity) and the conditions of the store, particularly RH and temperature. The consequence of seed deterioration is the production of physiologically aged seeds with symptoms of reduced vigour, and eventually increased seedling abnormality or death of the seeds.

There is an accumulated body of evidence which shows that deteriorated (low vigour) seed lots usually emerge and grow slowly and their growth rate is spread over a long period of time, while high vigour seed lots emerge and grow rapidly, uniformly and healthily (eg Roberts, 1972; Perry, 1980, TeKrony and Egli, 1991). While the latter are free from physiological dysfunctions, and are capable of expressing maximum growth rates, the former suffer from deterioration of vital systems and function of their cellular components (eg membranes, organelles, etc) and biochemical mechanisms (eg protein synthesis, etc) that control the physiological processes necessary for resumption (eg repair metabolism) and subsequent flourishment of the seedling or plant.

However, it is known that deterioration is always variable and inconsistent. Normally, individual seeds within a seed lot deteriorate at different rates. Consequently, a partially deteriorated seed lot may contain seeds of high vigour and low vigour or non-viable seeds (cf Priestley, 1986). The net effect of deterioration may affect the potential of the seeds to emerge or their speed and uniformity of growth, and may persist to influence the subsequent coleoptile/shoot length and/or synthesis of biomass, reflected as a reduction in the rate or total accumulation of dry weight. The work of Sanchez-Nieto *et al.* (1992) for example, showed that the activity of the tonoplast, plasma membrane and mitochondrial ATPase was lower in seeds of low vigour than that of high vigour. A corresponding difference in coleoptile growth rate was observed, and the accumulated dry weights were 2-3 times higher in the high vigour line than in the low vigour line. Seedling growth and/or dry weight content, estimated by the seedling growth test (section 3.4.1.4), is often used to determine physiological seed vigour (Perry, 1981; Catizone and Lovato, 1987), and has been recommended as a good criteria for evaluation of seed vigour (AOSA, 1983; ISTA, 1987). Evidence of positive association of seed vigour with early plant growth (Glenn *et al.*, 1974; Burris, 1975; Adegbuyi and Burris, 1989) or seedling dry weight (Burris, 1975; Woodstock, 1976; Mock and McNeil, 1979; Mashauri *et al.*, 1992) has been reported in maize.

However, some researchers (eg Glenn *et al.*, 1975; Adegbuyi and Burris, 1989) showed no significant effect of seed vigour on emergence rate, while others (Akintorin-Adegbuyi, 1985; Adegbuyi and Burris, 1989) have indicated that seed vigour can affect seedling growth only at the early stage of development but that as the plant grows, the influence of seed vigour decreases. This discrepancy may be due to experimental technique, environmental interactions and variability of the vigour level of the seed lots used (Egli and TeKrony, 1979) or most importantly, genotypic variation (refer section 2.4.1).

2.7.3 Principle based on cell membrane integrity

Cell membranes undergo dramatic changes when the seeds desiccate at maturity, and reorganise again when the cells rehydrate during imbibition (Simon, 1974; Edwards, 1976; Bryant, 1985). Simon (1974) suggested that membrane phospholipids in a dry seed are arranged in an "inverted hexagonal (H_{II}) phase". During the subsequent imbibition, membranes become reorganised into their typical bilayers, and solute leaks into surrounding medium when the membranes revert to their bilayer state. A recent theory (Crowe *et al.*, 1989) suggests that leakage occurs during the transition phase change from gel to liquid crystalline state. The way in which dehydration-rehydration proceeds determines how soon the membranes become reorganised. Leakage is greatest shortly after immersion in water

but decreases as membranes reorganise (Parrish and Leopold, 1977; Powell and Matthews, 1981). The time element is crucial because membranes must be reorganised before the cell is fully hydrated, otherwise some of the cellular inorganic ions such as K, Mg, Cl, Ca, etc (Loomis and Smith, 1980; Lott *et al.*, 1991) and organic compounds such as amino acids, sugars, enzymes, etc (including membrane components, phospholipids, proteins) (Duke *et al.*, 1983; Spath, 1987, 1989) may leak out (Larson, 1968; Simon and Raja Harun, 1972; Duke and Kakefuda, 1981). For successful germination and seedling establishment, cell membranes must reorganise in the shortest possible time (Abdul-Baki, 1980; Bryant, 1985).

There is much evidence indicating that seeds throughout their life history are subject to deterioration (see other sections) and various forms of membrane malfunctions may occur (Simon and Rajaharun, 1972; Powell and Matthews, 1981). Both ageing and imbibition damage lead to impaired membrane integrity, resulting in the leaching of greater amounts of electrolytes (Schoettle and Leopold, 1984; Powell, 1988; Bruggink *et al.*, 1991b), presumably due to incomplete reformation of the lipid bilayers of the membrane, or incomplete or incorrect re-alignment of proteins in the lipid bilayer on rehydration (Bryant, 1985). Membrane damage during ageing is discussed in section 2.4. The greater the leakage or the longer the period that a high leakage rate is sustained (because of poor membrane reorganisation), the greater the damage to the seed tissue (Simon, 1974; Simon and Mills, 1983), and this may cause poor performance of the seeds (Larson, 1968; Powell *et al.*, 1986). Usually, high vigour seeds reorganise their membranes at a faster rate with less electrolyte leakage than low vigour seeds (AOSA, 1983, Bruggink, *et al.*, 1991b). This leakage of intracellular materials during early imbibition can be a good indication of health of the seed tissues and therefore, by inference, seed vigour (Powell and Matthews, 1977) as determined by electrolyte conductivity testing (section 3.4.2.4). Conductivity test results have been used to predict seed vigour in maize, and have been significantly correlated with field emergence (Tao, 1980a,b; Joo *et al.*, 1980; AOSA, 1983).

Apart from differences resulting from vigour levels, there are other factors which can also affect electrolyte leakage. Pericarp or seed coat characteristics or integrity plays a significant role in regulating the supply of water reaching the embryo (McDonald *et al.*, 1988). Water is absorbed more slowly by embryos with intact testa than with a damaged pericarp (Bruggink *et al.*, 1991b). Damaged seedcoats increase leakage during imbibition (Duke *et al.*, 1983; Bruggink *et al.*, 1991b) presumably because of a too rapid movement of water into the dry seed that interferes with membrane reorganisation. Rapid imbibition results in rupture of some cells (Perry and Harrison, 1970; Duke *et al.*, 1983) and this may

result in extrusion of streams of cytoplasmic materials including starch grains and protein bodies (Spaeth, 1987, 1989).

Temperature and SMC have been shown to influence imbibition and leakage. Low temperatures during imbibition could be expected to prevent reorganisation of membranes, presumably because the membrane lipids are in a gel phase, or the formation of a continuous bilayer might not be possible or the bilayer formed might be functionally imperfect (Bochiocchio *et al.*, 1991). Alternatively, disruption of respiratory or repair metabolisms may occur as a result of altered activity of membrane-related enzymes (Larson, 1968; Crawford, 1977; Bochicchio *et al.*, 1991), causing a loss of membrane semi-permeability resulting in extensive leakage, severe tissue damage and reduced germination rates (Pollock and Toole, 1966; Simon, 1974; Simon and Mills, 1983; Powell *et al.*, 1986; Chozin, 1992) and low vigour seeds are often the victims of this circumstance (Woodstock and Tao, 1981; Styer and Cantliff, 1983). Cold tolerant cultivars, however, would leak small amounts of solute at lower temperatures (Chozin, 1992). The disruption of respiratory and repair metabolisms suggested by Larson (1968), Crawford (1977) and Bochicchio *et al.* (1991) probably does not occur to such a great extent in cold tolerant seeds. Seeds with initial low moisture content at the start of imbibition leak more electrolytes during imbibition and are more likely to be damaged (Becwar *et al.*, 1982; Ishida *et al.*, 1988). A combination of low SMC and low temperature imbibition is more damaging (Pollock and Toole, 1966; Pollock, 1969). This fact indicates that low temperature reduces the ability of the seed tissue to expand during wetting and the low initial SMC of the tissue magnifies the importance of the wetting event during the initial stage of water entering during cold stress (Zheng, 1991). Different tissue may leak electrolytes at different rates. Embryos leak more electrolytes than the remaining parts (Bruggink *et al.*, 1991b). Different cultivars also differ in leakage pattern and levels (Tao, 1980a,b; Powell *et al.*, 1986; Bruggink *et al.*, 1991b).

2.7.4 Principle based on respiration and adenosine tryphosphate (ATP) production

There is clear evidence that mature, dry, quiescent seeds, contain the whole range of metabolic and synthetic machinery for immediate renewal of metabolic activities after imbibition (Bryant, 1985). Most of the biochemical reactions involved that become active upon initiation of metabolic activities for initiation of germination, utilise chemical energy, adenosine tryphosphate (ATP), for which production mainly depends on the functional capacity of the respiratory apparatus (Abdul-Baki, 1980). Respiratory metabolism results in oxidation of food reserves and the production of intermediate compounds that serve as

a building block for synthesis of protoplasmic components (proteins, nucleic acids, lipids) and production of energy (ATP) for driving the synthetic processes (AOSA, 1983).

Ageing has been reported to decrease respiratory activity in seeds (Anderson, 1970; Abdul-Baki, 1980; Priestly, 1986) and many studies have shown that changes which reduced vigour, reduced respiration and activity of respiratory enzymes (Abdul-Baki, 1980). Changes involved in a deteriorated seed include decreased oxygen uptake, increased respiratory quotient (RQ) values or changes in ATP levels (Abdul-Baki, 1980; Woodstock *et al.*, 1984). Consequently, respiration measurements (gas exchange) (section 3.4.2.6) can be used to evaluate physiological seed vigour, and have shown to correlate with seed performance, measured as percent and speed of germination, and with seedling growth (Wilson and Grabe, 1967; McDaniel, 1969; Lunn and Madsen, 1981). However, other workers (eg Abdul-Baki, 1969; Byrd and Delouche, 1971) reported that changes in respiration have little or no correlation with vigour, presumably because not all tissues behave in the same way. While embryonic tissues are most sensitive to deterioration, cotyleodnary or endospermic tissues are less so (Anderson and Abdul-Baki, 1971; Buchvaron and Alekhina, 1984). Some studies have shown no significant changes in oxygen uptake, but a big decrease in ATP levels (Ching, 1973a; Anderson, 1977; Woodstock and Taylorson, 1981), an indication of mitochondrial damage resulting in partial uncoupling of oxidative phosphorylation (Abdul-Shakra and Ching, 1967; Woodstock *et al.*, 1984) or change of respiratory pathways from the cytochrome oxidase system (efficient in production of ATPs) to pentose phosphate pathway (PPP) (inefficient in production of ATPs) (Abdul-Baki, 1970; Leopold and Musgrave, 1980; Priestly, 1986) or complete loss of coordination between activity of glycolysis and the Krebs cycle (Woodstock *et al.*, 1984). Excess glycolysis may result in anaerobic respiration, producing toxic materials (ethanol and acetaldehyde) (Gorecki *et al.*, 1985) that may cause destruction of mitochondrial membranes (cf Crawford, 1977).

ATP content measurements can also be used to evaluate seed vigour and a close correlation has been suggested between loss of vigour and loss of ability to produce ATPs (Ching, 1973a; Lunn and Madesen, 1981). However, Perl (1987) argued that ATP production may be not necessarily related to vigour because the measurement is a function of both synthesis and utilisation. A greater proportion of the synthesised ATP is utilised immediately on synthesis and only < 5% is measurable. Hampton and Coolbear (1990) suggested that rather than recording ATP production, measurement of ATP synthesis may give a better indication of seed vigour.

2.7.5 Individual Vigour Testing Methods

An ideal vigour test should provide a more sensitive index of seed quality than the germination test, and should provide consistent information on the potential performance of seed lots. Additionally, it should be simple, rapid, inexpensive, interpretable, and reproducible (McDonald, 1980; Hampton and Coolbear, 1990). However, as earlier mentioned, there is no single test complying to all these requirements under all conditions. The major problems are the interpretation and reproduction of the test results, inevitably causing standardisation difficulties. The following is a brief discussion on the individual vigour test methods.

2.7.5.1 Cold test

Although the cold test has the advantage of being a more sensitive indicator of deterioration than the standard germination test, the test suffers a severe interpretation and reproduction problem. There are many factors which can influence its results, eg soil characteristics, type of substrate, temperature, duration of the cold test, seed treatment (dressing), and assessments (Burggink *et al.*, 1991a).

Soil differs in characteristics such as type, pH, activity of micro-organisms, water holding capacity, etc. The type of soil used in the cold test is important. In general, it is suggested that sandy loam to loam sand soils with a weakly acidic to neutral reaction should be used, and that soils with extreme values in their biological, physical and chemical characteristics should be avoided (Ader and Fuchs, 1978). Hooks and Zuber (1963) demonstrated variable results when soil was obtained from different sources and on different days and years. Svien and Isely (1955) presented data showing a significant difference in cold test results due to soil type and also in re-used and new soils.

Soil moisture content can also affect the cold test results. Normal seedlings decrease and dead seeds increase with increasing soil moisture content (Svien and Isely, 1955; Koehler, 1957; Nijenstein, 1985, 1988). In the AOSA soil cold test method, a water holding capacity (WHC) of 60-80% is used to distinguish vigour differences. However, Nijenstein (1988) observed that 60% WHC gave lower percentage of normal seedlings than 80% WHC, although Bruggink *et al.* (1991a) showed few vigour differences at soil WHC of 50-75%, and Hook and Zuber (1963) and Ader and Fuchs (1978) reported no significant influence of soil moisture content. Soil type and soil pathogen differences or subjective assessment may lead to reproducibility problems.

Many workers (eg Gill and Singh, 1970; Bruggink *et al.*, 1991a) have shown that cold test results are determined largely by the activity of soil micro-organisms. However, Rice (1960) showed that a medium consisting of either 1:1 or 2:1 sand and soil containing micro-organisms gave similar correlations with field emergence, and Burris and Navratil (1979) reported that the cold test using sterile substrate correlated as well with field emergence as a non-sterile cold test. It was found that much of cold test responses in maize appears to be influenced by temperature and a little by soil (Burris and Navratil, 1979). They suggested that using the cold test without soil could be advantageous in minimising the variation of test results between laboratories. The method is simple, reproducible and adequately sensitive, and has a good relationship with field emergence (Loeffler *et al.*, 1985), and the test results are not significantly different to those obtained from soil cold test (Cal and Obendorf, 1972). However, van de Venter and Lock (1991) argued that in seed lots where injury to the pericarp could result in substantial damage by soil pathogens, the cold test without soil would have less predictive value than the soil cold test. The protocol for the cold test without soil is given in section 3.4.2.2.

Temperature is one of the most important stresses simulated in the cold test. Different countries, however, employ different methods, temperature and duration. In USA, for example, the box method prescribed by AOSA (1983) is widely used, whereas in the European countries, the rolled towel paper prescribed by ISTA (1987) is widely employed. In the Netherlands, the cold test is usually conducted at 8.5°C for 17 days or 10°C for 14 days, whereas Germany and USA use 10°C for 7-10 days (Nijenstein, 1988). While AOSA and ISTA cold test methods use a temperature of 10°C, some workers (eg Bruggink *et al.*, 1991a) have reported 8.5°C to be the most effective temperature for detecting vigour differences in a range between 8.5-15°C, whereas others (eg Van de Venter and Grobbelaar, 1985; Van de Venter and Lock, 1991) have shown that rolled paper towel at 13°C was better to predict field emergence than the cold test. This disparity may be due to genotypic or vigour differences of the seed lots used and/or environmental factors.

Seed treatment and interpretation of test results may also affect cold test results. As the results may be greatly influenced by the activity of micro-organisms, it is clear that fungicide treated seeds may be better able to resist the effect of soil micro-organisms than untreated seeds (Clark, 1953). However, some reports (eg Bruggink *et al.*, 1991a) have shown no significant differences in cold test results between treated and untreated seeds. Mechanical damage levels, prevalence and virulence of micro-organisms may have a great influence on the test results. Variation of results can be simply because of interpretation problems. In the ISTA cold test method for exemple, the seedlings are classified into five

categories (strong, slightly weak, weak, abnormal and dead) according to stage of development (section 3.4.1.6). This assessment is a very subjective one, as the judgement depends mainly on the individual analyst, and thus the results are inevitably prone to variation.

2.7.5.2 Complex stressing vigour test

The test combines the results of the standard germination test with a complex stressing vigour test. After the period of both water and cold stress, seed performance is evaluated into high, medium and low vigour (section 3.4.1.5). The method is interpretable and reproducible, and its applicability has been tested over several years (Barla-Szabo and Dolinka, 1988; Barla-Szabo *et al.*, 1989). However, the test appears so far to have promise for only maize and wheat.

2.7.5.3 Seedling growth test

Seedling growth measurement is a sensitive indicator of deterioration. The test is convenient to perform in conjunction with the standard germination test, and is suitable for crops which can produce a single straight coleoptile, such as maize. However, growth assessments may not be a perfect criteria for measuring vigour because variability of results in this method can be significant, as seedling growth can be influenced by germination variables, particularly moisture and temperature. As germination, growth and vigour are under the control of genotype (section 2.4.1), strictly, this method cannot be used between different genotypes.

2.7.5.4 Electrical conductivity test

The conductivity test has the great advantage of being simple, quick and reproducible. However, the test results can be very difficult to interpret and some results may be misleading. For example, a small change in leakage of the embryo may be masked by enormous and uncontrolled leakage by the endosperm as a result of mechanical damage or cellular rupture due to rapid in-rush of water and not necessarily linked with membrane damage *per se* and hence vigour of the seed. High electrolyte leakage may merely be due to uncontrolled leakage resulting from a small number of dead seeds in the population, or an increase in the area of dead tissue in the individual viable seeds (Powell, 1986; Priestly, 1986). Additionally, a relationship between solute leakage and vigour does not exist in all species. There is evidence suggesting that in seeds with large inert endosperm (eg sorghum, wheat, tomato, etc) the conductivity of seed leachate does not always correlate with vigour. The Coolbear *et al.* (1984) study, for example, showed no associated increase in conductivity of leachates of aged tomato seeds, even though an increase in leakage of

amino acids and sugars was clearly evident. The permeability of the pericarp of the seed coat and cellular membrane integrity are different in different species and/or cultivars (section 2.4.1). These too may mask the effect of ageing *per se*.

2.7.5.5 Respiration test

This method is not popular because it is complicated and sophisticated and thus requires trained personnel. Although a decreased oxygen uptake is regarded as a measure of deterioration, there is also evidence suggesting that sometimes aged seeds take up increased levels of oxygen as they attempt to compensate for poor respiratory efficiency. Further than this, the respirometer does not measure chemical energy (ATP), only gas (carbondioxide and oxygen) exchange. The RQ indicate the likely type (aerobic or anaerobic) and substrate (glucose, fatty, protein) for respiration, but even though aerobic respiration is more efficient (approximately 10 times) than anaerobic, and a fatty substrate is more efficient than glucose or protein, it does not indicate how much ADP is being used to produce how much ATPs (Coolbear, 1990).

CHAPTER 3

MATERIALS AND METHODS

3.1 SEED MATERIALS AND PREPARATION

Two seed lots of super sweet corn (*Zea mays* L.) cv 'Illini Gold' were used in this course of study. These commercial seed lots were harvested in the same year but obtained from different sources (Table 3.1). Before commencing the experiments, each seed lot was divided into two equal portions, and each half was sub-divided into four sublots using a soil divider (ISTA, 1985). These sublots became replications. Samples in replicate I were from Sublot 1, replicate II from Sublot 2, replicate III from Sublot 3, and replicate IV from Sublot 4, respectively. In order to obtain differential vigour levels (high and low vigour), one half of each seed lot (with 4 replicates) was artificially aged.

Table 3.1 Super sweet corn seed lots used in the experiments.

Seed lot number*	Source[a]	Year of harvest	Initial germination	Initial SMC	Vigour status	Code
A5954	Watkins Seeds Limited	1991	88	13.2	High vigour	HV_1
A5954[1]	Watkins Seeds Limited	1991	60	15.3	Low vigour	LV_1
A5954[2]	Watkins Seeds Limited	1991	58	15.0	Low vigour	LV_3
BE 90	Webling & Stewart Ltd	1991	88	12.2	High vigour	HV_2
BE 90[1]	Webling & Stewart Ltd	1991	65	14.9	Low vigour	LV_2
BE 90[2]	Webling & Stewart Ltd	1991	50	15.2	Low vigour	LV_4

[1] Artificially aged at ~ 100% RH and 42°C for 24 hours
[2] Artificially aged at ~ 100% RH and 45°C for 24 hours
[a] Addresses: Watkins Seeds Ltd, New Plymouth, New Zealand
Webling & Stewart Ltd, Wellington, New Zealand
* Pre-storage history not known

3.2 THE RATIONALE OF ARTIFICIAL AGEING

Artificial ageing involves rapid deterioration of seeds by exposing them to harsh environmental (high RH and temperatures) conditions. This method was initially developed to predict seed storability under adverse ambient storage conditions (Delouche and Baskin, 1973). The technique was subsequently adapted by researchers as a means of hastening the deterioration process in seed ageing studies, which otherwise can extend over several years. In this respect, it was believed that physiological changes occurring during artificial ageing are similar to those occurring in natural ageing, except that the rate of deterioration is more rapid in artificial ageing compared to that occurring during natural ageing (Heydecker 1972; Matthews, 1985). Although critical questions have been raised about the

similarity of these two ageing methods (Priestly, 1986; Coolbear, 1990), the practicalities of research schedules often mean that the use of some form of accelerated ageing techniques may be inevitable (Nath, 1991). The different types of artificial ageing techniques and their procedures have been described in detail by Perry (1981), and that used in this study is presented in the next section.

3.3 ACCELERATED AGEING (AA) TEST

This technique involved incubating seeds in a high humidity atmosphere which allowed them to absorb moisture naturally, while being held at high temperatures for different lengths of time (Delouche and Baskin, 1973; Baskin, 1987). In this study, seeds were placed in 10 x 10 x 3 cm wire mesh baskets (Plate 3.1), and the wire baskets (with seeds) were then randomly placed onto shelves of a germinator (Plate 3.2). The AA treatment was carried out by holding seeds in approximately (~) 100% RH for 24 hrs at 42°C. A further AA test was conducted under more severe conditions, by raising the temperature to 45°C while maintaining the same RH and time. These conditions were chosen because previous AA work under similar conditions (Wahab, 1985; Taramai, 1985) with four seed lots of sweet corn (cv not known) decreased seed vigour, ie only a few seeds died, but normal seedlings declined with a corresponding increase in abnormal seedlings. This was later substantiated in a pilot AA test with the seed lots under investigation. Immediately after removing the aged seed samples (which in this study will be called low vigour seed lots) from the incubator, SMC was determined as in section 3.4.1.1. Details of all seed lots are shown in Table 3.1.

3.4 SEED QUALITY AND VIGOUR ASSESSMENTS

Extensive seed quality assessments were carried out in the laboratory in two experiments, before the subsequent field performance was monitored and correlated. In experiment I, a series of seed quality and vigour assays were employed, aimed to give an estimate of potential performance of seed lots under various environmental conditions. Experiment II was designed either to confirm and/or investigate further the results in experiment I so as to give clear and detailed information of seed planting value before sowing.

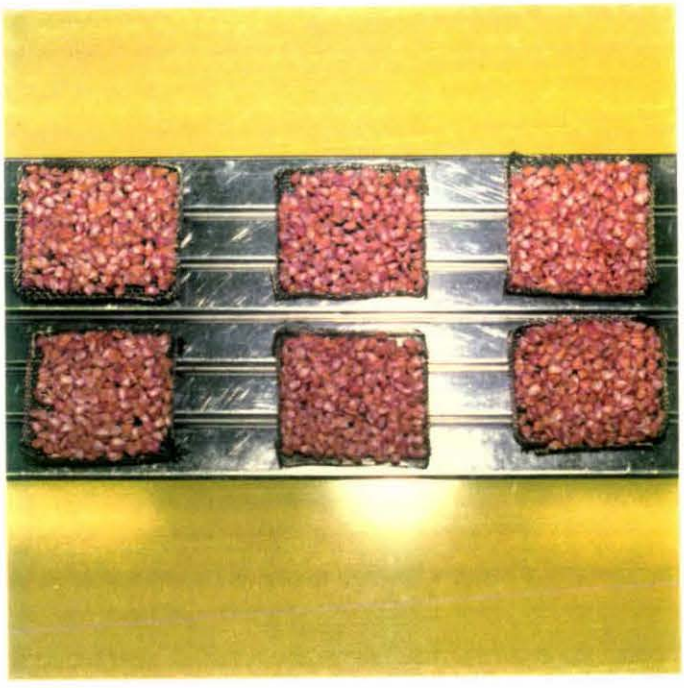

Plate 3.1 A photograph showing wire-mesh baskets with seeds before putting in a germinator.

Plate 3.2 A germinator with baskets placed onto shelves during accelerating ageing.

3.4.1 Experiment One

In this experiment, various components of seed quality; SMC, standard germination, thousand seed weight, mechanical damage and vigour, were determined using both unaged (high vigour) and aged (low vigour) seed lots (ie HV_1, HV_2, LV_1, LV_2, LV_3 and LV_4 - Table 3.1).

3.4.1.1 Seed moisture content

Seed samples were randomly hand drawn from the four replicates of each individual seed lot, and two 10 g replicates of a ground composite sample were placed in aluminium containers, covered, weighed, the cover removed, and dried using the high constant temperature oven method, ie 130°C for four hours (ISTA, 1985). The containers were then covered and placed in a desiccator to cool for 30 minutes before reweighing. Moisture content was calculated and expressed as a percentage on a fresh weight basis using the following formula:

$$\% \text{ SMC} = \frac{M_2 - M_3}{M_2 - M_1} \times 100$$

where:

M_1 = the weight in grams of the container and cover

M_2 = the weight in grams of the container, cover and contents before drying

M_3 = the weight in grams of the container, cover and contents after drying

3.4.1.2 Thousand seed weight and mechanical damage

The thousand seed weight (TSW) and mechanical damage were determined on HV_1 and HV_2 seed lots only. TSW for each seed lot was determined by counting eight replicates of 100 seeds (ISTA, 1985), and the mean TSW was calculated from the average of eight weights of 100 seed replicates multiplied by ten. Mechanical damage was determined using three replicates of 100 seeds for each lot. Evaluation was done by visual examination of the individual seeds for any physical damage such as pericarp crevices, spots and parts of pericarp or endosperm missing, collapsed pericarp etc. Total damage was expressed as a percentage regardless of size, type or position.

3.4.1.3 Standard germination test

The between paper (BP) method (ISTA, 1985) was used. Four replicates of fifty seeds from each seed lot were germinated at 25°C for 7 days. Normal, abnormal seedlings and dead seeds were counted and the results were expressed as a percentage (ISTA, 1985).

Normal seedlings in this study were seedlings which showed the potential for continued development into satisfactory plants when grown under favourable conditions (ISTA, 1985).

3.4.1.4 Seedling growth test

The logical basis for this test is presented in section 2.7.2. Seedling growth was recorded by measuring shoot length and root and shoot dry weight of normal seedlings at the end of the standard germination test (7th day after planting at 25°C). Length of individual coleoptiles were measured and mean length was calculated using the formula:

$$L = \Sigma (x_i \cdot n_i) \cdot \frac{1}{N}$$

where N is the number of normal germinants and n is the number of normal seedlings whose coleoptile terminate in the mid point x_i. Results were expressed as shoot length per normal seedling. Shoot and root dry weights were determined separately by removing the shoot and root from normal seedlings, putting them separately into aluminium tins and drying at 65°C to constant dry weights. Results were expressed as dry weight per normal seedling.

3.4.1.5 Complex stressing vigour test

Complex stressing vigour testing (CSVT) was done according to Barla-Szabo and Dolinka (1988), but with some modifications. The test is based on two days of water and two days of temperature stress, after which germination is carried out. The principle behind this test is given in section 2.7.1.

200 seeds of each seed lot were soaked for 48 hours at 25°C in 250 ml water containing 0.15% NaOCl. The soaked seeds were then removed and incubated at 5°C for a further 48 hours. After four days of stress treatment, seeds were germinated using the between paper (BP) method, with four replicates of fifty seeds at 25°C for 96 hours. Seedling evaluation was carried out according to ISTA (1985), but the seedlings were classified into:

I Normal seedlings (N)

- High vigour (HV): seedlings longer than a quarter of the mean of the 5 longest
- Medium vigour (MV): seedlings shorter than a quarter of the mean of the 5 longest

II Abnormal seedlings (Abn) were classified as low vigour

III Non-germinating, rotten seeds (Ng)

3.4.1.6 Soil cold test

The principle of the cold test method is described in section 2.7.1

Soil preparation:

The soil used in this study was an Ohakea silt loam, and was randomly sampled from the same field area where the crop was to be grown (section 3.5.2). The soil was screened through a 4 mm sieve, was then mixed thoroughly and put in a 40 x 62 x 21 cm plastic container with a tight lid.

Soil moisture content determination:

The soil moisture content was 19.7% (wet basis) and was determined as described by Perry (1987). Three replicates of 25 g were sampled randomly and dried in a 105°C oven for 3 hours. Soil moisture content was then calcualted (wet basis) using the formula:

$$\% \text{ moisture content} = \frac{\text{weight of moisture loss}}{\text{wet weight of soil}} \times 100$$

Soil maximum water holding capacity:

Maximum water holding capacity (WHC) is defined as the water held in the root-zone between the field capacity and permanent wilting point. WHC of the soil used in this study was 62.3% and was obtained using three replicates of soil samples placed in weighed cylindrical tin containers. The containers had small holes in the bottom to allow water to drain out. The soil samples were flooded with water until the water ran out the base, and the saturated soil was then left to drain out in a moist chamber for 24 hours to avoid evaporation. After that, the containers plus the saturated soil were weighed, placed in a 105°C oven for 24 hours, and then reweighed. WHC was calculated using the formula:

$$\% \text{ WHC} = \frac{\text{weight of moisture loss}}{\text{dry weight of soil}} \times 100$$

Achieving the required WHC:

The soil was adjusted to 60% of its WHC (Nijenstein, 1988) by adding 129 ml of water to 1250 g of soil. The amount of water required (ie 129 ml) to achieve this WHC (ie 60%) was calculated using the formula:

$$\text{Quantity of water required (ml)} = \frac{A \,.\, (100 - B) \,.\, C \,.\, D}{10^6} - \frac{A \,.\, B}{100}$$

where

A = weight of moist soil (ie 1250 g)

B = % moisture content of moist soil (ie 19.7%)

C = maximum water holding capacity of dry soil (ie 62.3%)

D = required % of WHC (ie 60%).

Cold test procedures:

Four replicates of 1250 g of soil were placed in 18 x 25 x 10 cm plastic boxes to a level of 2.5 cm, then 129 ml of water was added to bring the soil to 60% of its WHC. The soil was then mixed thoroughly before being levelled compactly. After that, fifty seeds of each replicate were placed onto the soil (one replicate/seed lot/box). The same amount of soil and water was mixed separately, placed over the seeds, and then levelled compactly. The boxes were covered with plastic bags and put in a germinator cabinet running at 10°C and 95% RH for 7 days, with alternatiing light (12 hrs darkness - 12 hrs light). After that period, the temperature was raised to 25°C for a further 6 days.

Seedling evaluation was carried out on the 13th day. Seedlings were classified into 5 groups according to their stage of development (ISTA, 1987). The first group included strong seedlings with no damage and two or more leaves present, while group 2 constituted strong seedlings but with development slightly delayed or with slight injuries and one or two leaves present. Group 3 included weak or short seedlings and only the coleoptile or one leaf present. Groups 4 and 5 contained abnormal seedlings and dead seeds, respectively. Shoot lengths from normal seedlings (groups 1-3) were measured and their dry weights determined. The procedure was similar to that employed in the seedling growth test (section 3.4.1.4).

3.4.2 Experiment Two

This experiment was designed on the basis of results in experiment I. The results from this experiment indicated that vigour was worth further investigation, in particular the metabolism of seeds under cold stress, to see if contradictory results (see Section 4.1) could be explained. This would give a clearer picture of the vigour status of seed lots before planting in the field.

3.4.2.1 Seed health testing

Samples from both high (HV_1 and HV_2) and low (LV_1 and LV_2) vigour seed lots were tested for fungal infection using potato dextrose agar (PDA). Preparation of PDA was done by suspending 45 g of PDA in 1 litre of tap water, and the solution was then autoclaved for 30 minutes before pouring the cooling agar into sterile plastic petri dishes in a sterile environment cabinet (Hill, 1991, Pers. comm.[1]). Seeds were placed in a muslin cloth bag and surface sterilised in a 1% NaOCl solution for 2 minutes, followed by a thorough rinsing in running water for 5 minutes. Ten replicates of 10 seeds from the composite sample of the four replicates of each seed lot were aseptically plated onto PDA, embryo down. The seeds were then incubated at 25°C for five days, and fungi present were isolated and identified, based on both colony and conidial characteristics using the dissection steriopic microscope and the compound microscope, respectively (Kabeere, 1991, pers. comm.[2]). Quantifying was made by counting the number of seeds infected by a particular fungus and expressing as a percentage.

3.4.2.2 Cold germination test

This test is a modification of the soil cold test (section 2.7.5.1). The difference is that the soil cold test is done with wet soils, and thus determines the effect of water, temperature and soil-borne micro-organism stresses, while the cold germination test measures the effect of cold (low temperature) stress *per se*. Four replicates of fifty seeds were planted between paper (BP) and the rolls were prepared as for the standard germination test (section 3.4.1.3) but incubated as for the soil cold test, ie put in a germinator at 10°C for 7 days before incubating at 25°C for a further 6 days. Seedling evaluation was done as for the standard germination test. Shoot lengths were also measured and shoot and root dry weight recorded as for the seedling growth test (section 3.4.1.4).

3.4.2.3 Soak germination test

This test is based on the principle that soaking (water) and low temperature stresses cause anaerobic respiration and the excretion of ethanolic and acetaldehyde compounds (Martin, 1986; 1987) depending on vigour levels (Pesis and Ng, 1984) both of which are toxic to seed performance. These may cause severe membrane disruption (Crawford, 1977) causing high leakage of solute. The test can be used to determine physiological vigour and

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has been shown to be as effective in predicting field emergence as the cold test (Martin *et al.*, 1988). In this test seeds were soaked for 24 or 48 hours at 10°C or 25°C, and then germinated. Three replicates of fifty seeds were sown between paper (BP) and the preparation of the rolls and evaluation was done as for the standard germination test.

3.4.2.4 Electrical conductivity test

The principle underlying this test is described in section 2.7.3, and the test was carried out according to Matthews and Powell (1987) with some modifications. Three replicates of fifty seeds from both high and low vigour seed lots were used. Each individual replicate of 50 seeds was weighed before being put into a 500 ml Erlenmeyer conical flask containing 250 ml distilled water at low (10°C) and optimal (25°C) temperatures. Flask tops were sealed with parafilm to prevent evaporation and contamination. Two flasks containing only distilled water were prepared as controls. Conductivity of leachate was recorded in intervals of 2, 12, 24, 36 and 48 hours, using a conductivity meter (CDM-83 Radiometer). The experiment was in two sets; the first terminated at 24 hrs while the second was prolonged to 48 hours, and the same seeds were used in all measurements. The conductivity readings were obtained by dipping the conductivity meter electrode in water with the seeds. The reading from the control (distilled water only) was subtracted from the reading obtained from the seeds, and the results were expressed in micro siemens per gram of seed (μSg^{-1} seed).

3.4.2.5 Imbibitional conductivity test

As mechanical damage and too rapid water uptake by seeds at high temperatures (eg 25°C) may jointly escalate leakage, and therefore confound or mislead the essence of the conductivity test, ie loss of membrane integrity (ageing) *per se* (section 2.7.5.4), seeds were imbibed at low temperature to reduce imbibition rates before vigour was determined. Absorbant papers wetted with tap water as used for normal germination testing (ISTA, 1985) were used for imbibition of seeds. Only seed lots HV_1 and LV_1 were used. Three replicates of fifty seeds were rolled in paper as for the standard germination test and kept in a plastic bag at 10°C for 24 or 48 hours. Imbibed seeds were blotted dry before determining the seed's fresh weight. After that, seed leachate conductivity at 25°C for 24 or 48 hours was determined. The preparation and measurements were as for the normal electrical conductivity test (section 3.4.2.2). At the end of the test, germinability performance was determined as for the normal germination test.

3.4.2.6 Respiration test

The principle of this test is described in section 2.7.4. This experiment used seeds from HV_1 and LV_1 only, and a time course of respiration studies was done manometrically in two sets of experiments using a Gilson differential respirometer with 12 Warburg flasks (Plate 3.3) similar to that employed by He and Burris (1991). The first was designed to measure respiratory metabolism occurring during stress conditions in the cold test with or without soil (ie 10°C, 7 days; 25°C, 6 days), while the second was designed to determine respiratory metabolism at optimal conditions in the standard germination test (ie 25°C, 7 days).

A composite sample of the four replicates of each seed lot (high or low vigour) was used. Before imbibition, seeds were first placed in a nylon bag and surface sterilised by completely submerging them in 1% NaOCl for 15 minutes to minimise any contaminant fungi which may develop during the imbibition and interfere with respiration measurements. After surface sterilisation, the seeds were washed with distilled water and transferred to 0.01 N HCl for 10 minutes to remove any residual chlorine as this substance affects metabolism (Abdulbaki, 1974). The seeds were then washed 3 times in distilled water for 5 minutes followed by imbibition at 10°C or 25°C on top of Whatman filter paper moistened with distilled water. The 12 flasks were randomly selected and arranged in pairs; +1 vs -10, +12 vs -2, +8 vs -3, +7 vs -11, +5 vs -9 and +4 vs -6. The first three pairs were used for the high vigour seed lot, while the rest were for the low vigour seed lot, and each pair represented one replicate. Only internal replicates were used. The seeds were put into Warburg flasks (4 seeds in each flask) either with (+) or without (-) 0.2 ml of 20% KOH in the centre well. In each pair, a flask containing KOH (+) would measure oxygen (O_2) uptake while those without KOH (-) measured net gas evolved (CO_2 - O_2); thus CO_2 evolved could be calculated. In seeds imbibed at optimal (25°C) conditions, respiration measurements were recorded every eight hours until radicle emergence. For the low temperature (10°C) stress respiration studies, measurements were recorded daily for 7 days, and after transferring to optimal (25°C) temperature, measurements were taken at intervals of 6 hours until radicle emergence. Respiration measurements were recorded afer 20-30 minute equilibration in the Gilson differential respirometer where the flask containing seeds were shaken at 130 oscillations per minute. The seeds were used once, and at the end of each record, seed fresh weight of each replicate was determined. Respiratory quotients (RQs) were calculated on the basis of the ratio between CO_2 evolved and O_2 uptake.

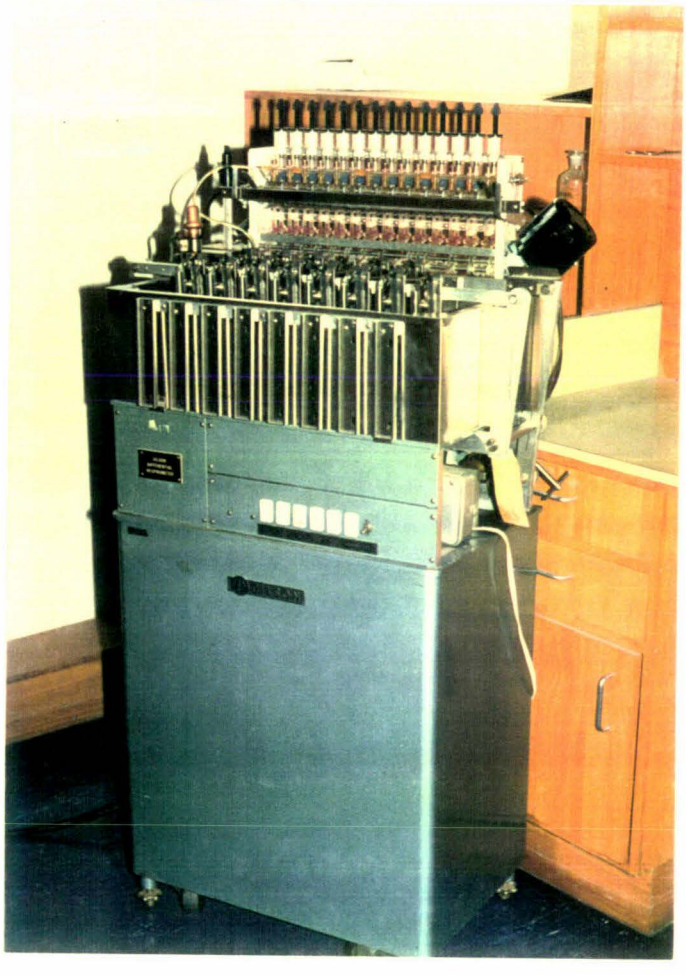


Plate 3.3 A Gilson differential respirometer.

3.4.2.7 Tetrazolium test

A quick viability (tetrazolium) test was carried out at the end of the cold stress respiration, ie 7th day of imbibition at 10°C. This test measures the activity of dehydrogenase enzymes. The importance of these enzymes is based on the assumption that they are involved in a number of metabolic events. Many of them are known as mitochondrial dehydrogenase enzymes which could be linked to respiration metabolisms. These enzymes reduce tetrazolium chloride salt to a red water insoluble compound, formazan. Formazan stains living cells a red colour, whereas dead cells remain colourless (AOSA, 1983; ISTA, 1987). One replicate of 50 seeds from high (HV_1) or low (LV_1) vigour seeds were dissected longitudinally and soaked in a 1% aqueous solution of 2,3,5-tryphenyl tetrazolium chloride (TTC) and held at 20°C for 17 minutes. The staining process was monitored until approximtely 3/4 of the seeds were completely stained. After that, the TTC was decanted off and the seeds were washed in running water. Individual seeds were then examined for staining intensity and pattern. Results were expressed as a percentage.

3.5 FIELD EXPERIMENTS

3.5.1 Experimental field

The first study in the field was carried out at the Seed Technology trial field, Massey University, Palmerston North, New Zealand (40° 23' S, 175° 37' E) in the 1991/1992 seasons. The field had previously been cropped with barley (1989/1990) and maize (1990/1991). The soil type was an Ohakea silt loam with a maximum water holding capacity (WHC) of 62.3%. A full description of this soil is presented in Appendix 3.1.

3.5.2 Weather data

Soil moisture content and temperature at 5 or 10 cm depth were determined every 24 hours from sowing to emergence, while temperature and rainfall data during the growing season were obtained from a site 1 km from the trial area (at the AgResearch Grasslands CRI in Palmerston North, New Zealand) Data are presented either daily or as means for rainfall, evaporation, relative humidity, maximum and minimum air temperature, and 10 cm earth temperature.

3.5.3 Seed bed preparation

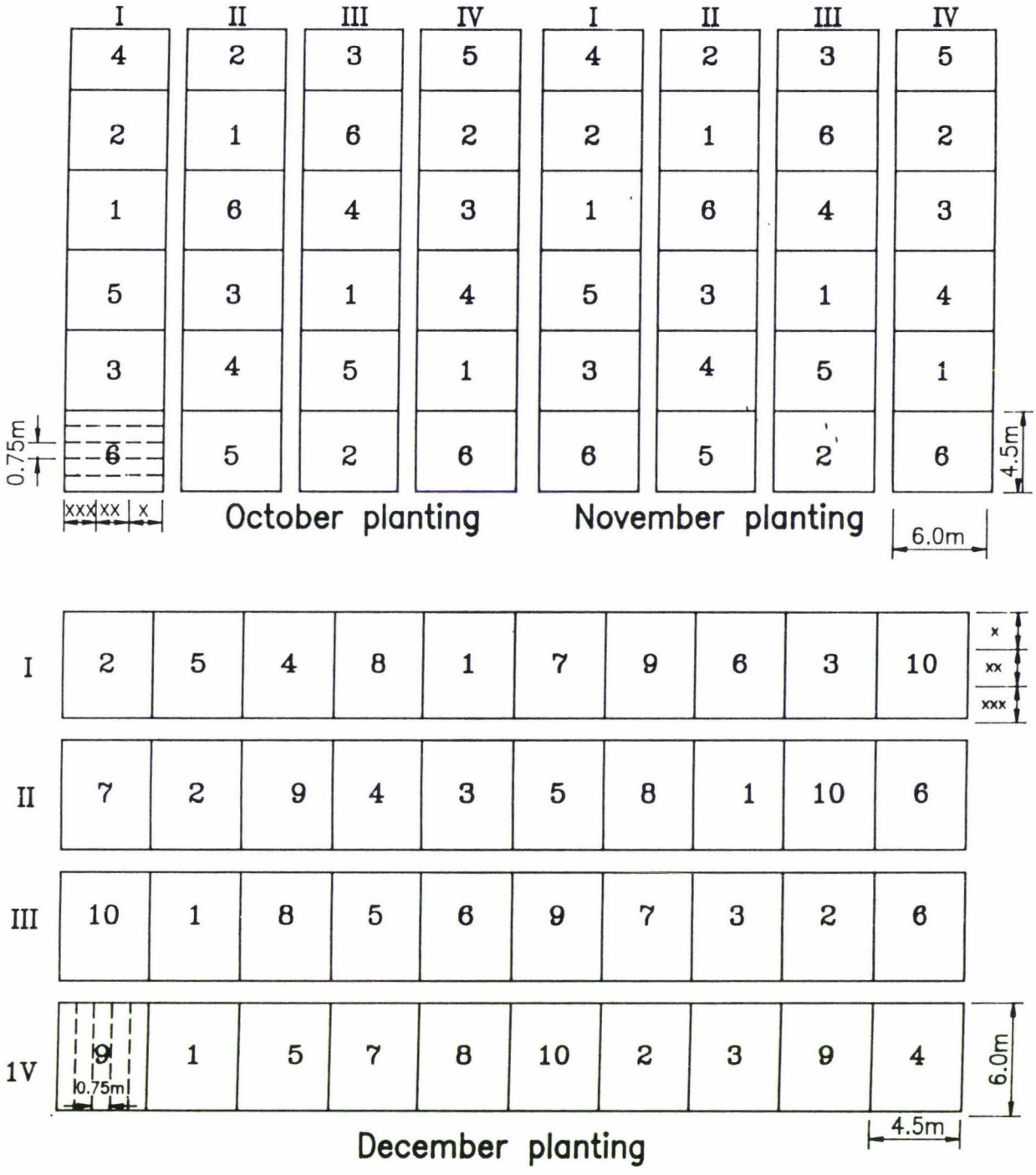
The field was first sprayed with Roundup (glyphosate) herbicide on 7 October 1991 (360 g a.i./litre) using a boom sprayer mounted on a tractor, to kill weeds. Roundup is a non-selective systemic herbicide for control of most annual and perennial grass and broadleaf weeds (Anon., 1990). It is taken up only through foliage and translocated to all parts of the plant, including roots and rhizomes. It is inactivated on soil contact and therefore has no residual activity. On 14 October 1991, the block was ploughed out of desiccated plant materials and harrowed thoroughly on 17 October 1991 using a Mashio power harrow. A further harrowing took place three days before the first planting.

3.5.4 Experimental design and field layout

The experiments were arranged in a randomised complete block design for each planting with four blocks of 6 m x 27 m, and a one metre border between blocks (Figure 3.1). This study was carried out using three different planting dates. For the first two plantings, six plots of 4.5 x 6 m with 0.75 m between plots were replicated four times per block. HV_1, HV_2, LV_1 and LV_2 seed lots were planted at 12-14 kg ha^{-1} to achieve a theoretical optimum population of 88888 plants ha^{-1}. On the assumption that LV_1 and LV_2, because of poor vigour may emerge poorly, these two seed lots were also planted at double this rate (24-28 kg ha^{-1}) and then thinned to 88888 plants ha^{-1}. The intention was to determine whether low vigour seed affected subsequent plant performance when population density was not a factor. In the last planting, everything was similar to the first two plantings except that seed lots LV_3 and LV_4 were also planted at the rate of 88888 plants ha^{-1}, and at double this rate, thus making ten plots. Each plot consisted of six rows of a particular seed lot but measurements were taken only from the two middle rows and the other four were used as border plant rows. The gap between rows was 0.75 m and the distance between hills was 0.15 m, so that there were 40 plants/row.

3.5.5 Time and method of sowing

The first planting was on 29 October 1991, representing an adverse sowing, because although late October planting is recommended for the Manawatu region (Hardacre *et al.*, 1991) mid spring (October) soils may be wet and cold depending on the season, and this may affect emergence. The second and third plantings were on 28 November and 23 December, respectively. These represent an ideal sowing period for good germination and establishment as soil temperatures are expected to have increased (Hardacre *et al.*, 1991).



x = Vegetative samples
xx = reproductive samples
xxx = Seed yield samples
I, II, III, IV = Replicates

1 & 2 = HV_1 & HV_2
3 & 4 = LV_1 & LV_2
5 & 6 = LV_{11} & LV_{22} (LV_1, LV_2 overplanted then thinned)
7 & 8 = LV_3 & LV_4
9 & 10 = LV_{33} & LV_{44} (LV_3, LV_4 overplanted then thinned)

Figure 3.1 **The field layout for experiments conducted in the 1991/1992 season.**

Seeds were hand planted at a depth of 2-3 cm, with one seed planted per hill for all seed lots, except in plots where population was intentionally inflated.

3.5.6 Crop management

For the October sowing Nitrophoska (12N:10 P: 10 K:1 S:6Ca) was applied at sowing time as a starter fertiliser by evenly broadcasting at a rate of 230 kg ha^{-1}. A mixture of Alachlor EC and Atrazine (500 FW) herbicides was applied three days after planting, using a boom sprayer mounted on a motorcycle. The former is a selective pre-emergence herbicide and was applied at the rate of 2.88 kg a.i. ha^{-1}, while the latter is a selective post-emergence herbicide applied at the rate of 1.5 kg a.i. ha^{-1}. Hand weeding was employed to remove weeds that survived the Alachlor/Atrazine herbicide application. Hallmark 5EC insecticide was sprayed thirteen and twenty-one days after seeding, at the rate of 19 g esfenvalerate a.i. ha^{-1} and 179 g xylene a.i. ha^{-1}. Hallmark is an insecticide active on a wide range of foliar feeding pests, particularly caterpillars (Anon., 1990), and was applied with the use of a knapsack sprayer. Mesurol snail and slug bait was applied twenty-four days after seeding, by banding at the rate of 2.34 kg Methiocarb a.i. ha^{-1} to control snails and slugs. Forty-five days after planting, urea was applied as a side-dressing at the rate of 290 kg ha^{-1}.

In the November sowing, as in the October planting, a compound fertiliser, Nitrophoska (12 N:10 P:10 K:1 S:6 Ca) was applied before planting by evenly broadcasting at the same rate as that used in the October planting. A mixture of pre- and post-emergence (Alachlor EC and Atrazine 500 FW) herbcides was applied five days after planting. One week later (twelve days after sowing), Mesurol baits were spread to control snails and slugs. Application rates were the same as those employed in October. Hallmark 5EC was applied twice at the rate of 19 g esfenvalerate a.i. ha^{-1} and 179 g xylene a.i. ha^{-1}, the first application was twelve days after planting and the second was twenty-one days after planting. After symptoms of diseases such as wilting and post-emergence damping-off (thought to be *Fusarium* diseases) were found, a mixture of Benlate and Thiram fungicides was sprayed twenty-seven days after planting in an attempt to control the pathogen(s). Benlate has good fungicidal properties against a wide range of plant diseases through protective and systemic activity, while Thiram fungicide has a broad spectrum, multi-site action for control of many plant diseases (Anon., 1990). These were applied twenty-seven days after sowing with the use of a knapsack sprayer (Plate 3.4). Application rates were 1 kg a.i. ha^{-1} and 3.2 kg a.i. ha^{-1} for Benlate and Thiram respectively. Twenty days after sowing, Buster herbicide was sprayed at the rate of 1 kg a.i. ha^{-1} to control weeds which

Plate 3.4 An application of fungicide with the use of a knapsack sprayer.

had escaped the activity of Alachlor/Atrazine. Buster is active against a wide range of grass and broadleaf weeds and clovers (Anon., 1990). It is a non-selective herbicide, but was applied using knapsack sprayer with a shield to avoid reaching the sweet corn plants. Persisting weeds were hand removed. Fifty-five days after sowing, urea was applied as a band application at the rate of 290 kg ha^{-1}.

In the December planting a mixture of pre-emergence (Alachlor) and post-emergence (Atrazine) herbicide was applied three days before planting. The application rates were similar to those applied in the October planting. Ten days after sowing, signs of infection such as wilting and post-emergence damping-off (Plate 3.5) (presumably *Fusarium*) were noted and in response to this, Benlate and Thiram fungicides (mixture) were applied at the rates similar to those used in the November planting. One day later (eleven days after sowing) Hallmark 5EC was sprayed at rates similar to those applied previously. Benlate fungicide was again applied as a drench using a watering can and during rain for good penetration at the rate of 1 kg Benomyl a.i. ha^{-1} to control *Fusarium* diseases. Dicamba (Banvel 200) herbicide was applied thirty-seven days after planting to clear weeds not killed by Alachlor/Atrazine. Dicamba is a selective herbicide for the control of difficult to kill broadleaf weeds, and was applied at the rate of 329 g dicamba a.i. ha^{-1}. Thereafter, any surviving weeds were hand removed. Forty-two days after sowing, urea fertiliser was banded at the same rate as to that employed in the October planting. Thinning of seedlings in plots planted at double rate was done immediately after completion of emergence.

3.6 GROWTH MEASUREMENTS

As these experiments involved destructive sampling, care was taken to avoid the effects of plant density on seedling vigour caused by sampling. The sampling rows were divided into x, xx and xxx segments (Figure 3.1). Portion x was for vegetative samples only, xx for reproductive samples, and the xxx segment was for seed yield and seed yield components alone. As random sampling will inevitably violate spacing patterns within the plant populations, which in turn may conceal any seed vigour effects, samples were taken sequentially, rather than randomly.

3.6.1 Field emergence and rate

The potential and rate of emergence in the field were estimated using the four replicates of either high or low vigour seed lots. Overplanted plots were not counted.

Plate 3.5 A photograph showing damaged plants resulting from fungal infection.

Emerging seedlings were counted daily from two rows of each replicate until no further increase was noted. Total emergence was expressed as a percentage. The daily emergence values were used to compute the estimated field emergence rate in days.

3.6.2 Vegetative growth and development

During vegetative growth, stem and leaf dry weights were determined at different stages of mature leaves (3, 5, 7 and 9 mature leaves). Only above ground parts from the x section were measured. Five or three plants were harvested from each plot (including compensated plots), and stems and leaves were separately dried in a 65°C oven until constant dry weight before their dry weights were recorded. Total plant dry weight at each particular leaf stage was measured by summing stem and leaf dry weights and results were expressed as dry weight per plant.

As the activity of pathogens can potentially influence seed and plant performance, and hence conceal any seed or plant vigour effects, an attempt was made to identify those present. Diseased plants were randomly sampled, and damaged parts (mainly shoot) were cut, sterilised and placed onto PDA agar plates. Sterilisation, preparation of agar and plating procedures were the same as that used in section 3.4.2.7. The plates were then incubated at 25°C for 5 days. Suspected pathogens were cultered on PDA agar plates, and then sub-cultured and pure cultures of each strain were identified based on both colony and conidial characteristics using the dissection stereoscopic microscope and the compound microscope, respectively. At every 5 leaf stage in each planting, losses of plants as a result of post-emergence damping-off were estimated by counting the remaining plants and then subtracting from the emerged number and expressing as a percentage of emerged plants.

The mean number of days to 50 percent silk emergence was determined by visual inspection of plants in the xxx segment in each plot of high (H_1 and HV_2) or low (LV_1 and LV_2) vigour seed lots only. At this stage, stunted plants were counted and expressed as a percentage of emerged plants. These were plants showing severely retarded growth, and which had become covered by the plant canopy to the extent that they had no chance of producing a cob. Final plant stand in the field was also calculated by counting all surviving plants (including stunted) and expressing them as a percentage of emerged plants.

3.6.3 Seed development

Due to a shortage of plants caused by high mortality of plants post-emergence in the October and December plantings, reproductive development studies were done in the November planting only. These were assessed as changes in seed moisture content and fresh and dry weights from 27 days after silking to mass maturity. As the performance of HV_1 and HV_2 or LV_1 and LV_2 seeds lots were the same from emergence to maturity, one high vigour (HV_1) seed lot and its corresponding low vigour (LV_1) seed lot were used with three replicates instead of four. Two cobs were harvested from the xx portion of each replicate at intervals of 7 days, hand shelled, and thoroughly mixed before any measurements.

3.6.3.1 Determination of seed moisture content, fresh and dry weights

For seed moisture content > 65%, triplicate samples of 200 whole seeds from two cobs were placed in tins with lids and weighed before drying in an oven at 103°C for 17 hours. The tins were cooled, and then reweighed and SMC was calculated as described in section 3.4.1.1. For SMC ≤ 65%, a two stage method was employed. In the first stage (S1) the seeds were pre-dried by first weighing and then put on the top of a 130°C oven to dry overnight before reweighing, and % SMC was computed as previously described. In the second stage (S2), seeds were ground by machine (Cemotec 1090 sample mill), put in tins and weighed before being dried at 103°C for 4 hours. After that, the samples were cooled in a desiccator, reweighed, and % SMC calculated normally. Finally, the original % SMC of the sample was calculated using the following formula:

$$\% \text{ SMC} = S_1 + S_2 - \frac{S_1 \cdot S_2}{100}$$

where

S_1 = percent seed moisture content in stage 1

S_2 = percent seed moisture content in stage 2.

While seed fresh weight was determined directly with the use of weighing scales, seed dry weight was computed using the following formula:

$$\text{SDW} = \text{SFW} \cdot \frac{100 - \text{SMC}}{100}$$

where

SDW = seed dry weight (g)

SFW = seed fresh weight (g)

SMC = seed moisture content (%)

3.6.4 Seed yield and seed yield components

Seed yield was determined from one high vigour (HV_1) seed lot and its corresponding low vigour (LV_1) seed lot, and also the low vigour (LV_1) planted at high density and thinned. Three plants were used in each replicate to measure the seed yield component; number of plants per unit area, cobs per plant, seeds per cob and seed dry weight. Finally, seed yield was calcualted using the formula:

$$\text{Seed yield (tonnes)} = P \cdot E \cdot N \cdot S$$

where

P = Plant density ha^{-1}

E = Number of cobs $plant^{-1}$

N = Number of seeds cob^{-1}

S = Seed dry weight adjusted to 15% SMC.

3.7 TIME AND METHOD OF HARVESTING AND DRYING

Hand harvesting was employed in all plantings, and all the four replicates from each seed lot (including the compensated plots) were harvested and dried, except for the December planting where only three replicates were used. The October planting was harvested on 20 June 1992 when seed moisture content was 30.9%. Approximately 8 cobs were harvested from each replicate, dehusked and then spread in the glasshouse to dry naturally for 32 days, under ambient temperature and relative humidity of 6-16°C and 69-98%, respectively. After drying, all ears from each replicate were hand shelled and put in paper bags individually, and then placed at room temperature until the evaluation of seed quality and vigour characteristics. In the November and December sowings, ears were harvested on 28 June and 10 July 1992, when seed moisture content was 50.8% and 63.9%, respectively. As in the October sowing, about 8 cobs were harvested. Because of elevated moisture content of seeds in these treatments, seeds were mechanically dried immediately after harvest using a heated air-system mini driers (Kiwi mini drier, Seed Technology Centre, Massey University, New Zealand).

3.7.1 Equipment and procedure

The physical configuration of a Kiwi mini drier is shown in Plate 3.6, and its basic functional components include a heating unit (up to 1,000 watt) which heats up ambient air to a desirable level before blown being through the seeds. Next is a fan, which can create a constant air flow of up to 12 m s^{-1}, and a condensation component with a chilling capacity of up to 0.8790 kWh (but not used in this study).

Due to desiccation problems of the crop under investigation (Chapter 2), slow and low temperature drying was employed for both the November and December sowings, as suggested by Wilson and Trawatha (1991). During drying, three mini driers were used, situated in a big room of about 12 x 6 x 3 m. The room is fitted with three HECO heaters with heating capacity of up to 2000 Watts. Because of the lower ambient temperature (4.9-12.2°C) and high humidity (79-98%) at the time of drying (particularly during the night), these heaters were turned on throughout to increase the ambient temperature and reduce the RH in the drying room for effective drying. Additionally, doors and windows were kept closed most of the time to minimise flow of cold and humid air from the macro-atmosphere (space) into the micro-atmosphere (drying room). Ears from each replicate were randomly placed in a rectangular tray of 0.35 x 0.23 x 0.09 m and identified. The trays were then randomly stacked on the perforated rectangular air outlet duct base of the drier (Plate 3.7). Eight trays were piled on each drier.

During drying of cobs harvested from the November planting, the driers were set to heat the ambient air at 20°C and force it to move at a constant airflow of 12 m s^{-1} for 86 hours, under mean ambient temperature and RH of 14-28°C and 54-81%, respectively. The ambient temperature was recorded using a dry and wet bulb thermometer and the ambient relative humidity (RH) was computed using a psychometric chart. After drying, both dry and wet cobs were tested for moisture content. Dry cobs were shelled by hand and kept in paper bags. Cobs which resisted drying were counted in each replicate and expressed as a percentage. In cobs harvested from the December sowing, arrangement of the replicates and trays were similar to that in November planting, but because of uneven drying experienced in cobs harvested from the November sowing, drying temperatures in this treatment was raised to 25°C and the drying period prolonged to 126 hours, but maintaining the airstream velocity at 12 m s^{-1}. Ambient air temperature and RH both outside and inside the drying room were more or less the same as those recorded during drying of cobs harvested from the November sowing. After 126 hours of drying, dry and the still wet

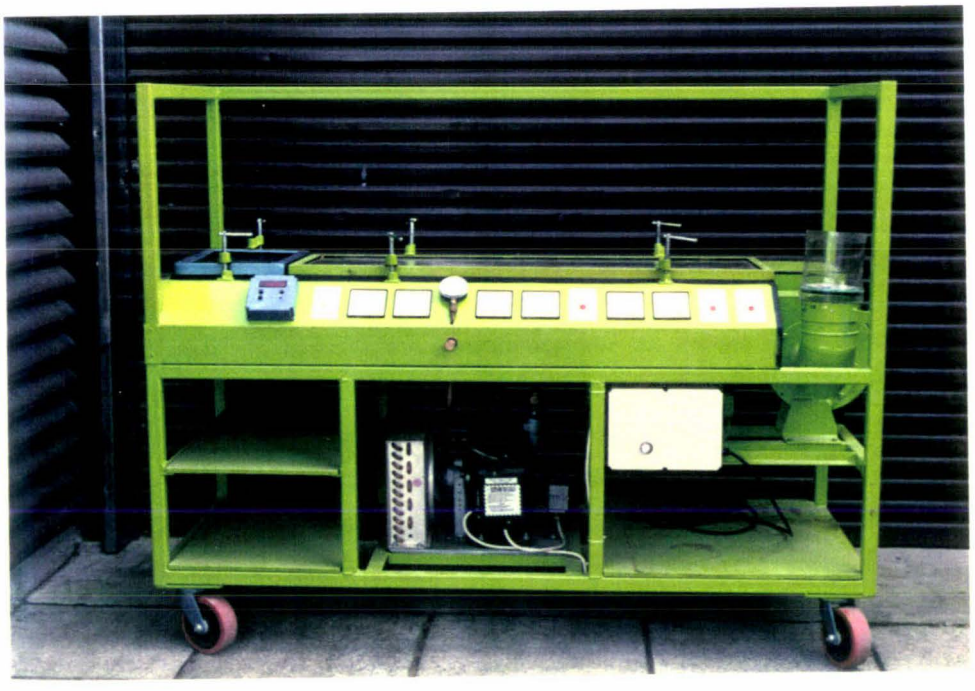


Plate 3.6 A Kiwi mini drier.

Plate 3.7 A Kiwi mini drier with piled rectangular trays.

cobs were determined for SMC. After that dry cobs were shelled by hand, and shelled seeds were put in paper bags. Wet cobs were counted in each replicate and expressed as a percentage.

3.8 SEED QUALITY AND VIGOUR TESTING

3.8.1 Seed lots

Seed germination, vigour and health status of both original (F_2) and fresh harvested (F_3) seed lots were determined. One original (F_2) high vigour (HV_1) seed lot had previously been divided into two sub-groups. One group was stored for 14 months at 5°C, and the other was stored for 14 months at 25°C. The remainder of the original low vigour (LV_1) seed lot was stored for 14 months at 5°C. Details of all seed lots used are presented in Table 3.2.

3.8.2 Measurements

The seed qualities determined were seed moisture content, standard gemination, seedling growth test, conductivity test, soak germination, health test, cold germination test, thousand seed weight (fresh harvested seeds only), soil cold test (fresh harvested seeds only). Protocols employed for each of these tests are as described in experiments I and II. Details of replications and any special approach will be indicated alongside the appropriate data in the subsequent chapters.

3.9 FIELD EMERGENCE TRIALS

3.9.1 Site and land preparation

Field emergence trials were conducted in the 1992/1993 season, about 60 m from the previous field experimental area, and thus the soil type was the same. The land had previously been grown with peas (1991/1992). It was sprayed with Roundup herbicide on 7 October 1992 (section 3.5.3), and stubble weeds were cleared using a forage harvester three days later. The land was first ploughed on 14 October, and disced on 21 October. Due to rapid weed growth, the block was harrowed and rotary hoed twice before planting on 17 November 1992.

Table 3.2 **Original and fresh harvested super sweet corn seed used in the experiments.**

Seed lot	Year harvested	Description	Code
A5954[1a]	1991	HV_1 stored 5°C; 12.9% SMC; 14 months	A-1
A5954[a]	1991	HV_1 stored 25°C; 11.6% SMC; 14 months	A-2
A5954[a]	1991	LV_1 stored 5°C; 13.7% SMC; 14 months	A-3
A5954[2]	1992	HV_1 fresh harvested stock	B-1
A5954	1992	LV_1 fresh harvested stock	B-2
A5954	1992	LV_{11} (LV_1 overplanted/thinned)	B-3
BE90	1992	HV_2 fresh harvested stock	B-4
BE90	1992	LV_2 fresh harvested stock	B-5
BE90	1992	LV_{22} (LV_2 overplanted/thinned)	B-6
A5954[3]	1992	HV_1 fresh harvested stock	C-1
A5954	1992	LV_1 fresh harvested stock	C-2
A5954	1992	LV_{11} (LV_1 overplanted/thinned)	C-3
BE90	1992	HV_2 fresh harvested stock	C-4
BE90	1992	LV_2 fresh harvested stock	C-5
BE90	1992	LV_{22} (LV_2 overplanted/thinned)	C-6
A5954[4]	1992	HV_1 fresh harvested stock	D-1
A5954	1992	LV_1 fresh harvested stock	D-2
A5954	1992	LV_{11} (LV_1 overplanted/thinned)	D-3
A5954	1992	LV_3 fresh harvested stock	D-4
A5954	1992	LV_{33} (LV_3 overplanted/thinned)	D-5
BE90	1992	HV_2 fresh harvested stock	D-6
BE90	1992	LV_2 fresh harvested stock	D-7
BE90	1992	LV_{22} (LV_2 overplanted/thinned)	D-8
BE90	1992	LV_4 fresh harvested stock	D-9
BE90	1992	LV_{44} (LV_4 overplanted/thinned)	D-10

[1] Original seed lots stored at different conditions.
[2] Fresh harvested seed lots from October planting.
[3] Fresh harvested seed lots from November planting.
[4] Fresh harvested seed lots from December planting.
[a] Samples stored in polyethylene packages.

3.9.2 Weather data

Weather data were obtained from a site 1 km from the trial area, at the AgResearch Grasslands CRI in Palmerston North, New Zealand, and are presented as daily rainfall, evaporation, RH, maximum air temperature and 10 cm soil temperate, for fourteen days from the planting date.

3.9.3 Experimental design and seed lot

As all measurements showed no significant differences between high and low vigour seed lots forl planting dates (section 6.1.1), field emergence trials were carried out to

determine the effects of harvesting dates, rather than vigour differences. Field emergence trials were run using only original and fresh harvested (A1-A3, B1-B3, C1-C3 and D1-D3) HV_1 and LV_1 seed lots. Three replicates of 100 seed from each seed lot were planted in a randomised complete block design of three blocks (Figure 3.2). A total of 36 plots (replicates) of 50 x 70 cm (12 plots per block) were planted on 17 November 1992, each consisting of five rows, 10 cm apart. Plots were covered with a wire mesh cage to prevent bird damage to emerging seedlings. Twenty seeds were sown 2-3 cm deep in each row, and the distance between hills was 3 cm. Seedling emergence was estimated 14 days after planting, by counting the total germinants and results expressed as a percentage.

3.10 DATA ANALYSIS

Statistical Analytical Systems (SAS, 1991) software packages were used for all statistical procedures. Several models of analysis of variance (ANOVA) were employed because different characters had different data structures. Most of the characters were analysed separately (according to a Randomised Complete Block Design (RCBD)) using the general linear model (GLM) procedure, and Duncan's multiple range test or least significance difference (LSDs) were used to compare the means. Alternatively, mean values of the parameters along with the standard errors of each mean were calculated, and the T-test procedure of SAS was used to distinguish the means.

The different seed lots and planting dates were regarded as a RCBD factorial experiment, and subjected to analysis of variance by the use of the GLM procedure to determine the significance of differences of the main factors of vigour levels and planting dates and the interaction between them. Mean treatment values were distinguished using the least squares means of effect of vigour level planting date or a pooled least significance difference (LSDs) at $P = 0.05$. Data from the different characters from the December planting were anlaysed separately and treatment mean comparisons were performed by using least significant differences (LSDs) at $P = 0.05$. Simple regression and correlation analysis was used in determining the relationship between seed quality characters and field emergence. Graphs were prepared using AutoCAD Version 12 computer software and tables were made using WordPerfect Version 5.1 software.

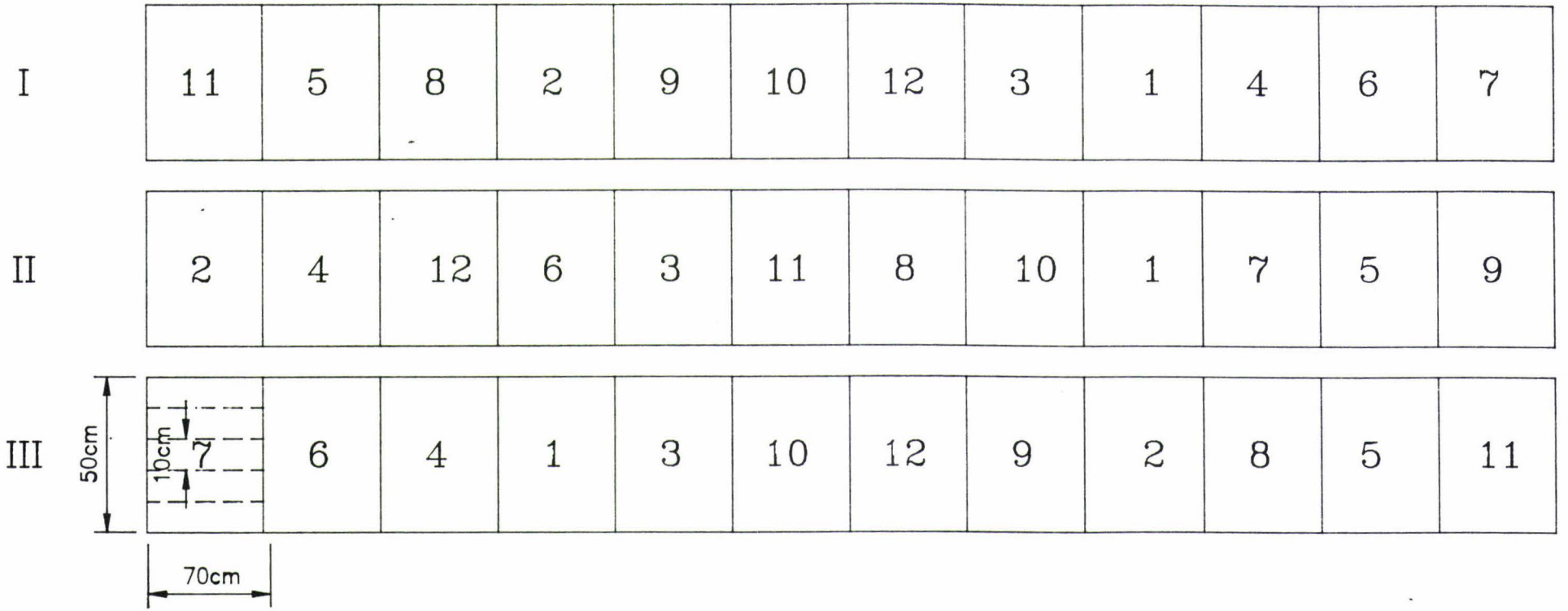


Figure 3.2 **The field layout for emergence trials conducted in the 1992/1993 season.**

CHAPTER 4

RESULTS

4.1 EXPERIMENT ONE

For ease of presentation and reference, a summary of results is presented in Table 4.1. These results are detailed in the following sections.

Table 4.1 Summary of results in experiment one.

Seed lots	Test conducted	Testing conditions*	Differential performance	References
HV_1 and HV_2	TSW[1] and MD[2]		Significant	Table 4.2
HV_1, LV_1, LV_3 HV_2, LV_2, LV_4	Standard germination test	Optimum conditions	Significant	Figure 4.1
HV_1, LV_1, LV_3 HV_2, LV_2, LV_4	Soil cold test	Pathogens, water and temperature stresses	Not significant	Table 4.3 Table 4.4
HV_1, LV_1, LV_3 HV_2, LV_2, LV_4	Seedling growth test	Optimum conditions	Significant	Table 4.5
HV_1, LV_1, LV_3 HV_2, LV_2, LV_4	Complex stress vigour test	Water and temperature stresses	Significant	Table 4.6

[1] TSW = Thousand seed weight
[2] MD = Mechanical damage
* For details of test methods, refer section 3.4.1.

4.1.1 Seed moisture content, thousand seed weight and mechanical damage levels

Initial seed moisture contents (SMC) of the different seed lots are shown in Table 3.1. SMC was generally low in all seed lots, ranging from 12.2 to 15.3%, with the high vigour (HV_1 and HV_2) seed lots having the lowest SMC of 13.2 and 12.2%, respectively. However, through the exposure of these dry seed lots to high humidity (>90% RH) at 42°C for a short period of time (24 hours) during the accelerating ageing treatment, the low vigour (LV_1 and LV_2) seed lots absorbed moisture and had SMC of 15.3 and 14.9%, an increase of approximately 15 and 22%, respectively. By extending the incubation temperature to 45°C at >90% RH for the same time, the corresponding low vigour (LV_3 and LV_4) seed lots attained SMC of 15.0 and 15.2%, an increase of about 14 and 25%, respectively.

The HV_1 and HV_2 seed lots differed in their thousand seed weight (TSW) and mechanical damage level (Table 4.2). There was a small but significantly ($P < 0.05$) greater TSW in seed lot HV_1 (162 g) than in seed lot HV_2 (138 g). Although seed lot HV_1 had a higher SMC (13.2%) than HV_2 (12.2%), this difference did not account for the differences in TSW because the thousand seed dry weight in seed lot HV_1 (140.4 g) was also significantly ($P < 0.05$) greater than that of seed lot HV_2 (121.3 g). Visible mechanical damage levels were significantly ($P < 0.05$) greater in seed lot HV_1 (66%) than in seed lot HV_2 (52%). The type of damage in these seed lots ranged from spots and parts of the pericarp or endosperm missing, collapsed or cracked pericarps to pest damaged and broken seeds. However, the most predominant type of injury were spots, portions of the pericarp missing, and collapsed and cracked pericarps (Plate 4.1 a,b).

Table 4.2 **Thousand seed weight and mechanical damage levels of different seed lots of super sweet corn (cv. Illini Gold). Data are means of eight or three replicates of 100 seeds. Figures in brackets are a thousand seed dry weight (g).**

Seed lot	Thousand seed weight (g)	Mechanical damage (%)
HV_1	162[1] ±[4] 3.85[a3] (140.4[a])[2]	66 ± 3.24[a]
HV_2	138 ± 5.59[b] (121.3[b])	52 ± 2.65[b]
lsd (0.05)	5.15 (2.43)	6.73

[1] At ambient SMC
[2] At 0% SMC
[3] Mean values in the same column followed by the same letter are not significantly different at $P < 0.05$, according to the lsd test.
[4] ± = standard deviation of individual means.

4.1.2 Standard germination test

Standard germination test results for the different seed lots are presented in Figure 4.1a,b. Normal seedlings showed a clear cut differential performance between unaged (high vigour) and aged (low vigour) seed lots. High vigour (HV_1 and HV_2) seed lots did not differ ($P < 0.05$) for normal seedlings (88% in seed lot HV_1 and 86% in seed lot HV_2 (Figure 4.1a)). However, the harsh treatment imposed by the artificial ageing reduced germinability significantly ($P < 0.05$) to below 70% in low vigour (ie LV_1, LV_2, LV_3 and LV_4) seed lots with LV_4 (50%) seed lot being significantly ($P < 0.05$) lower than the rest (58-68%). This reduction in normal seedlings following accelerating ageing was because of a corresponding increase in levels of abnormal seedlings, rather than dead seeds. While high vigour seed lots yielded a significantly lower number (about 10% only) of abnormal seedlings, low vigour

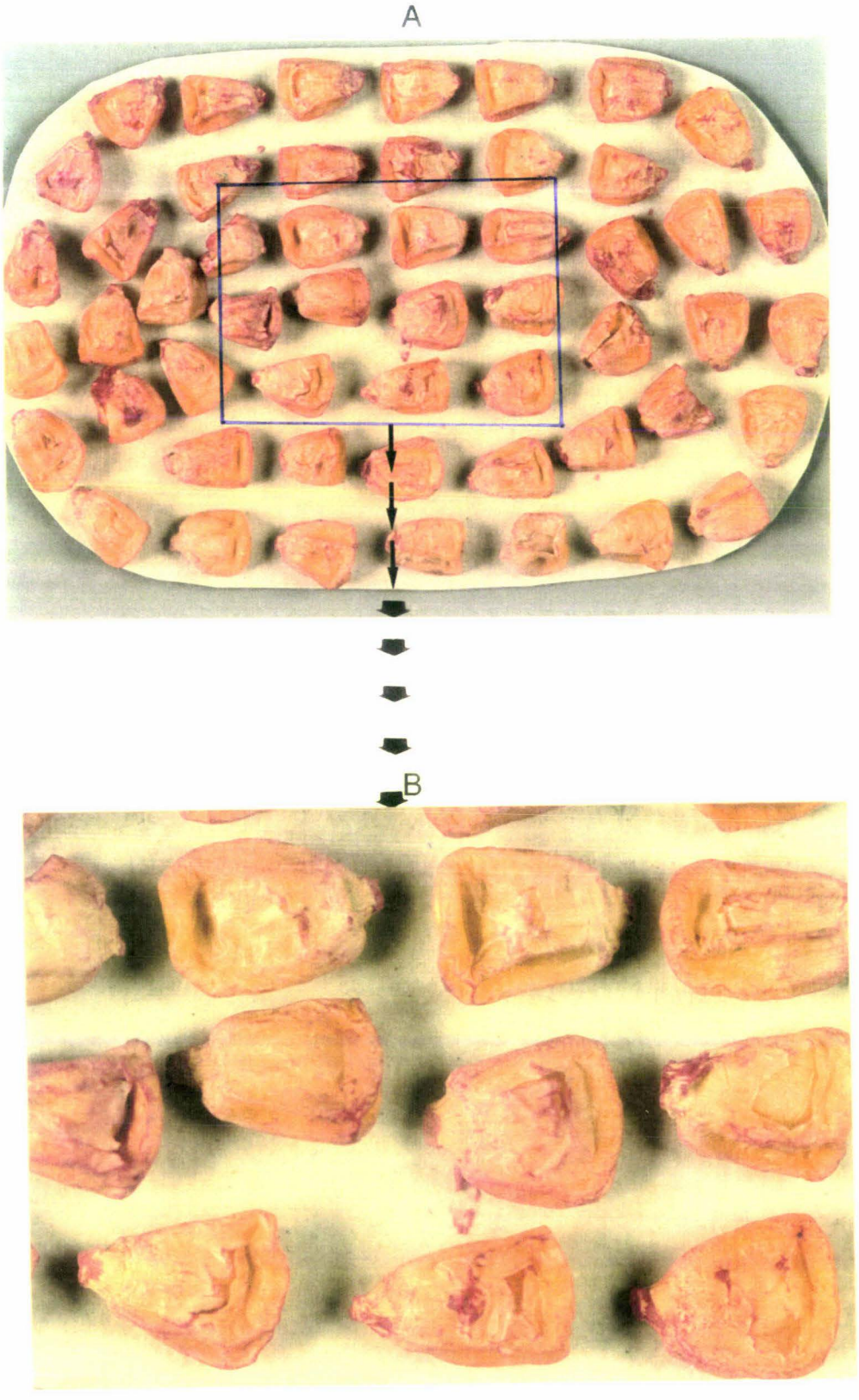


Plate 4.1a,b Photographs showing some of the mechanical damage.

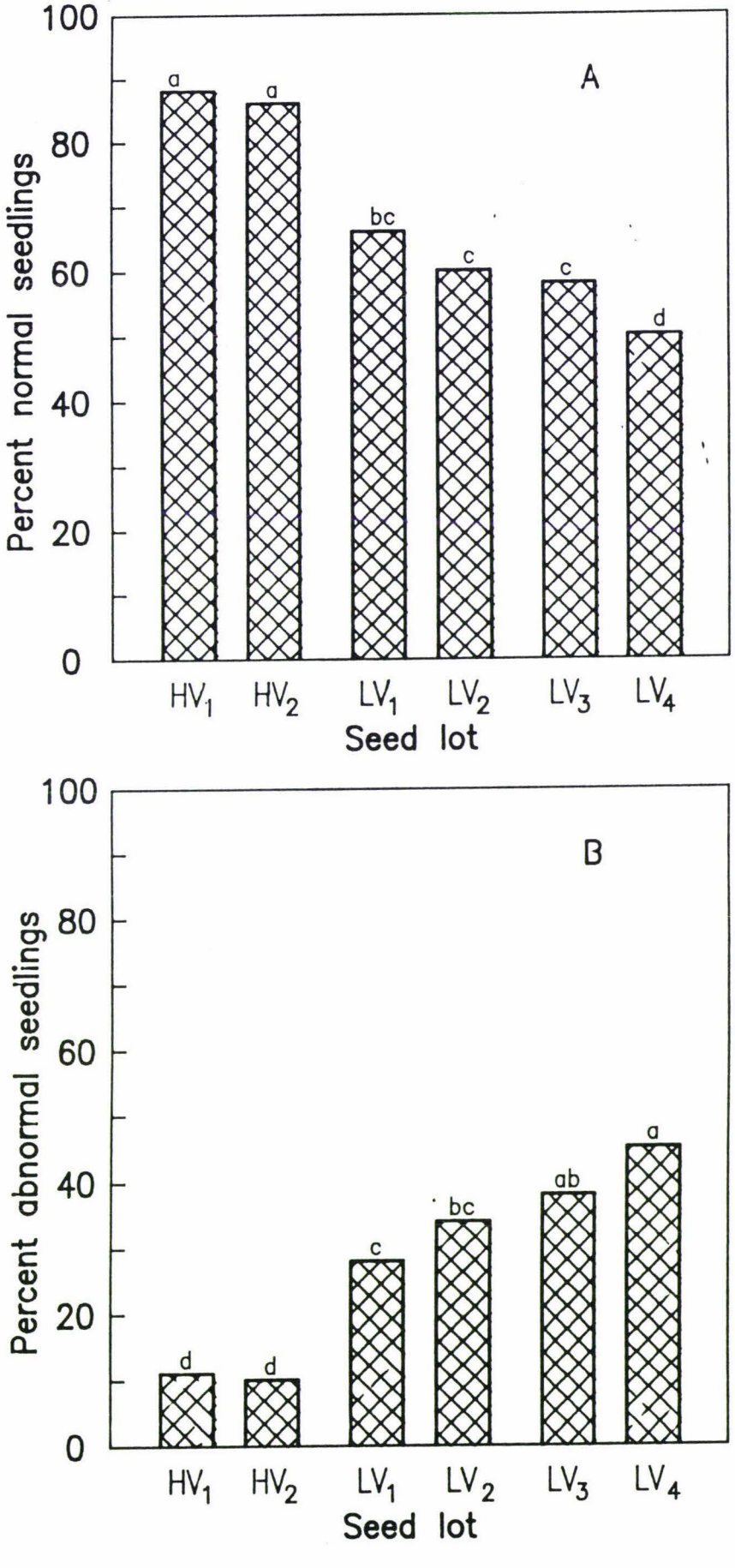


Figure 4.1 Percentage normal (4.1a) and abnormal (4.1b) seedlings of unaged and aged seed lots of super sweet corn (cv. Illini Gold) from the standard germination test. Data are means of four replicates. Values capped by the same letter are not significantly different at $P < 0.05$.

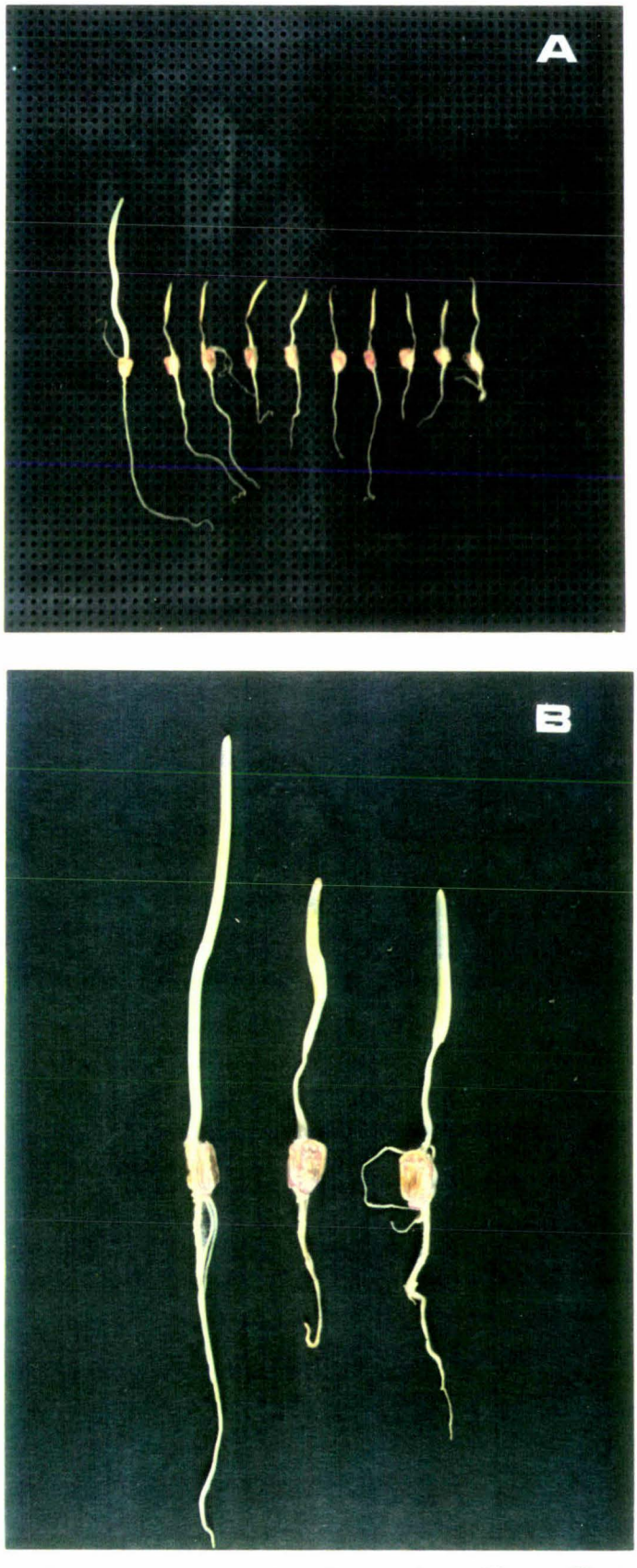
seed lots produced considerably more, ranging from 28-45%, with seed lot LV_1 having the lowest (24%) and seed lot LV_4 the highest (45%). LV_1 (28%) and LV_2 (34%) or LV_3 (38%) and LV_4 (45%) did not differ ($P < 0.05$) significantly (Figure 4.1b).

The type of seedling abnormalities included decayed seedlings resulting from fungal infection. These seedlings showed a severe rotting of shoot bases, covering mostly the whole of the mesocotyl and part of the coleoptile areas, and occasionally the root system (Plates 4.2 a,b). This category of abnormality was the most predominant, contributing 61 to 74% of the abnormal seedlings in low vigour seed lots, and up to 52% in high vigour seed lots. The other types of seedling abnormality recorded resembled that resulting from physiological or biochemical deterioration as a consequences of interactions of diseases, adverse storage conditions or mechanical damage, eg deformed or unbalanced development, stunted seedlings etc.

4.1.3 Soil cold test

The previous test (standard germination test) showed a clear differential performance between high vigour (unaged) and low vigour (aged) seed lots under optimum conditions (Figure 4.1a). In contrast, in the soil cold test, which was conducted under unfavourable conditions, there were no significant differences ($P < 0.05$) in percentage normal seedlings among high and low vigour seed lots (Table 4.3). Even the classification of normal seedlings into strong, slightly strong and weak seedlings indicated that almost all the seedlings fell in to the "strong seedlings" group, and as in normal seedlings, there was no significant difference ($P < 0.05$) in strong seedlings among high and low vigour seed lots (Table 4.3).

There was no consistent difference in abnormal seedlings and dead seeds among high and low vigour seed lots. The levels of abnormal seedlings were generally low (up to 15%), with seed lot HV_1 having the highest (15%) and LV_3 having the lowest (3%), while seed lots HV_2 and LV_1, LV_2 and LV_4 or HV_1, HV_2 and LV_2 were not significantly ($P < 0.05$) different (Table 4.3). Dead seed ranged from 7-9% in the high vigour seed lots and 14-24% in the low vigour seed lots, although HV_2, LV_1 and LV_2 did not differ significantly ($P < 0.05$).



Plates 4.2a,b Photographs showing diseased seedlings. The seedling on the left in each photograph is a healthy seedling.

Table 4.3 **Percentage normal, high vigour and abnormal seedlings and dead seeds of unaged and aged seed lots of super sweet corn (cv. Illini Gold) from the soil cold test. Data are means of four replicates.**

Seed lot	% Normal seedlings	% Abnormal seedlings	% Dead seeds	% High vigour seedlings
HV_1	78^{a}	15^{a}	7^{d}	75^{a}
HV_2	80^{a}	11^{ab}	9^{cd}	77^{a}
LV_1	77^{a}	9^{b}	14^{bcd}	72^{a}
LV_2	74^{a}	10^{ab}	16^{abc}	73^{a}
LV_3	73^{a}	3^{c}	24^{a}	69^{a}
LV_4	73^{a}	7^{bc}	20^{ab}	71^{a}
lsd (0.05)	9.40	5.39	8.01	9.01

Means values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, based on Duncan's multiple range test.

The comparison of the soil cold test results (Table 4.3) with that of the standard germination test results (Figure 4.1) was of particular interest. Although more dead seeds were recorded in the soil cold test in low vigour seed lots (up to 24%), and there was a minor reduction in normal seedlings (up to 10%) in high vigour seed lots, there was a considerable increase in normal seedlings in low vigour seed lots (up to 46%) because abnormal seedlings were reduced.

Shoot length and normal seedling dry weight from this test are shown in Table 4.4. There was no significant difference ($P < 0.05$) in shoot length between high vigour (10.30 - 10.60 cm) and low vigour (9.75 - 10.10 cm) seed lots. As for shoot length, shoot dry weight also did not differ significantly ($P < 0.05$) among seed lots.

Table 4.4 **Shoot length (cm normal seedling^{-1}) and shoot dry weight (mg normal seedling^{-1}) of unaged and aged seed lots of super sweet corn (cv. Illini Gold) from the soil cold test. Data are means of four replications.**

Seed lot	Shoot length (cm normal seedling^{-1})	Shoot dry weight (mg normal seedling^{-1})
HV_1	10.60[a]	28.38[a]
HV_2	10.30[a]	28.58[a]
LV^1	9.93[a]	28.05[a]
LV_2	10.10[a]	28.57[a]
LV_3	9.75[a]	28.13[a]
LV_4	9.75[a]	28.68[a]
LSD (0.05)	1.01	3.30

Mean values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

4.1.4 Seedling growth test

Seedling growth analysis results (root dry weight, shoot length and shoot dry weight) are shown in Table 4.5. The root dry weights of high vigour (HV_1 and HV_2) seed lots did not differ ($P < 0.05$) (6.18 versus 6.21 mg normal seedling^{-1}). Low vigour (LV_1 and LV_2) seed lot dry weights (5.83 and 5.89 mg normal seedling^{-1}), did not differ, respectively, and also did not differ significantly from those of high vigour seed lots. However, the ability of the root system of low vigour (LV_3 and LV_4) seed lots to grow and deposit dry mass was significantly ($P < 0.05$) inferior from those of all other lots as demonstrated by the lowest root dry weights of 4.37 and 4.50 mg normal seedling^{-1}, respectively (Table 4.5).

Unlike the root system, the shoot system, assessed as shoot length and shoot dry weight differed in performance between unaged and aged seed lots. Both shoot length and shoot dry weight declined as the severity of the ageing conditions increased (Table 4.5), ie HV_1 and HV_2 > LV_1 and LV_2 > LV_3 and LV_4. Within vigour classes, seed lots did not differ.

Table 4.5 **Shoot length (cm normal seedling^{-1}), root and shoot dry weights (mg normal seedling^{-1}) of unaged and aged seed lots of super sweet corn (cv. Illini Gold) from the seedling growth test. Data are means of four replications.**

Seed lot	Root dry weight (mg normal seedling^{-1})	Shoot length (cm normal seedling^{-1})	Shoot dry weight (mg normal seedling^{-1})
HV_1	6.18^a	8.25^a	27.69^a
HV_2	6.21^a	8.01^a	28.04^a
LV_1	5.83^a	6.50^{bc}	22.99^b
LV_2	5.89^a	7.00^b	23.35^b
LV_3	4.37^b	6.10^c	17.37^c
LV_4	4.50^b	5.89^c	14.78^c
lsd (0.05)	0.72	0.87	3.63

Mean values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

An interesting observation was that although the seedling growth test was done under optimum conditions, shoot lengths and shoot dry weights were much lower than those produced from the soil cold test (Table 4.4). Shoot length in high vigour seed lots was lower by over 20% in the seedling growth test, and shoot length in low vigour seed lots was decreased by up to 40%. Although shoot dry weight for high vigour seed lots remained more or less constant, low vigour seed lot shoot dry weights dropped considerably (by 48%).

4.1.5 Complex stressing vigour test

The results from the complex stress vigour testing (Table 4.6) indicated that high vigour seed lots (HV_1 and HV_2) had a significantly greater germination than low vigour seed lots, but germination by low vigour seed lots did not differ, ranging from 40-46%. Abnormal seedlings were similar among seed lots, ranging from 21-24%, except for the LV_2 (15%) and LV_4 (16%) seed lots which showed significantly ($P < 0.05$) lower levels than the rest. Dead seeds were significantly higher in low vigour seed lots than in high vigour seed lots. Grouping of normal seedlings into high, medium and low vigour seedlings showed that almost all the normal seedlings were of high vigour, with seed lots HV_1 and HV_2 showing a significantly ($P < 0.05$) higher values of 53 and 54%, respectively, compared to 38-41% in low vigour seed lots.

Table 4.6 **Percentage normal, abnormal, and high vigour seedlings and dead seeds of unaged and aged seed lots of super sweet corn (cv. Illini Gold) from complex stress vigour test. Data are means of four replications.**

Seed lot	% Normal seedlings	% Abnormal seedlings	% Dead seeds	% High vigour seedlings
HV_1	61^a	21^{ab}	18^b	53^a
HV_2	57^a	24^a	19^b	54^a
LV_1	40^b	21^{ab}	39^a	40^b
LV_2	46^b	15^b	39^a	41^b
LV_3	41^b	21^{ab}	38^a	39^b
LV_4	43^b	16^b	41^a	40^b
lsd (0.05)	7.47	7.29	8.71	6.28

Mean values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, according to Duncan's multiple range test.

Normal seedling percentages were lower for all seed lots in this test than for the two other tests, mainly because the conditions of the complex stress vigour test killed more seeds.

4.2 EXPERIMENT TWO

In Experiment One, the influence of fungi on test results became evident. Contrasting results were recorded because tests done at favourable temperatures showed a clear differential of seed lot performance, while those conducted at unfavourable low temperatures showed no germinative/vigour differences among seed lots. Accordingly, another series of germinative and health tests were established to identify and estimate the prevalence and importance of the disease-causing fungi, and to confirm or investigate further the observed differences/similarities. Additionally, since germination and seedling growth are the end results of a series of biochemical and physiological changes in seeds, biochemical/physiological approaches were employed so as to gain a closer look at the germinability or vigour differences/similarities recorded in the previous experiment.

4.2.1 Seed health testing

The incidence of fungi in both high vigour (HV_1 and HV_2) and low vigour (LV_1 and LV_2) seed lots is presented in Table 4.7. All seed lots were heavily contaminated with *Fusarium subglutinans* (60-82%) and also contained lower levels of *Penicillium* (6-8%) and *Rhizopus* (8-20%) species. Although seed lots LV_1 and LV_2 were subjected to high temperatures (42°C) during the accelerating ageing, there was no significant difference in infection among seed lots.

Table 4.7 Percentage of fungal infection from different seed lots of super sweet corn (cv. Illini Gold). Data are means of five replications of twenty seeds.

Seed lot	*F. subglutinans* (%)	*Penicillium* spp. (%)	*Rhizopus* spp. (%)
HV_1	60 ± 8.00[a]	8 ± 5.83[a]	18 ± 5.83[a]
HV_2	72 ± 9.70[a]	6 ± 4.00[a]	12 ± 3.74[a]
LV_1	82 ± 5.83[a]	6 ± 4.00[a]	8 ± 3.74[a]
LV_2	76 ± 9.80[a]	6 ± 4.00[a]	20 ± 5.48[a]
lsd 0.05	25.44	13.57	14.38

Mean values in the same column followed by the same letter superscript are not significantly different at lsd 0.05 level. ± are standard errors of individual means.

4.2.2 Cold germination test

Cold germination test results are shown in Table 4.8. Seed lots did not differ significantly ($P < 0.05$) with the exception of seed lot LV_4 which had a significantly lower germination (63%) than seed lots of high vigour (71-72%). Neither abnormal seedlings (17-24%) nor dead seeds (5-13%) differed significantly ($P < 0.05$) among seed lots.

A comparison of these results with that of the soil cold test (section 4.1.3, Table 4.3) clearly showed that although the test conditions counterbalanced the differences between high and low vigour seed lots, there was a minor but noticeable decrease in normal seedlings (5-12%) and dead seeds decreased by 19-64% while abnormal seedlings increased by over 80% in some seed lots.

Table 4.8 **Percentage normal, abnormal and dead seeds of different seed lots of super sweet corn (cv. Illini Gold) from cold germination test. Data are means of four replicates.**

Seed lot	% Normal seedlings	% Abnormal seedlings	% Dead seeds
HV_1	72^a	17^a	11^a
HV_2	71^a	19^a	10^a
LV_1	71^a	24^{ab}	5^a
LV_2	70^{ab}	17^a	13^a
LV_3	65^{ab}	24^a	11^a
LV_4	63^b	24^a	13^a
lsd (0.05)	8.00	8.39	7.78

Mean values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, based on lsd test.

Root and shoot dry weight of normal seedlings from the cold germination test (Table 4.9) also did not differ significantly ($P < 0.05$) between high and low vigour seed lots. Root dry weight ranged from 4.18 to 5.28 mg normal seedling^{-1}, while that of shoot dry weight was 17.97 to 22.97 mg normal seedling^{-1}. Over all, shoot dry weight from this test was lower by over 30% compared to that of the soil cold test.

Table 4.9 **Root and shoot dry weight (mg normal seedling^{-1}) from different seed lots of super sweet corn (cv. Illini Gold) from the cold germination test. Data are means of four replicates.**

Seed lot	Root dry weight (mg normal seedling^{-1})	Shoot dry weight (mg normal seedling^{-1})
HV_1	4.80^{ab}	22.32^a
HV_2	5.28^a	22.79^a
LV_1	4.72^{ab}	20.69^a
LV_2	5.24^a	21.85^a
LV_3	4.18^b	17.97^a
LV_4	5.15^a	21.15^a
lsd 0.05	0.82	6.60

Mean values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, based on lsd test.

4.2.3 Soak germination test

The soak germination test results for seeds soaked for 24 or 48 hours at 10°C are presented in Appendix 4.1a,b. Soaking of seeds for 24 hours resulted in a dramatic reduction in normal seedlings (Appendix 4.1a). High vigour seed lots (HV_1 and HV_2) did not differ (about 33% normal seedlings) but had significantly ($P < 0.05$) more normal seedlings than that of deteriorated (LV_1, LV_2, LV_3 and LV_4) seed lots, which ranged from 9-19, with LV_4 having a significantly ($P < 0.05$) lower percentage of normal seedlings than the rest. Abnormal seedlings and dead seeds were generally high ranging from 22-34% and 51-66%, respectively (data not shown).

Prolonging the soaking period to 48 hours caused a further reduction in normal seedlings in high vigour seed lots (24-26%) with minor changes in normal seedlings in low vigour seed lots (Appendix 4.1b). The differential performance between high and low vigour seeds remained significant ($P < 0.05$). The levels of abnormal seedlings were almost similar in all seed lots, ranging from 15-25%, while dead seeds were significantly higher (64-69%) in low vigour seed lots than in high vigour seed lots (49-55%) (data not shown).

Percentage normal seedlings, abnormal seedlings and dead seeds for the seeds soaked for 24 or 48 hours at 25°C are shown in Tables 4.10 and 4.11. Soaking of seeds for 24 hours at 25°C (Table 4.10) showed higher levels of normal seedlings compared to that soaked at 10°C (Appendix 4.1a), but high vigour (HV_1 and HV_2) seed lots had a significantly ($P < 0.05$) higher number of normal seedlings compared to low vigour seed lots. Abnormal seedlings, which ranged from 22-35%, were not significantly different ($P < 0.05$), but dead seeds were significantly lower in high vigour seed lots than in low vigour seed lots. Surprisingly, following soaking of seeds for a longer period of time (48 hours) at 25°C, no significant differences were found between high and low vigour seed lots, except for seed lots LV_1 and LV_3 which differed significantly (Table 4.11). Although normal seedlings in high vigour seed lots declined by around 20%, there was an increase in normal seedling in low vigour seed lots. Abnormal seedlings were similar, although seed lot HV_1 had a significantly higher number of abnormal seedlings than seed lot LV_4. Dead seeds were also the same, although seed lot LV_4 recorded a significantly higher level than seed lot HV_1 or HV_2.

Table 4.10 **Percentage normal and abnormal seedlings, and dead seeds from different seed lots of super sweet corn (cv. Illini Gold) soaked for 24 hrs at 25°C. Data are means of three replications.**

Seed lot	% Normal seedlings	% Abnormal seedlings	% Dead seeds
HV_1	70^{a}	26^{a}	4^{c}
HV_2	73^{a}	22^{a}	5^{c}
LV_1	53^{b}	23^{a}	24^{ab}
LV_2	50^{b}	24^{a}	26^{a}
LV_3	48^{b}	29^{a}	23^{ab}
LV_4	45^{b}	35^{a}	20^{ab}
lsd (0.05)	9.89	14.47	10.48

Mean values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, based on lsd test.

Table 4.11 **Percentage normal and abnormal seedlings, and dead seeds from different seed lots of super sweet corn (cv. Illini Gold) soaked for 48 hrs at 25°C. Data are means of three replications.**

Seed lot	% Normal seedlings	% Abnormal seedlings	% Dead seeds
HV_1	55^{ab}	27^{a}	18^{b}
HV_2	58^{ab}	23^{ab}	19^{b}
LV_1	59^{a}	20^{ab}	21^{ab}
LV_2	56^{ab}	21^{ab}	23^{ab}
LV_3	53^{b}	24^{ab}	23^{ab}
LV_4	57^{ab}	16^{b}	27^{a}
lsd (0.05)	5.69	10.67	7.11

Mean values in the same column followed by the same letter superscript are not significantly different at $P < 0.05$, based on lsd test.

4.2.4 Electrical conductivity test

The time courses of electroconductivity measurements at either 10°C or 25°C from unaged (HV_1) and aged (LV_1 and LV_3) seed lots are shown in Figures 4.2 and 4.3. Solute leakage at low (10°C) temperature in all seed lots was initially very low (3.38-3.68 μsg^{-1} seed), and increased with soaking time. There was no significant difference ($P < 0.05$) among seed lots for the first 24 hours. After 36 hours of soaking high vigour (HV_1) seeds had leaked significantly ($P < 0.05$) less (11.59 μsg^{-1} seed) than low vigour seed lots (15.54-16.07 μsg^{-1} seed). By 48 hours, seed lot LV_3 had leaked a significantly higher level of electrolytes (22.88 μsg^{-1} seed) than the rest, but seed lots HV_1 and LV_1 released similar amounts of solute (18.46 vs 20.42 μsg^{-1} seed). Electroconductivity at 25°C (Figure 4.3) was much higher than that observed at 10°C (Figure 4.2). As in conductivity recorded at 10°C, initial solute leakage at 25°C was low (5.32-5.69 μsg^{-1} seed) and increased with time (Figure 4.3). No significant differences were recorded between high and low vigour seeds for the first 12 hours. Differences in leakage became apparent after 12 hours and by 24 hours, seed lot HV_1 leaked a significantly ($P < 0.05$) lower (19.62 μsg^{-1} seed) amount of leachate than seed lots LV_1 (24.05 μsg^{-1} seed) and LV_3 (26.22 μsg^{-1} seed). However, these differences diminished and by 36 hours conductivity did not differ among seed lots (range from 27.10-27.69 μsg^{-1} seed).

4.2.5 Imbibitional conductivity

Allowing seeds to imbibe at low (10°C) temperature for 24 hours before soaking for conductivity at 25°C for 24 hours produced varying results. No significant difference ($P < 0.05$) in conductivity was recorded between high vigour (16.90 μsg^{-1} seed) and low vigour (25.24 μsg^{-1} seed) seeds following imbibition (Figure 4.4a). However, conductivity levels decreased significantly ($P < 0.05$) in high vigour (HV_1) seeds from 19.62 μsg^{-1} seed in unimbibed seeds to 16.90 μsg^{-1} seed in imbibed seed (Figure 4.4a). There was no significant difference ($P < 0.05$) in solute leakage in low vigour seeds between unimbibed (24.05 μsg^{-1} seed) and imbibed (25.24 μsg^{-1} seed) seeds. Interestingly, imbibition for 48 hours before soaking for 24 hours offset the difference in conductivity between high (16.92 μsg^{-1} seed) and low (19.88 μsg^{-1} seed) seed lots. A significant ($P < 0.05$) decrease in electrolyte leakage was evident in imbibed high vigour (16.92 μsg^{-1} seed) compared with unimbibed (19.62 μsg^{-1} seed) seeds. Similarly, low vigour imbibed seeds leaked significantly ($P < 0.05$) less solute (19.88 μsg^{-1} seed) than unimbibed (24.05 μsg^{-1} seed) seeds (Figure 4.4b). However, imbibition for 48 hours before soaking for 48 hours at 25°C showed no advantage of the treatment as illustrated by higher solute leakage than for seeds soaked for 24 hours

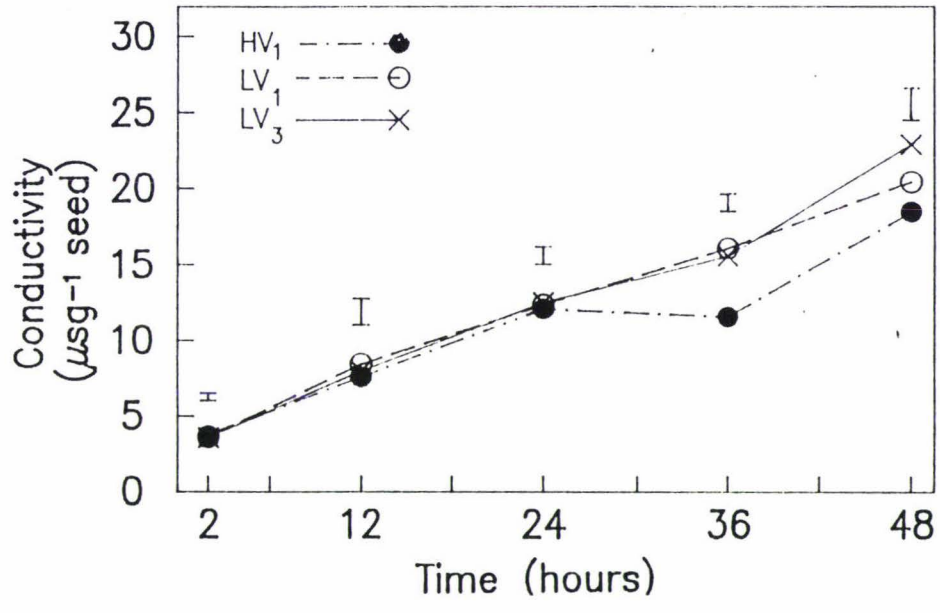


Figure 4.2 Time course of electrolyte leakage (μsg^{-1} seed) from different seed lots of super sweet corn (cv. Illini Gold) recorded at 10°C. Data are means of three replicates. Bar represents least significance different (P = 0.05) among any three means.

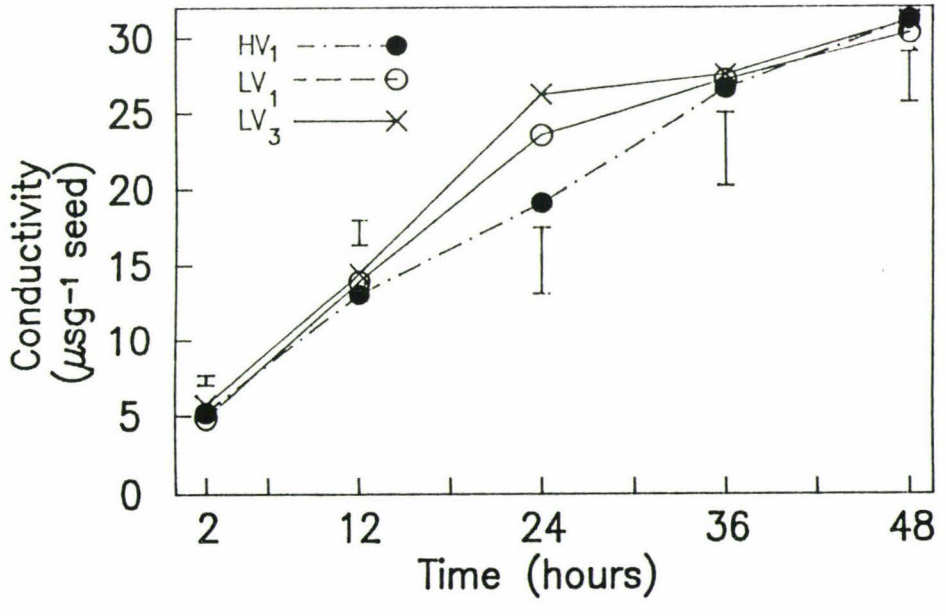


Figure 4.3 Time course of electrolyte leakage (μsg^{-1} seed) from different seed lots of super sweet corn (cv. Illini Gold) recorded at 25°C. Data are means of three replicates. Bar represents least significance different (P = 0.05) among any three means.

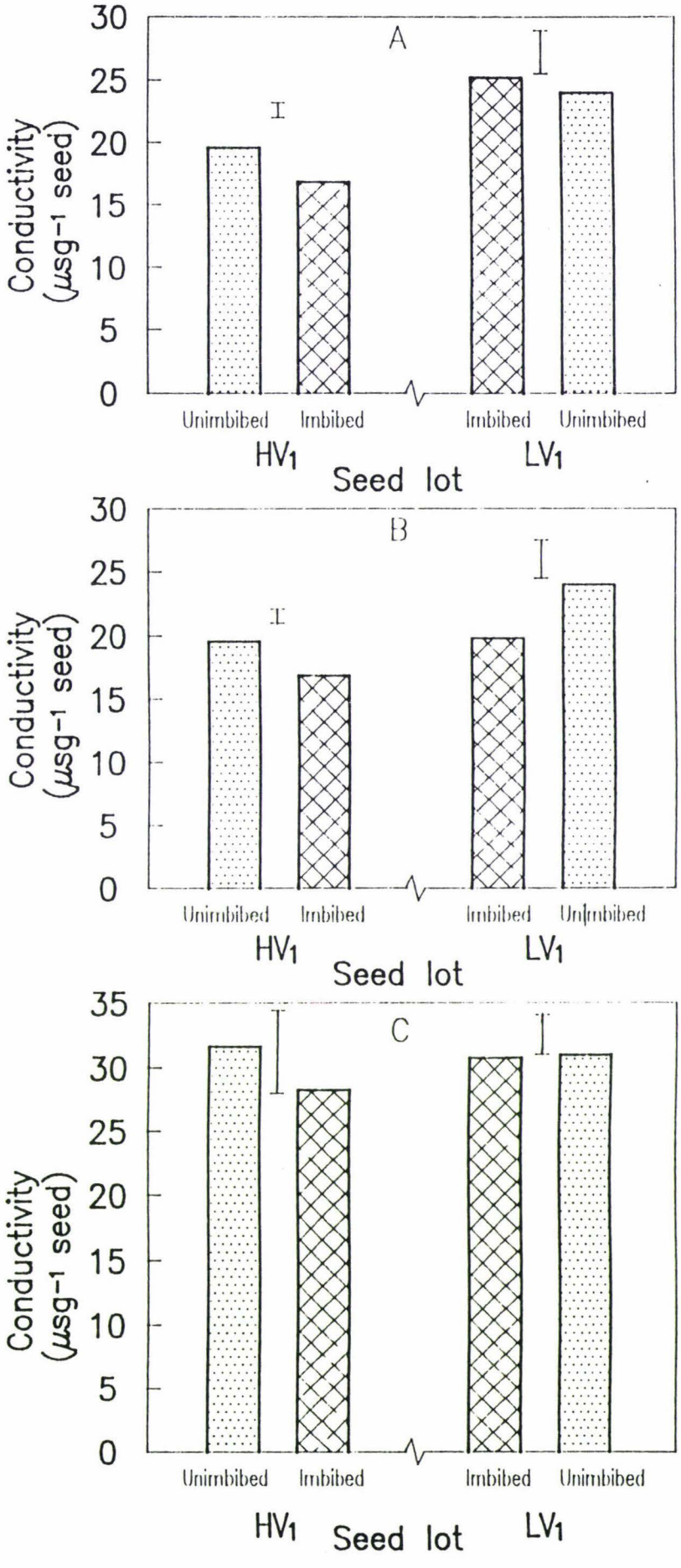


Figure 4.4 **Electrolyte leakage from unaged and aged seeds of super sweet corn (cv. Illini Gold) imbibed at 10°C for 24 h and leakage measured 24 h at 25°C (a), seeds imbibed at 10°C for 48 h and leakage measured 24 h at 25°C (b), seeds imbibed at 10°C for 48 h and leakage measured 48 h at 25°C (c). Bar represents least significant (P = 0.05) between any two means. Data are means of three replicates.**

(Figure 4.4b) and similarity in conductivity readings between high and low vigour seed lots (Figure 4.4c). As for unimbibed seeds, there was no significant difference ($P < 0.05$) in conductivity levels between imbibed high (28.30 μsg^{-1} seed) and low (30.99 μsg^{-1} seed) vigour seed lots (Figure 4.4c). Electro-conductivity from imbibed high (28.30 μsg^{-1} seed) or low (30.99 μsg^{-1} seed) vigour lots did not differ significantly with that of unimbibed high (31.67 μsg^{-1} seed) and low vigour (30.78 μsg^{-1} seed) seeds.

4.2.5.1 Germinability of seeds imbibed at 10°C for 24 or 48 hours before soaking at 25°C for 24 or 48 hours

Germination of seeds imbibed/soaked in Figures 4.4a-c was assessed at the end of the conductivity measurements and results collated with those unimbibed but soaked for the same period of time. Normal seedlings from seeds imbibed at 10°C for 24 hours and then soaked for 24 hours at 25°C showed no significant difference ($P < 0.05$) between high vigour (HV_1) imbibed (67%) and unimbibed (70%) lots, while imbibed low vigour (LV_1) attained a significantly lower (35%) germination than unimbibed (50%) lots (Figure 4.5a). Following imbibition for 48 hours and soaking for 24 hours at 25°C, percentage normal seedlings differed significantly between imbibed high (68%) and low (53%) seed lots (Figure 4.5b). Neither imbibed high vigour (68%) nor low vigour (53%) lots differed significantly with their counterpart unimbibed high vigour (70%) and low vigour (50%) lots. Soaking of seeds for 48 hours following imbibition for 48 hours produced no significant difference between imbibed high (61%) and low (46%) vigour seed lots (Figure 4.5c). Imbibed high vigour seeds yielded a significantly ($P < 0.05$) greater (61%) germination (normal seedlings) than unimbibed seeds (52%). Conversely, imbibed low vigour seeds produced a significantly lower (46%) germination than unimbibed (54%) seeds.

4.2.6 Respiration and tetrazolium tests

4.2.6.1 Oxygen uptake

The time courses of respiration measurements at optimal (25°C) and stressful (10°C/25°C) temperatures are shown in Figure 4.6a,b. Gas exchange assessments at 25°C (Figure 4.6a) indicated that oxygen uptake in both high and low vigour seeds was generally high throughout the imbibition period (8-48 hours). Seed imbibed for 8 hours consumed 12.80 and 16.91 $\mu l/seed^{-1}$ $hour^{-1}$ for high and low vigour lots, respectively. The rate of oxygen uptake increased with increasing imbibition time, and by 48 hours, high vigour seeds were able to consume 160.14 $\mu l/seed^{-1}$ $hour^{-1}$, while low vigour seeds took up 146.24 $\mu l/seed^{-1}$ $hour^{-1}$. There was no significant difference in respiratory metabolism between high and low vigour seeds during the early stage of imbibition as demonstrated by similarities

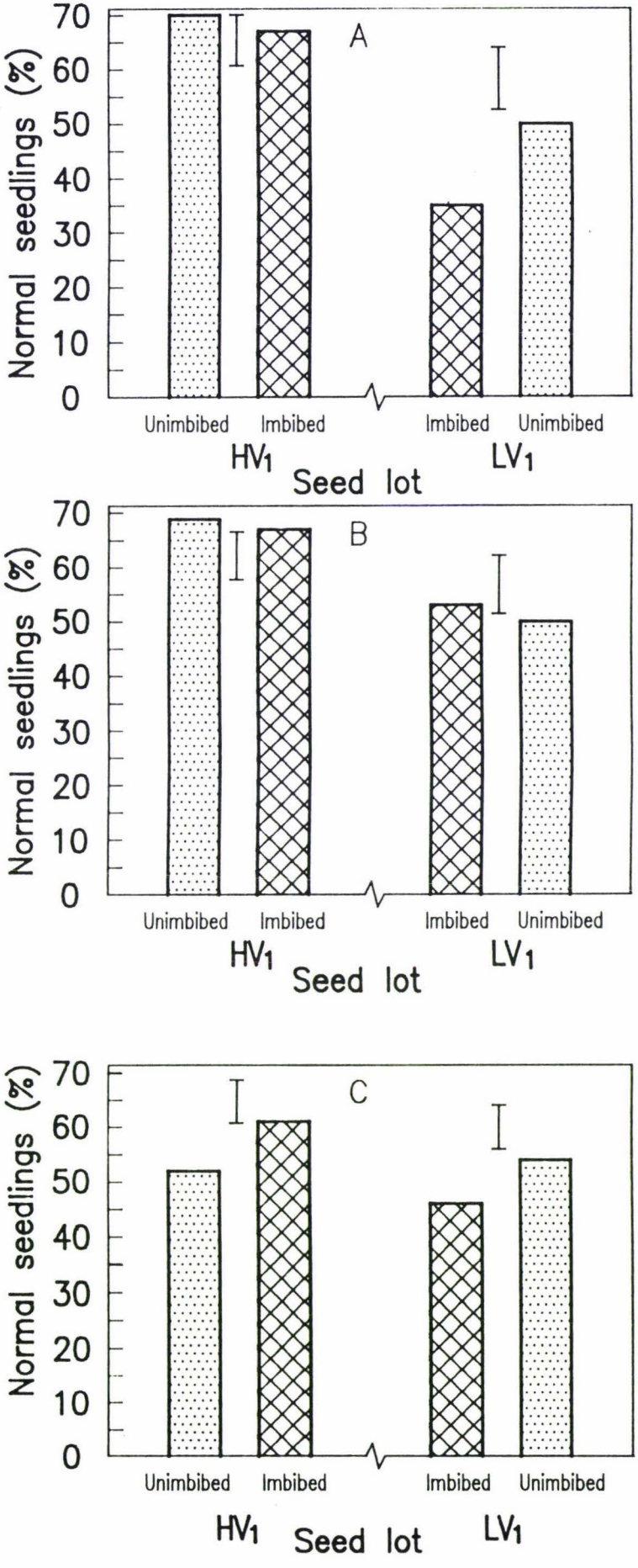


Figure 4.5 **Germinability of super sweet corn (cv. Illini Gold) seeds imbibed at 10°C for 24 h and soaked for 24 h at 25°C (a), seeds imbibed at 10°C for 48 h and soaked for 24 h at 25°C (b), seeds imbibed at 10°C for 48 h and soaked for 48 h at 25°C (c). Bar represents least significance (P = 0.05) between any two means. Data are means of three replicates.**

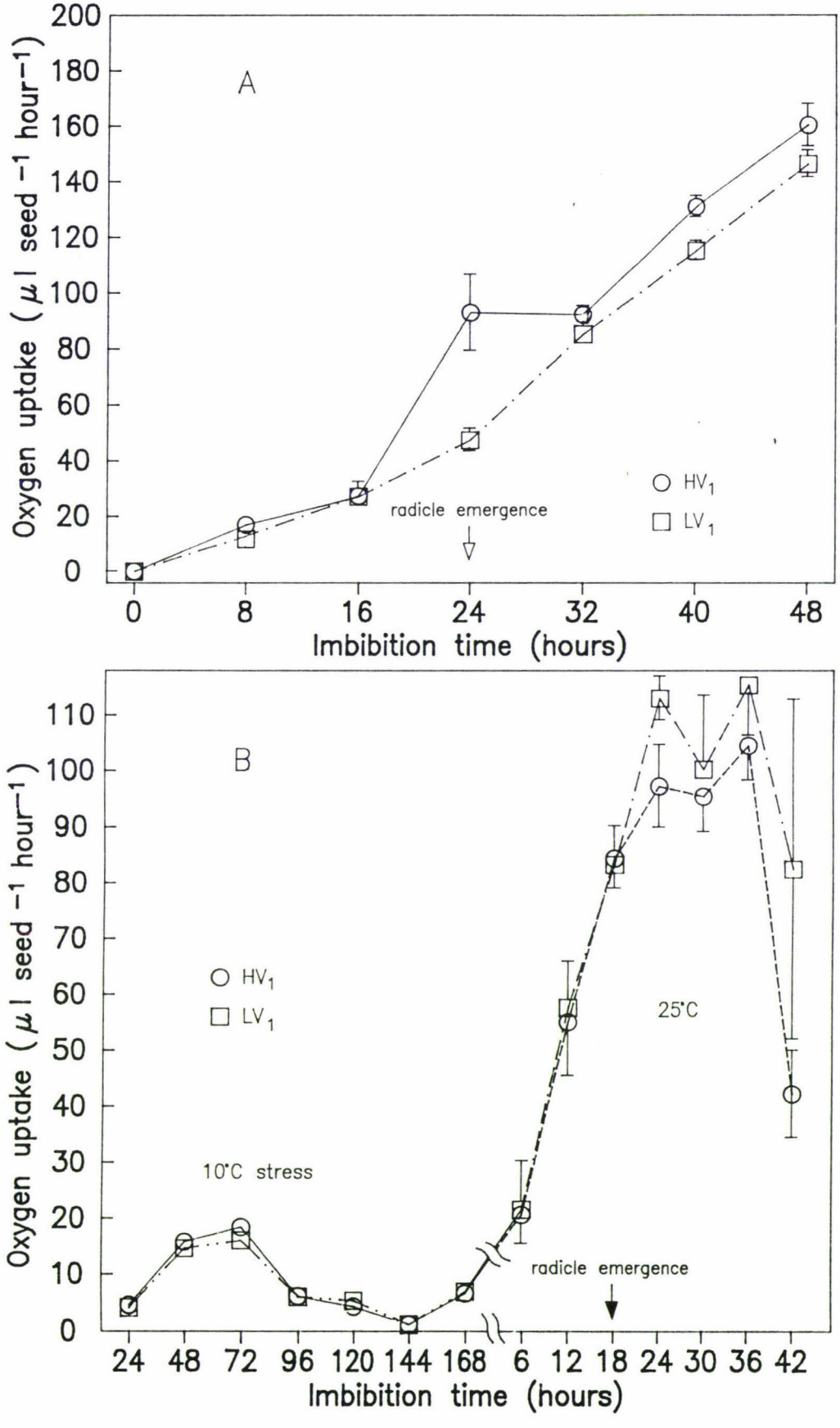


Figure 4.6 **Time course of oxygen uptake under optimum (25°C, 48 h) temperature (a) and low (10°C, 7 d) temperature stress/optimum (25°C, 42 h) temperatures (b) of super sweet corn (cv. Illini Gold). Data are means of three replicates, vertical bars are ± se of individual means. Arrows indicate the time of the onset of radicle emergence.**

in their oxygen uptake levels during the first 16 hours of imbibition. By 24 hours deteriorated (LV_1) seeds had consumed a significantly lower level of oxygen (47.30 μl/seed^{-1} hour^{-1}) than unaged (92.73 μl/seed^{-1} hour^{-1}) seeds (HV_1), a period that coincided with the beginning of radicle protrusion (Figure 4.6a). However, the former showed less radicles than the latter. Throughout the radicle emergence period (24-48 hours), low vigour seeds consumed a relatively lower amount of oxygen (though not always significantly lower) than high vigour seeds.

Oxygen uptake under stressful low (10°C) temperature for seven days before measured at 25°C for 42 hours are shown in Figure 4.6b. There was no significant difference in oxygen uptake between seed lot HV_1 and LV_1 throughout the low temperature respiration. An interesting observation was that the dramatic increase in oxygen uptake during the early stage of imbibition (48-72 hours) at low (10°C) temperature. Oxygen consumption increased from 4.61 μl/seed^{-1} hour^{-1} in seed lot HV_1 and 4.05 μl/seed^{-1} hours^{-1} in seed lot LV_1 at 24 hours to 15.78 and 14.61 at 48 hours and 18.38 and 16.02 at 72 hours, respectively. Oxygen uptake then dropped to as low as 1 μl/seed^{-1} hour^{-1} at 144 hours before rising again to above 6 μl/seed^{-1} hour^{-1} at 168 hours.

After shifting the cold (10°C, 7 days) stressed seeds into optimum (25°C) temperature, both HV_1 and LV_1 seed lots initially showed a very rapid increase in oxygen uptake, before finally decreasing to 42.18 and 82.38 μl/seed^{-1} hour^{-1} in high and low vigour seeds, respectively after 42 hours of imbibition. Difference in oxygen consumption between high and low vigour seeds started to occur after 18 hours of imbibition at 25°C, coinciding with the onset of the radicle emergence period (18-42 hours) (Figure 4.6b). Contrary to oxygen uptake with unstressed seeds during radicle emergence (Figure 4.6a), the rate of oxygen consumption during radicle emergence of cold stressed seeds was relatively higher in low vigour seeds than in high vigour seeds (Figure 4.6b).

4.2.6.2 Respiratory quotients

During the early stage of imbibition (< 16 hours) at optimum (25°C) temperature, respiratory quotients (RQ) were high in both high vigour (up to 2.18) and low vigour (up to 2.52) seeds (Figure 4.7a). As imbibition progressed RQ values decreased, with seed lot HV_1 showing a relatively faster rate of decrease compared to that of seed lot LV_1 throughout the imbibition period, with the exception of 32 hours where both showed similar RQ values. During the start of radicle protrusion (24 hours) the high vigour seed lot had an RQ below 0.9, while the low vigour seed lot had an RQ of about 1.5. RQ values in high vigour seeds

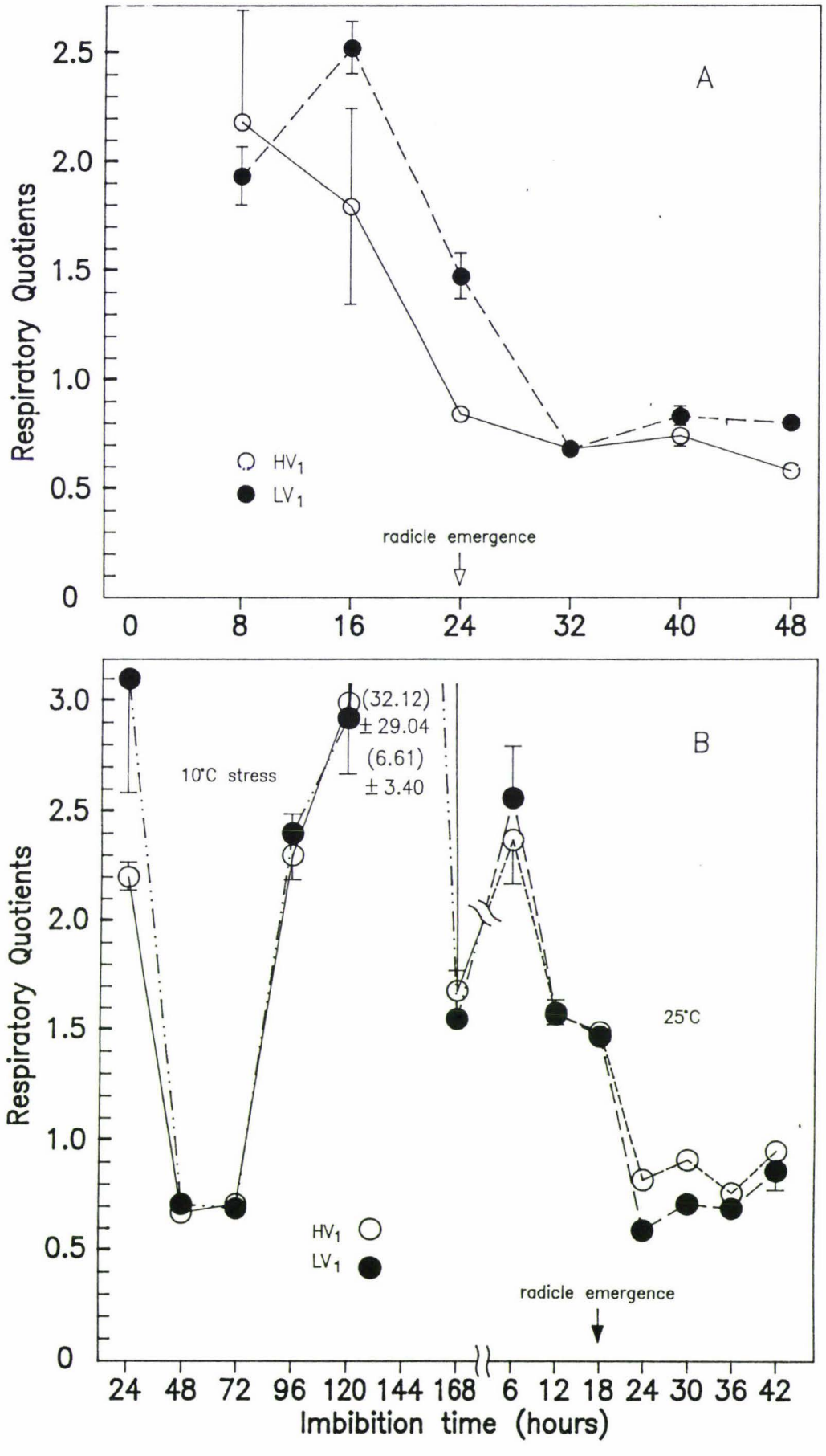


Figure 4.7 **Time course of respiratory quotients under optimum (25°C, 48 h) temperature (a) and low (10°C, 7 d) temperature stress/optimum (25°C, 42 h) temperatures (b) of super sweet corn (cv. Illini Gold). Data are means of three replicates, vertical bars are ± se of individual means. Arrows indicate the time of the onset of radicle emergence.**

became relatively stable at > 24 hours, ranging from 0.58-0.84, while that of low vigour seeds started later at > 32 hours of imbibition, ranging from 0.68-0.83.

Respiratory quotients recorded during low temperature respiration and subsequently at 25°C are presented in Figure 4.7b. Early imbibition (24 hours) at low temperature produced RQs of 2.20 and 3.10 in high and low vigour seeds, respectively. These values declined dramatically after 48 or 72 hours of imbibition ranging from 0.67-0.71 and 0.69-0.71 in high and low vigour seed, respectively, before increasing again to over 2 at > 96 hours imbibition. Surprisingly, extremely high RQ values (32.12 in HV_1 and 6.61 in LV_1) were observed following imbibition for six days (144 hours) but these then dropped again to 1.68 in high vigour seeds and 1.55 in low vigour seeds imbibed for 168 hours.

Early imbibition (6 hours) of seeds at 25°C following a cold stress for seven days showed similar RQ values (2.37 vs 2.56), and decreased with increasing imbibition time. Contrary to RQs in unstressed seeds (Figure 4.7a), RQ values from stressed aged and unaged seeds became relatively stable at the same time (> 24 hours) ranging from 0.59-0.95 (Figure 4.7b).

4.2.6.3 Tetrazolium (TT) test

Viability of seed lots HV_1 and LV_1 following cold respiration, ie 7th day of imbibition at 10°C measured by the TT test indicated no difference in the pattern and intensity of embryo staining (data not shown).

4.3 DISCUSSION

4.3.1 Experiment One

4.3.1.1 Seed Moisture Content after Accelerated Ageing

During the AA treatment, seeds were exposed to high temperatures (42°C or 45°C) and humidity (> 90% RH) for 24 hours. Due to their "hygroscopic" properties, exposure of seeds to such conditions for a short period causes an increase in seed moisture content (Table 3.1). Super sweet corn seeds in particular, have high levels of sugars (section 2.2) which are highly "hygroscopic". This increased moisture level, coupled with the higher temperature employed during the AA treatment may have deleterious consequences on the physiological performance of seeds as demonstrated later in sections 4.3.1.3 and 4.3.1.5.

4.3.1.2 Thousand seed weight and mechanical damage

Thousand seed weight (TSW) and mechanical damage (MD) assessments (Tabel 4.2) indicated that although seed lots HV_1 and HV_2 were of similar genetic (sh-2) merit, they showed a significant difference in both the TSW and MD levels. As discussed in section 2.4, the genetical expression or potential quality of seed going into storage may be influenced by environmental factors in the seed production field, harvesting or processing technology and management technology.

As the seed lots were obtained from different sources (Table 3.1), the environmental factors which affect seed weight (eg water supply, temperature, plant density) probably differed between the two seed production fields. These factors can influence the physiological status of the plant, especially during seed development, resulting in smaller sized or light seed (Hall *et al.*, 1981; Schussler and Westgate, 1991; Odimah, 1991). Temporary, but acute, water deficiency during seed development may affect photosynthesis and the reduction of carbohdyrate reserves (Westgate and Boyer, 1986) resulting in smaller or light seeds. This is because once seeds are set, the crop has little or no capacity to adjust seed number to the available assimilates (Delouche, 1980). The seed sink capacity or potential to accumulate dry weight is determined by the number and size of endosperm cells formed during the growth phase (stage I) of seed development (Bingham, 1969; Jenner, 1979), which in turn may determine the size and number of sites for starch deposition during the food accumulation phase (stage II) of seed development (Jones *et al.*, 1985). However, extended periods of extreme ambient temperatures during stage I of seed development may impair the cell division process, causing a reduction in the number and/or size of endosperm cells or starch granule formation (Radley, 1978; Jones *et al.*, 1985) which may subsequently reduce seed weight. Further than this, both inter-plant and intra-plant competition for light or nutrients as a result of high plant population or tillers may subsequently cause differences in seed size, and hence, seed weight.

The difference in mechanical damage levels (Table 4.2) between seed lots HV_1 and HV_2 may be attributed to both genotypic and processing technology management. The shrunken-2 genes increase sugar levels by inhibiting the sucrose-starch conversion (Ferguson *et al.*, 1978; Soave and Salamini, 1984). Styer and Cantliffe (1983) reported that this dramatic reduction in starch content may result in collapsed endosperm, cracks in the pericarp and air pockets which may leave crevices on drying. Additionally, the sh-2 mutant genes slow the rate of moisture loss as maturity progresses (Soberalske and Andrew, 1978' Churchill and Andrew, 1983), and as a result, seeds remain with very high SMC (above 50%) at harvest. Mechanical harvest at such elevated moisture levels makes the seeds prone to

mechanical damage. The work of Mashauri (1991) showed that dent maize (*Zea mays* L.) seeds threshed at high SMC suffered high levels of bruising and internal damage. SMC determines the viscoelastic property of seeds (eg tensile strength), which in turn may influence the type and magnitude of damage during harvesting and processing.

4.3.1.3 Standard germination test

Despite the fact that seed lots HV_1 and HV_2 differed significantly in TSW and MD levels (Table 4.2), their capacity to germinate did not differ significantly, as demonstrated by the similarity in percentage normal seedlings (Figure 4.1a). This suggests that seed weight is not necessarily associated with seed performance as reported by Delouche (1980) and TeKrony and Egli (1991), but contrasts with the findings of Ueyama (1975) for rice, Odiemah (1985) for wheat and Odiemah (1991) for maize. The other extreme, the type, position and magnitude of damage are important factors for seed quality. Mashauri *et al.*, (1992) observed that bruised maize seeds with internal disruption had a more severe reduction in germinability and vigour than cracked seeds, and Bartsch (1979) and Escasinas (1986) demonstrated that the extent of cracking damage *per se* bears little relation to loss of germinability; rather, it is the position of the damage in relation to the embryo which is more important. Mashauri *et al* (1992) pointed out that the effects of mechanical damage on seed quality may be obscured by the ways seed performance is subsequently evaluated, because deleterious effects on potential performance may not be apparent from an initial standard germination test.

Following artificial ageing, germinability of aged seed lots (LV_1, LV_2, LV_3 and LV_4) declined significantly (Figure 4.1a) with a corresponding increase in abnormal seedlings (Figure 4.1b) compared to unaged (HV_1 and HV_2) seed lots. This technique is useful in producing a deteriorated sub-seed lot from a single seed lot in a short period of time, and facilitates simultaneous collation of aged and unaged seed lots under identical conditions. The harsh treatment (high humidity [hence raised SMC] and temperatures) imposed by the artificial ageing can induce rapid deterioration (Harrington, 1978). The importance of these two factors (SMC and temperature) on seed quality is well documented. Increased SMC leads to the availability of water as a solvent for biochemical reactions in seeds which in turn can cause increased deterioration rates, particularly when accompanied by high temperatures (Harrington, 1978; Priestley, 1986; Leopold and Vertucci, 1989).

The sites of some of the consequences of high heat and humidity (SMC) imposed by AA include membrane damage (Basavarajappa *et al.*, 1991), changes in respiratory and DNA enzymes (Vazquez-Ramos *et al.*, 1988; Basavarajappa *et al.*, 1991) and damage to RNA

and protein synthesis ability (Priestley, 1986; Helm *et al.*, 1989), and the seeds exhibit symptoms of deterioration such as slower rate of germination and emergence, poor seedling growth, lowered germination levels and consequently increases in abnormal seedlings and dead seeds, lower respiration rate, enhanced leakage and decreased tolerance to sub-optimal conditions (Priestley, 1986; Powell, 1988; Hampton and Coolbear, 1990).

Diagnosis of seedling abnormalities (Figure 4.1b) indicated that extrememly high levels of abnormal seedlings in aged seed lots were the result of decay by fungi, particularly *Fusarium subglutinans* and *Penicillium* species (see section 4.3.2.1). This was a consequence of seed deterioration. As mentioned earlier, physiologically aged seeds loses their tolerance to sub-optimal conditions. It apears that resistance to fungi depends upon the maintenance of certain degrees of physiological vigour which relies upon the functional and efficient biochemical and physiological processes of the seeds. When vigour drops below a certain level because of stress-induced damage, the seeds become more susceptible. Helm *et al.*, (1989) suggested that deteriorated embryos lack the capacity to synthesise a specific set of proteins, the so-called heat shock proteins (HSP) which are produced as a protective response to sub-optimal conditions such as heat, cold, ethanol, anoxia and several other forms of stress (Lindquist, 1986; Van de Venter and Lock, 1992). This reduction or loss of stress protective system in aged seed lots may make the seeds more vulnerable to stress-induced damage (Helm *et al.*, 1989), predictably including that caused by fungi. It is obvious that the AA conditions did not kill the internally-borne fungi and the subsequent hydration of seeds at 25°C for 7 days during the standard germination test favoured their growth, as reflected by the reduction in percentage normal seedlings with consequent increases in the levels of abnormal seedlings.

4.3.1.4 Soil cold test

In contrast to the standard germination test (Figure 4.1a), normal and high vigour seedlings from the soil cold test (Table 4.3) indicated no signfiicant differences between unaged (HV_1 and HV_2) and deteriorated (LV_1, LV_2, LV_3 and LV_4) seed lots. These results are surprising in that despite the combined effect of AA treatment (section 4.3.1.1), severe pericarp damage (section 4.3.1.2), heavy infection by seed-borne pathogens (section 4.3.2.1) and the stress conditions of the soil cold test (soil-borne pathogens, wet and cold soils), germination remained relatively high.

The conditions of the test suppressed the activity and growth of the seed-borne pathogens, and therefore allowed normal seedling development. The fact that the seed is contaminated with pathogens does not automatically ensure establishment of the disease

in the soil environment, due in part to antagonists (Burpee, 1990) but also to the soil environment. Biological contol involves one or more natural processes such as competition, parasitism, antibiosis, predation, induced host resistance, etc (Baker, 1968; Cook and Baker, 1983). The processes are complex, being influenced by a myriad of soil environment factors, both biotic (soil-borne micro-organisms) and abiotic factors such as pH, temperature, moisture, soil type, organic and inorganic constituents or the soil, etc (Kloepper *et al.*, 1988; Burpee, 1990). This complex interaction may prevent infection of the seedling, limit spread from an initial infected seedling, or allow a complete attack of the seedling. The suppression of disease establishment is proposed to be via inactivation of the pathogen propagule, reduction in their numbers, or adverse interruption with their sporulation, ability to colonise substrates saprophytically, initiate infection or pathogenesis (Burpee, 1990). Additionally, the optimum growth temperatures for *Fusarium subglutinans* has been reported to be in the range of 20-25°C (Gonzalez *et al.*, 1988), suggesting the conditions (cold and wet soils) for the soil cold test inhibited the biological activity of this fungus (Gilbertson *et al.*, 1985; Shurtleff, 1980), and thus predisposed it to soil-borne antagonist pathogens (Burpee, 1990).

Although seed-borne pathogens were inactive under the conditions of the soil cold test, the effects of wet and cold soils (low temperature and water stresses) for as long as 7 days also did not seem to influence the performance of low vigour seed lots, as illustrated by high and similar percentages of normal seedlings, shoot length and normal seedling dry weight (Tables 4.3 and 4.4). This result suggested that the cultivar is cold tolerant. This is in agreement with the work of Mashauri (1991) who observed a substantial enhanced performance (measured as germination %) of deteriorated cold resistant dent mazie seed lots. For cold tolerant cultivars, incubation at low temperature (10°C) before holding at favourable temperatures (25°C) for germination can show a marked stimulatory effect on seed germinability performance (Van de Venter and Grobbelaar, 1985). Cold tolerant cultivars can exploit the low temperature incubation period to initiate germinative metabolisms and allow some cellular repair and reorganisation processes to become more active after subsequent imbibition (Heydecker, 1974; Villier and Edgcumbe, 1975; Roberts, 1981; Mashauri, 1991) and improve their germinability as a result (Ward and Powell, 1983; Mashauri, 1991).

Percentage abnormal seedlings decreased and dead seeds increased in the soil cold test (Table 4.3) compared to that observed in the standard germination test (Figure 4.1a) because a bigger proportion of seedlings were free of *Fusarium* infection in the former, and

could therefore be classified as normal seedlings. Seed-borne and/or soil-borne pathogens, and the effects of cold and water stresses increased the level of dead seeds (Table 4.3).

4.3.1.5 Seedling growth rate

Shoot length and root and shoot dry weights (Table 4.5) showed a clear cut difference between high vigour (HV_1 ad HV_2) and low vigour (LV_1, LV_2, LV_3, and LV_4) seed lots (eg Plate 4.3a versus 4.3b). As the severity of ageing conditions increased, there was a corresponding decrease in both shoot length and shoot dry weight. This is in line with Van de Venter (1988) who reported a significant reduction in shoot length in some cultivars of maize in response to AA (> 90% RH, 42°C, 96 hours). Apart from affecting the various cellular components necessary for resumption of growth, AA (eg 42° ± 1°C, 100% RH, 24-96 hours) has been reported to cause a decrease in total content of food reserves (eg carbohydrates, reducing sugars and proteins) in maize seeds for a short time (≥ 24 hours) of treatment (Basavarajappa *et al.*, 1991), presumably because of increased respiratory activity when the seeds are incubated at high humidity (SMC) and temperature, or because of increased activity of hydrolytic enzymes (eg amylase) at elevated SMC and temperatures, which in turn may have an important consequence on the growth and development capability of the seedlings, and be reflected as a reduction in shoot length or shoot dry weight (Basavarajappa *et al.*, 1991). Roberts (1972) considered the depletion of essential metabolites, including loss of food reserves, as one of the important factors responsible for loss in seed performance.

On the other hand, the activity of pathogens can have a considerable impact on seed performance (Mashauri *et al.*, 1992). Invading pathogens utilise the hydrolysed starch or sugar for their metabolisms and secrete enzymes of their own which in turn may cause a rapid decomposition of the endosperm reserves. The produced hydrolytic enzymes (eg protease) and phytotoxins (Cherry, 1983; St Angelo and Ory, 1983; Agarwal and Sinclair, 1987) can erode the structural integrity of cell membranes, or impair the biochemical systems and functions of cellular components, and the combined effect of these factors may render the seeds incapable of growing normally, synthesising and depositing dry weight.

Another salient feature which emerged from this test was that the decrease in root dry weight was evident only at the most severe ageing conditions (LV_3 and LV_4 - Table 4.5), while shoot length and dry weight delcined as the severity of ageing conditions increased. This suggests that the root and shoot systems were not affected similarly by the AA/pathogen interaction. In this study, it appears that plumule primordia were much more sensitive to the damaging effects of AA/pathogens than the radicle primordia. *Fusarium*

subglutinans in particular, tended to affect mostly the shoot system and only occasionally the root system (see section 4.1.2, Plates 4.2a,b; 4.3a,b).

Shoot length and normal seedling dry weight of low vigour seed lots from this test was much lower than that produced from the soil cold test (Table 4.4). This demonstrates the adverse effect of ageing but particularly microflora-induced damage on seed quality and performance.

4.3.1.6 Complex stressing vigour test

Normal and high vigour seedlings from the CSVT (Table 4.6) demonstrated a differential performance betwen high and low vigour seed lots, with a correspondingly higher level of dead seeds in low vigour seed lots. In this test, efforts were made to eliminate or reduce the effect of microflora by mixing the soaking water with sodium hypochlorite disinfectant. The observed differential performance between unaged and aged seed lots may reflect the impact of AA treatment, because sodium hypochlorite has been used as a seed disinfectant in maize (Schoen and Kulk, 1977). Similarly, Anderegg and Guthore (1981) found seedlings of sweet corn were less infected with *Fusarium moniliforme* after applying sodium hypochlorite seed disinfectant. May be some of the sodium hypochlorite solution was absorbed during imbibition and suppressed the internally-borne *Fusarium*.

There are factors which can account for the escalated level of dead seeds in the low vigour seed lots. The seeds used in this test had severe pericarp damage (Plate 4.1a,b). Adding this to the effect of AA, this may have made the seeds more prone to imbibition damage during soaking. Extended periods of soaking (eg 96 hours) make the seeds prone to anoxia injury. Furthermore, the soaking water contained sodium hypochlorite, which probably penetrated through the damaged parts of the seeds and killed not only the pathogens but also the already deteriorated embryo, thus increasing the dead seeds percentage.

4.3.2 Experiment Two

4.3.2.1 Seed health testing

Pathological studies indicated that although aged (LV_1 and LV_2) seed lots were subjected to high temperatures and RH, all were heavily contaminated with *Fusarium subglutinans* at similar levels, and also harboured lower amounts of *Penicillium* and *Rhizopus* species.

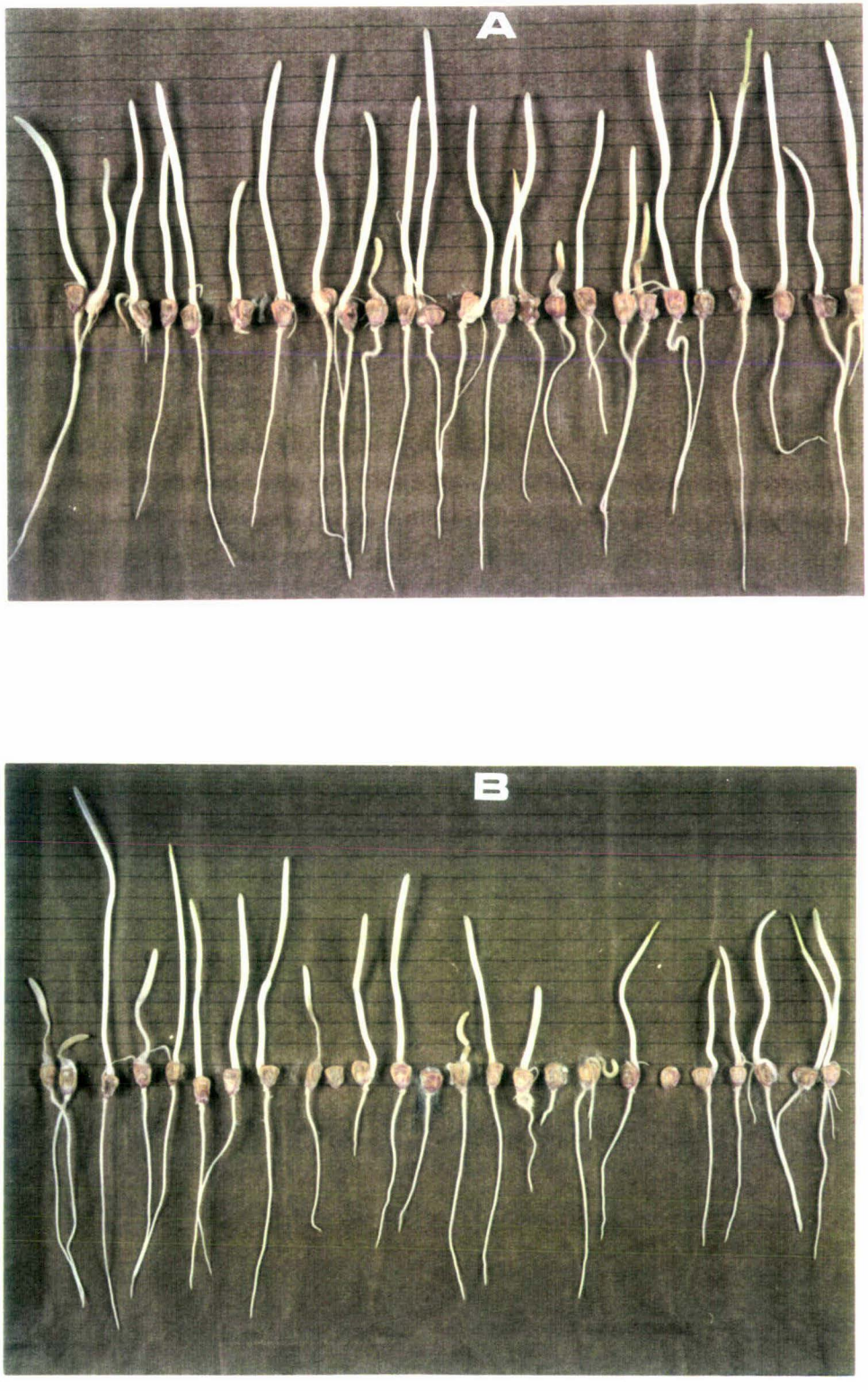


Plate 4.3 A photograph showing seedling growth from high vigour (HV_2) seed lot (a) and low vigour (LV_2) seed lot (b).

Fusarium subglutinans has recently been raised to species status by Nelson *et al.*, (1983). It is equivalent to *Fusarium sacchari* (Butler) W. Goms Var. *subglutinans* (Wollenweber and Reinking). Because of the complexity of *Fusarium* taxonomic systems, variation (mutations) of cultures, and various media and environmental conditions, most microbiologists and pathologists in the past (< 1983) were not able to distinguish *Fusarium subglutinans* (Wollenweber and Reinking) Nelson, Toussoun and Marasas Comb. from *Fusarium moniliforme* Sheldon var. *subglutinans* (Wollenweber and Reinking). Others recognised and named *Fusarium sacchari* (Butler) Gams var. *subglutinans* (Wollenweber and Reinking) (eg Marasas *et al.*, 1979; Kommedahl and Windel, 1981; Neish *et al.*, 1983). Some of the distinguishing characteristics observed in *Fusarium subglutinans* included the presence and formation of microconidion polyphialides and absence of microconidial chains as described by Nelson *et al.* (1983). Due to this confusion, much work on this newly described species "*Fusarium subglutinans*" may be reported in the name of *Fusarium moniliforme* or *Fusarium moniliforme* var. *subglutinans*, etc.

Fusarium subglutinans is one of the most virulent species of *Fusarium*, reported to affect the germinating seed or growing seedling causing seed rot and pre- and post-emergence damping-off etc (eg Gilbertson *et al.*, 1985; Dey *et al.*, 1989 - see also section 2.6). Similarly, *Penicillium* spp. can influence seed germination and seedling growth. *Penicillium oxalicum* for example has been reported to reduce germination, inhibit seedling growth, cause seedling blight, cob rot and death of seeds, seedling or plants of sweet corn and other crops (Johann and Holbert, 1931; Caldwell *et al.*, 1981; Halfon-Meiri and Solel, 1990). Johann and Holbert (1931) suggested that although *Penicillium oxalicum* is saprophytic in habit, the toxic substance (oxalic acid) released by this fungus may kill the cells in advance of the fungus. The presence of *Fusarium subglutinans* and *Penicillium* species in the seed tissue at such high levels is likely to have been responsible for decreased germinability and vigour in deteriorated seed lots, as demonstrated by the decline in normal seedlings, coleoptile length, root and coleoptile dry weights. The optimum growth temperatures for *Fusarium subglutinans* (20-25°C: Gonzalez *et al.*, 1988) and *Penicillium* species (25-30°C: Halfon-Meiri and Solel, 1990) are similar to that used for standard germination and seedling growth tests (25°C). With availability of substrates (food) leaking from the damaged cell membranes and pericarps from aged seed lots (section 4.3.1.2), the fungi were able to flourish and subsequently do extensive damage to the seeds.

4.3.2.2 Cold germination test

As for the soil cold test (section 4.3.1.4), normal seedlings, root and shoot dry weights from the cold germination test showed no significant difference between unaged

(HV_1 and HV_2) and aged (LV_1, LV_2, LV_3 and LV_4) seed lots (Tables 4.8 and 4.9). This lends support to the idea presented earlier that the cultivar used in this study is cold tolerant. Cold tolerant seed crops do not suffer any physiological disorder (chilling injury) caused by exposure to temperatures in the chilling range (0° - 15°C) for long periods of time (Van de Venter and Grobbelaar, 1985; Herner, 1986). It is possible that imbibition at 10°C for 7 days allowed the seeds to imbibe slowly, avoiding imbibition damage and leakage of electrolytes and start the germination processes (Parera and Cantliffe, 1991, 1992), but the actual germination (radicle emergence) could not occur because the temperature was too low (Heydecker, 1974). This process 'advanced' or 'primed' seeds, so when they were moved into favourable temperature (25°C, 6 days) for growth or germination, they had already completed a portion of the germination process, ie imbibition, repair and reorganisation of cellular components, which gave the aged seed lots an advantage on the subsequent performance, and offset the vigour differences observes in other treatments (sections 4.3.1.3 and 4.3.1.5). Low temperature hydration seed treatment or priming has been used for sweet corn to enhance its potential performance in recent years, and has been reported to improve germination and vigour (eg Bennett *et al.*, 1988; Barbato *et al.*, 1993; Sung and Chang, 1993).

Another interesting feature of this test was that although the test counterbalanced the difference between high and low vigour seed lots, there was some decrease in normal seedlings and dead seeds, with a consequent increase in percentage abnormal seedlings, in comparison with the soil cold test data (Table 4.3). This may highlight the proposal given earlier (section 4.3.1.4) that the soil used for the soil cold test was suppressive or contained some soil-borne micro-organisms antagonistic to seed-borne fungi, which might survive the low temperature stress *per se* during the cold germination test and attacked the otherwise normal seedlings and so increasing the number of abnormal seedlings. The lower levels of dead seeds in the cold germination test than in the soil cold test may be explained by the fact that the rolled paper cold germination test medium is free from non-seed-borne diseases, while the soil environment can harbour a great number of insects and microflora and viruses (Agarwal and Sinclair, 1987). Some may therfore have gained access to the seed's internal tissues via the damaged parts of the seeds and killed the embryo.

4.3.2.3 Soak germination

Soaking of seeds at 10°C for 24 hours resulted in a decrease in normal seedlings (Appendix 4.1a) with a corresponding higher level of abnormal seedlings and dead seeds (data not shown), although the performance of unaged seed lots remained superior than that of aged seed lots. These data suggest that the combined effect of chilling injury, anoxia

injury and imbibition injury had a severe effect on the seed's tissues, especially aged ones. Pericarp integrity plays an important role in regulating the supply of water reaching the embryo (McDolnard *et al.*, 1988). Seeds with damaged pericarps are more liable to imbibition damage that can result in poor performance (Simon and Mills, 1983). The severe seed coat damage observed in seed lots used in this study might have increased leakage due to too rapid an influx of water into the dry seed (Bruggink *et al.*, 1991b). Perry and Harrison (1970) and Duke *et al.* (1983) have suggested that rapid imbibition may cause rupture of some cells causing the extrusion of a stream of starch grains and protein bodies (Spaeth, 1987, 1989) which may affect seed performance.

Further to this, soaked seeds respire (anoxia) anaerobically and release ethanol and acetaldehyde (Marting, 1986, 1987). Initially seeds take up water and oxygen but the oxygen content of the water is depleted within 4 hours (Martin *et al.*, 1988). Similarly, low temperature causes anaerobic respiration, producing similar toxic metabolites, which in turn may damage the vital systems and function of cell membranes, organelles, nuclear materials and biochemical mechanisms, causing death or abnormalities. The inferior performance of low vigour seed lots compared to high vigour lots is probably because low vigour seed lots produced more toxic materials than their counterpart high vigour seed lots (Presis and Ng, 1984), or because deteriorated embryos detoxification and repair systems were impaired and could not withstand the presence of toxic metabolites (Martin *et al.*, 1988).

Prolonging the soaking period to 48 hours at 10°C caused a further reduction in normal seedlings in unaged seed lots. However, the differential performance between high and low vigour remained significant (Appendix 4.1b). This is because individual seeds within a population deteriorate at different rates. Thus a partially deteriorated seed lot (HV_1 and HV_2) may contain seeds of high vigour, medium and low vigour, or those that are non-viable (Priestley, 1986) that in turn may respond differently to severity of stress. Martin (1987) reported a linear rate of toxic material (eg ethanolic compounds) production between 10 and 48 hours of soaking. In addition to synthesis of toxic metabolites, the soaking treatment and low tempertaure incubation also induce changes in free amino acid content of the seeds compared to seeds germinated aerobically (Martin, 1986, 1987).

Soaking of seeds in non-aerated water at 25°C for 24 or 48 hours (Tables 4.10 and 4.11) showed higher normal seedlings compared to those soaked at low temperature (Appendix 4.1a,b). This is apparently the influence of severity of treatment conditions. Seeds in the latter received a more harsh treatment (temperature and water stresses) than

did the former (water stress), and therefore more severe tissue damage would occur in the latter treatment than in the former.

An interesting feature of the soak germination at 25°C for 24 or 48 hours was that seeds soaked for 24 hours showed a significant difference between high and low vigour seed lots, while those soaked for 48 hours did not. The possible explanation for this is that as fungi respire aerobically, the extended period of soaking (48 hours) was too long for them to survive under chronic oxygen deficiency, and therefore they died out, giving the low vigour seed lots an opportunity to repair during the germination period. Alternatively, it may be because of dramatic increases in biochemical and physiological activities in response to stress, the so-called "adaptive protective reactions" which in this cultivar have been shown to occur during 48-72 hours of stress (see Figure 4.3, Figure 4.4b,c, Figure 4.6b and 4.7b). However, the reason why this event did not occur during the CSVT (Table 4.6), conductivity at 10°C (Figure 4.2) and soak germination at 10° C (Appendix 4.1a,b) is not known. The first and third of these treatments produced very high abnormal and dead seeds, because of the severe streses (in combination). These severe stresses may have impaired the seeds capability to respond to stimulus (stresses).

4.3.2.4 Electrical conductivity test

Electroconductivity at low temperature (Figure 4.2) showed no significant differences between unaged (HV_1) and aged (LV_1 and LV_3) seed lots for the first 24 hours of soaking. Differences in leakage between unaged and aged seed lots became apparent after 36 hours of soaking, when low vigour seed lots leaked more than the counterpart high vigour seed lot. As mentioned earlier, both soaking and low temperature cause anaerobiosis and the release of the end products of anaerobic metabolism (ethanol, acetaldehyde, CO_2). The production of these deleterious materials increases with time (Martin, 1987). The accumulation of these toxic substances probably induced further damage to the membranes of the deteriorated embryos, causing a loss of membrane permeability resulting in extensive leakage. This suggests that the ability of aged embryos to limit the accumulation of these toxic metabolites, excrete them or withstand their presence diminished with time. Furthermore, these seed lots had pericarp damage, making them more prone to imbibition damage. Low vigour seeds may have high conductivity because some cells near the area of damage cannot cope with too rapid an influx of water, particularly at low temperature, since low temperature reduces the ability of the seed tissue to expand upon rehydration. These tissues eventually burst or undergo autohydrolysis, thus losing their cytoplasmic contents into water (Matthews and Powell, 1986; Bruggink *et al.*, 1991b).

Leakage of electrolytes at 25°C (Figure 4.3) were much greater than that recorded at low temperature. The increase in electrolyte leakage during imbibition theoretically could be due to a slower re-alignment of membrane phospholipids into their bilayer state (presumably because the phospholipids are in the gel phase) and/or due to disruption of respiratory or repair metabolisms as a result of altered activity of membrane related enzymes as the temperature decreases (Bochiocchio *et al.*, 1991). According to this theory, results reported here confirm the suggestion given earlier that the cultivar used in this study is cold tolerant. Cultivars of cold tolerant genotypes would leak smaller amounts of solute when incubated at low temperature (Chozi, 1992; Hardacre, 1993, Pers. comm.[1]).

Soaking of seeds at 25°C showed no significant difference in solute leakage during the early stage of soaking (Figure 4.3). Differences in leakage became evident between 12 to 32 hours of soaking. However, significant differences between high vigour (HV_1) and low vigour (LV_1, LV_3) seed lots occurred only at 24 hours. It can be observed from these data that the rates of membrane reorganisation were the same at > 32 hours for all seed lots, but low vigour seed lots lost much more solute than the high vigour seed lot. Priestley (1986) suggested that loss of seed performance may be caused by a matrix of interrelated events such as protein and RNA damage, respiratory and hormonal changes, genetic damage, enzyme changes, etc. Further to this, micro-organisms are considered to be an important factor affecting the potential performance of seeds. In these seed lots in particular, the observed massive leakage in low vigour seed lots not only may have depleted the limited levels of reserve for embryo/seedling growth, but also may have stimulated both internally and externally borne micro-organisms which substantially affect seed performance (eg Mashauri *et al.*, 1992).

4.3.2.5 Imbibitional conductivity

A starting point of this low temperature hydration tratement was the conception that seed coat or pericarp damage (eg Plate 4.2) and too rapid water uptake by seeds at high temperature (eg 25°C) may act in concert to escalate the electroconductivity recorded in section 4.3.2.4, and thus confound or mislead the essence of the test (section 2.7.5.4), ie deterioration of cell membranes *per se*; hence the employment of low temperature imibibition to minimise imbibition rates. Imbibition of seeds at 10°C for 24 hours before soaking for conductivity at 25°C for 24 hours showed no significant differences in leakage between high vigour (HV_1) and low vigour (LV_1) seed lots. However, the imbibed high vigour seed lot leaked a significantly lower amount of solute than the unimbibed high vigour seed

[1] Mr Alan K Hardacre, Crop and Food Research Institute, Palmerston North, New Zealand.

lot, while the leakage between imbibed and unimbibed low vigour seed lots did not differ significantly (Figure 4.4a). These results are of interest since they detected some physiological differences between the two seed lots as demonstrated by differences in repair and reorganisation rates. Usually, high vigour seeds repair and reorganise their membranes and other cellular components at a faster rate with less leakage than low vigour seeds (AOSA, 1983; Bruggink *et al.*, 1991b).

Interestingly, imbibition for 48 hours at low temperature before soaking for 24 hours offset the differences in conductivity leakage between high and low vigour seed lots, and both imbibed high and low vigour seed lots leaked significantly less solutes compared to unimbibed high and low vigour seed lots (Figure 4.4b). Several biochemical changes such as reduced imbibitional leakage have been observed in sweet corn seeds as they are pre-conditioned (Sung and Chang, 1993). Disruptions of the cellular membrane and organelle integrity due to maturation-dyring (and AA-induced damage in low vigour seed lot) were probably able to be repaired and returned to their normal configuration during the low temperature hydration treatment, resulting in less leakage after soaking. However, whether this enhancement performance is due to the beneficial effects of low temperature hydration treatment *per se* or in concert with the dramatic changes in the physiological/biochemical processes (measured by the respiratory oxygen uptake) which occurred at 48-72 hours of imbibition at low temperature (section 4.3.2.7), is not clear.

Imbibition for 48 hours before soaking at 25°C for 48 hours showed no advantage for the treatment (Figure 4.4c) as demonstrated by higher solute leakage than for seeds soaked for 24 hours (Figure 4.4b), and similarity in electroconductivity between high and low vigour seed lots (Figure 4.4c). The basic idea behind low temperature hydration treatment is to bring the seed tissue to an optimal moisture and temperature level for an optimum period of time. Based on this principle, it seems possible that prolonging the soaking period to 48 hours caused tissue damage regardless of vigour status (possibly membrane damage) and escalated solute leakage.

4.3.2.6 Percentage germination of seeds imbibed at 10°C for 24 or 48 hours before soaking at 25°C for 24 or 48 hours

Seeds imbibed and then soaked for conductivity (Figure 4.4a-c) were assessed for germinability at the end of conductivity measurements, and results collated with those of unimbibed seeds soaked for the same period of time (section 4.3.2.3) to see if the observed beneficial effects (less leakage) would have any influence on germination performance.

Although significant differences in solute leakage existed between high vigour (HV_1) imbibed and unimbibed seeds and similar leakage in imbibed and unimbibed low vigour (LV_1) seeds (Figure 4.4a), high vigour (HV_1) imbibed and unimbibed seeds showed no significant differences in normal seedlings but low vigour (LV_1) imbibed seeds showed significantly less normal seedlings compared to unimbibed seeds (Figure 4.5a). Similarly, while imbibition for 48 hours counterbalanced the difference in conductivity between high and low vigour seed lots, and both seed lots leaked less solute than their unimbibed counterparts (Figure 4.4b), imbibed low vigour seeds had inferior germinability compared to imbibed high vigour seeds (Figure 4.5b). Also imbibed high or low vigour seeds did not differ in normal seedlings with that of unimbibed seeds. These data strongly suggest that the beneficial effects of low temperature hydration were greatly influenced by the effects of micro-organisms on the subsequent germination. It is most likely that the conditions during imbibition at 10°C for 24 or 48 hours allowed the pathogens to initiate degradative activity. Field fungi, including *Fusarium subglutinans*, survive well in hydrated plant/seed tissue, became more active during seed germination at 25°C and suppressed the beneficial priming effects observed in section 4.3.2.5.

Due to this fungal problem, a new approach to pre-sowing treatments to enhance performance in sweet corn has been derived. It is the use of a solid matrix priming (SMP) technique with disinfection and/or microbial (antagonists) seed inoculation. SMP is a primary method wherein seeds are moistened for a given time at constant temperature in an organic or inorganic solid matrix carrier to which water has been added, and the osmotic and physical characteristics of the solid carrier are used to restrict water absorption (cf low temperature hydration treatment) (Parera *et al.*, 1992); this carrier can then be mixed with disinfectants (eg fungicides, sodium hypochlorite) to decrease the seed-borne fungi population and/or in combination with microbial antagonist inoculation to further restrict seed-borne and possibly soil-borne pathogens with the application and colonisation of the seed micro-organisms used for biocontrol (see also 4.3.1.4). This type of treatment has been shown to substantially improve germination and vigour (Parera and Cantliffe, 1991; Parera and Cantliffe, 1992; Kubik *et al.*, 1992; Parera and Cantliffe, 1992).

Soaking of seeds for 48 hours following imbibition for 48 hours produced an interesting result. Although there was no significant difference in conductivity between either vigour levels or imbibed and unimbibed seeds (Figure 4.4c), high vigour (HV_1) imbibed seeds showed a significant improvement in germinability compared with unimbibed seeds (Figure 4.5c), but imbibed low vigour (LV_1) seeds showed inferior germination over unimbibed seeds. As earlier suggested, prolonged anoxia (soaking) may have detrimental

effects on the micro-organisms, and therefore, the repair of membranes, and build-up of germinative metabolisms resulting from priming in the high vigour (HV_1) seed lot could have been the reason for the increased normal seedlings. Although pathogens were suppressed, imbibed low vigour (LV_1) seed performed poorly, presumably because the combined effect of prolonged chilling imbibition and subsequent soaking for a longer period caused tissue damage to the already deteriorated embryos, reduced or reversed the benefit gained during pre-conditioning, and was thus reflected as a lowered germination.

4.3.2.7 Respiration and tetrazolium tests

4.3.2.7.1 Oxygen uptake and dehydrogenase activity

Gas exchange measurements at 25°C (Figure 4.6a) were very high throughout the imbibition period for both high (HV_1) and low (LV_1) vigour seed lots, and increased with increasing imbibition time. Normally, respiration rates, which tend to be very low in dry seeds (Harrington, 1973), increase as seeds rehydrate. The first hours of imbibition are accompanied by considerable mitochondrial differentiation either by modifying the existing ones or by synthesising new organelles (Morohashi *et al.*, 1981). However, the first increase in oxygen consumption may be due to hydration and oxygenation of mitochondrial enzymes (Kolleffell, 1967).

The higher respiration rates observed in this study support the work of He and Burris (1991) who observed similar respiration rates of super sweet corn (sh-2) cv. Jubille. It is well known that embryos and endosperms of the sh-2 genotype contain higher levels of soluble sugars, eg sucrose, glucose, fructose, raffinose, sorbital, etc (He and Burris, 1991; Aung *et al.*, 1992, 1993), with the embryo containing more soluble sugars which provides a readily available substrate. He and Burris (1991) have suggested that this unique pattern of respiration may be associated with the low vigour potential in shrunken-2 seeds. High respiration rates may rapidly use up the low levels of reserves available to the sh-2 genotype, an event which may have a severe consequence to the growing embryo or seedling. However, other factors appear to be responsible for the loss of vigour of the sh-2 seeds. Ineffective membranes and poor pericarp integrity (section 4.3.1.2) may result in loss of soluble sugars and electrolytes (section 4.3.1.4) and potential promotion of seed-borne micro-organisms (section 4.3.1.3).

A comparison of oxygen uptake betwen high and low vigour seed lots (Figure 4.6a) indicated no significant difference during the early hours (< 16) of imbibition. However, during radicle emergence (24-48 hours), which is often accompanied by extensive operation of continued repair metabolism and other physiological/biochemical processes, the high

vigour (HV_1) seed lot demonstrated a relatively higher (though not always significant) respiratory oxygen uptake than the low vigour (LV_1) seed lot. This lower rate of oxygen uptake in the deteriorated seeds was because of a delay in radicle emergence, suggesting that the physiological integrity (eg mitochondrial activity) in low vigour seeds was impaired compared to that of high vigour seeds. Respiration plays a very important role in providing biochemical energy (ATP) which is essential for the metabolic processes (eg protein synthesis, repair metabolism) during early germination and subsequent growth. Aung *et al.* (1993) observed a substantial increase of embryo glucose in super sweet corn during radicle emergence, glucose can be phosphorylated to sustain early embryo growth (Humphreys and Garrard, 1964) and increase the level of ATP (Priestley, 1986). Lower respiratory oxygen uptake caused by impaired mitochondrial functions, particuarly during the event of radicle emergence may compromise the ability of low vigour seed tissue to synthesise and accumulate the metabolites necessary for growth, such as protein synthesis. This was illustrated by Klein *et al.*, (1971) who showed that exposure of the germinating embryos to inhibitors of protein synthesis prevented normal elongation of the embryonic axis.

Oxygen uptake at low (10°C) temperature (Figure 4.6b) showed no significant difference between unaged and aged seed lots. An interesting salient feature emerging from this treatment was the dramatic increase in oxygen uptake during the early stage of imbibition (48-72 hours) at low temperature. It is generally recognised that temperature and moisture conditions are an essential requisite for normal functioning and survival of living organisms (Barrington, 1968). Extreme drastic deviation of temperature and moisture from normal state for an organism invariably leads to cellular dysfunction, damage and death. However, under less extreme conditions of temperature and moisture, the organism can cope by exhibiting various adaptive mechanisms (Leopold, 1986).

One such adaptive mechanism involves a temporary increase of some physiological and biochemical processes (eg respiration rates etc) in the initial stage of imbibition. Results from this study lend support to that of Gu *et al.* (1981) who demonstrated a temporary dramatic increase in oxygen uptake of cold stressed seeds in the initial stage of chilling imbibition. Zheng *et al.* (1981) showed that cold stressed seeds (3°-5°C) exhibited a temporary increase in activity of dehydrogenase, peroxidase and Na^+_ K^+_ ATPase enzymes. Other adaptive mechanisms involve the change in carbohydrate which serves as a source of basal metabolic energy for biochemical processes and a substrate for polysaccharide biosynthesis (Aung *et al.*, 1992, 1993). This adaptive protection reaction occurred in the early stage of chilling imbibition because as the chilling imbibition was prolonged, oxygen uptake decreased to as low as 1 μ/seed^{-1} hour^{-1} at 144 hours, probably

because of accumulation of toxic substances (eg ethanol, etc) resulting from anaerobic metabolism (Coolbear, 1993, pers. comm.[2]). The duration of occurrence of adaptive protective mechanisms varies with species and cultivars (Zheng, 1991). In their work in different cultivars, Zheng *et al.* (1981) found that the period of dramatic changes in the activity of dehydrogenase enzymes (measured by the tetrazolium test) was longer in cultivars with moderate cold resistance than in cold susceptible cultivars.

Interestingly, incubation of cold stressed seeds at favourable (25°C) temperatures showed no significant difference in oxygen uptake between aged and unaged seed lots. Both seed lots showed very rapid respiration metabolisms and time to the onset of radicle protrusion was reduced by 6 hours compared to seeds imbibed directly at 25°C (Figure 4.6a). It seems possible that the observed dramatic increase in physiological activity at 48-72 hours gave the aged seed lot an opportunity to reorganise and repair, and subsequently be able to resist the continued cold stress conditions as the high vigour seed lot, and after shifting the seed lots into 25°C, both performed similarly. This enhanced performance, as demonstrated by much earlier and uniform radicle emergence after low temperature incubation, is typical of a low temperature hydration treatment. Priming induces quantitative changes in the biochemical contents of the seeds. Many studies have related to pre-conditioning-induced performance enhancement to the improvement in membrane integrity (via repair mechanisms) as well as increases in protein and nucleic acid synthesis. Membrane re-configuration in particular, would lead to reactivation of membrane-associated enzymes and subsequently result in an enhanced protein and nucleic acid synthesis (Sung and Chang, 1993). This occurs because hydrated sweet corn seeds accumulate more sugars that would be readily available to support the energy and carbon metabolisms operating during seed imbibition and consequently result in faster germination rates (He and Burris, 1991; Sung and Chang, 1993).

Tetrazolium test results (data not shown) indicated no difference in the pattern and intensity of staining between aged and unaged embryos, suggesting that the activity of dehydrogenase enzymes were similar in both seed lots following chilling imbibition for 7 days. As many dehydrogenase enzymes are believed to be involved in the respiration pathway, this result may further explain the observed similarities in respiratory oxygen uptake results after cold stress (Figure 4.6b). Dehydrogenase enzymes such as pyruvate dehydrogenase, isocitrate dehydrogenase, oxoglutarate dehydrogenase, cuccinate dehydrogenase and malate dehydrogenase are the most important in the tricarboxylic acid

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(TCA) pathway (Wiskich and Dry, 1985), being involved mainly in complex reactions to produce reduced NAD and FAD and finally producing chemical energy in the form of ATP (Ting, 1982).

Surprisingly, however, there was a considerable decrease in respiratory oxygen uptake after 42 hours of imbibition at 25°C (Figure 4.6b), presumably because of depletion of food reserves. Considering the high leakage of sugars and electrolyte caused by poor membrane and pericarp integrity, consumption of soluble sugars by prolonged respiratory metabolism at low (10°C) temperature and/pr higher respiration rates at high (25°C) temperature and the low level of initial endosperm reserves to provide a sustained energy supply, may be caused by a limitation on the rates of respiratory metabolism. He and Burris (1991) observed a dramatic decrease in sucrose levels in the endosperm of sh-2 between day 4 and 5 of imbibition. Lack of energy may result in depression of respiratory rates and so retard the synthetic metabolism needed for seedling growth.

4.3.2.7.2 Respiratory Quotients

Respiratory quotients (RQ) is the ratio of carbon dioxide (CO_2) evolved to oxygen (O_2) taken up. RQ indicates the type of respiratory activity taking place in seeds and also the type of respirable substrate. An RQ of around 1 suggests aerobic respiration using carbohydrate as a substrate, but an RQ above 1 indicates some anaerobic respiration. Protein and fat or lipid metabolism gives an RQ below 1 (Mayer and Poljakoff-Mayber, 1982; Coolbear, 1990).

In this study, RQ values during the early stage of imbibition ($\leq$ 16 hours) at 25°C were high (over 2.5) in both high (HV_1) and low (LV_1) seed lots, and decreased as imbibition progressed (Figure 4.7a). This was a period of anaerobic respiration because of moisture and oxygen limitations at the early stage of imbibition. Woodstock *et al.* (1984) showed a linear relationship between axis/seed moisture cotnent and rates of oxygen uptake. They suggested that this was due to hydration of adjacent tissues as the water front moved towards the interior regions of the axis/seed. These conditions may cause low respiratory oxygen uptake and an increase in anaerobic respiration with the production of high levels of CO_2 and ethanol, and thereby increase the RQ value as a result.

As rehydration of seeds progressed, seeds were better reorganised and changed the type of respiration from anaerobic to aerobic. However, the rate at which low vigour seeds reorganised and changed the type of respiration was behind that of high vigour seeds. This suggests that the functional capacity of the respiratory component or its enzyme complex

in the low vigour seed lot was damaged, and this may have had detrimental consequences on the RNA, protein and DNA synthesis which are among the important physiological processes during the early stage of imbibition. By ≥ 32 hours of imbibition, both seed lots showed unexpectedly low RQ values (0.58 - 0.84), presumably because the accumulated toxic byproducts of anaerobic respiration (ethanol, lactic acid, etc) during ≤ 24 hours of imbibition were metabolised. Under the conditions of increased aerobiosis this resulted in decreased RQ values (Bewley and Black, 1978; Coolbear, 1993, pers. comm.).

During the early stage of imbibition at low temperature seeds respired anaerobically, but because of dramatic changes in the physiological and biochemical activity caused by the adaptive protection reaction in response to cold stress, there was a shift of respiratory type to aerobiosis at 48-72 hours of imbibition. However, because of continued cold stress during imbibition at ≥ 96 hours, the seeds resumed anaerobic respiration (Figure 4.7b). Eagles (1982) reported that maize seeds generally do not germinate and grow when imbibed below 10°C, but imbibition at 10°C allowed the seeds to perform anaerobic respiratory metabolism and the release of ethanol into the environment. This may have occurred because of an increase in the activation energy (Ea) of the mitochondrial oxidative system at low temperature relative to the constancy of the activation energy (Ea) of glycolysis (Rasion, 1980). The extremely high RQ values at 120 and 144 hours of imbibition are probably due to the accumulation of endproducts of anaerobic metabolism during the prolonged period of cold stress; some of these (eg acetaldehyde) were probably released as volatile gases, inflating the CO_2 collected and raising the RQ values as a result (Coolbear, 1993, pers. comm.). Exposure of seeds at 25°C following cold imbibition for 7 days showed a rapid decrease in RQ values, and by ≥ 24 hours, RQ in both HV_1 and LV_1 seed lots was around 1, suggesting aerobic respiration using glucose.

CHAPTER 5

RESULTS

5.1 FIELD EXPERIMENTS

5.1.1 Weather data at sowing

The daily ambient air and soil temperatures from the planting date to maximum emergence for the October, November and December plantings are shown in Figure 5.1. In general, air and soil temperatures increased from October to December. The maximum and minimum temperatures in the October planting ranged from 14-17°C and 7-11°C, respectively, while soil temperatures (10 cm depth) ranged from 11.9-15.9°C (Figure 5.1a). In the November planting, maximum and minimum ambient air temperature ranged from 13.4-21.1°C and 4.3-11.3°C, respectively, but soil temperatures (10 cm depth) ranged from 13-15°C (Figure 5.1b). For the December planting maximum and minimum ambient air temperatures ranged from 16.5-24.7°C and 4.5-16.6°C, respectively, while the soil temperature ranged from 13.3-19.7°C (Figure 5.1c). The daily rainfall for the October planting ranged from 0-29.6 mm with a total of 61.9 mm for the period from planting to maximum emergence (Figure 5.2). Rain fell (29.6 mm) on the planting day with a further 6.6 mm on the day following planting. Three days later, another rainfall was recorded (18.0 mm) followed by three days of showers of 0.6-1.8 mm. The daily moisture loss, recorded as evaporation was generally low (0-3.4 mm), although it occasionally rose to over 4 mm, particularly on days with no rain (Figure 5.2). The total rainfall for the November planting (59.3 mm) was similar to that recorded in the October planting. Rain fell (26.6 mm) one day before planting and this was followed by 5.1 mm on the planting day. One day later, 4.3 mm of rainfall was recorded, followed by some showers (0.3-1.0 mm). More rain fell in the second week following planting. The daily evaporation was low, ranging from 0.2 to above 4 mm (Figure 5.2). The total rainfall for the December planting was low (34.8 mm) compared with that recorded in the October and November plantings. However, rain fell (0.1-15.2 mm) almost every day for the first ten days after planting. Evaporation was low, ranging from 0.6 to 7.3 mm.

5.1.2 Field emergence and emergence rate and its relationship with seed quality characters

No significant differences in either the percent field emergence or emergence rate were recorded among vigour levels in all planting dates. The factor of planting dates was very significant but the interaction between vigour levels and planting dates was not significant (Appendix 5.1a,b).

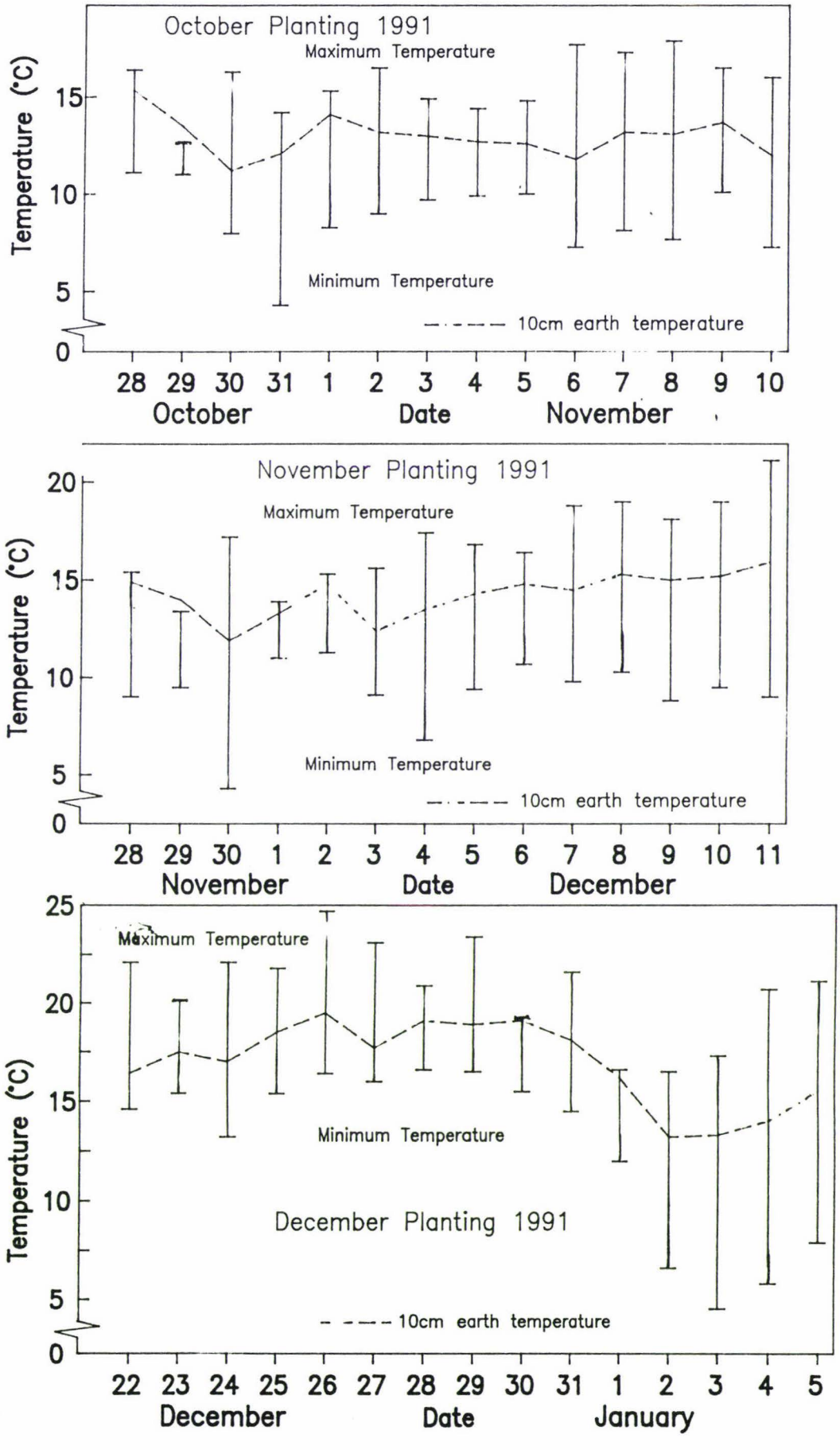

Figure 5.1 **Daily maximum and minimum air and soil (10 cm depth) temperatures from planting to maximum emergence for October, November and December plantings.**

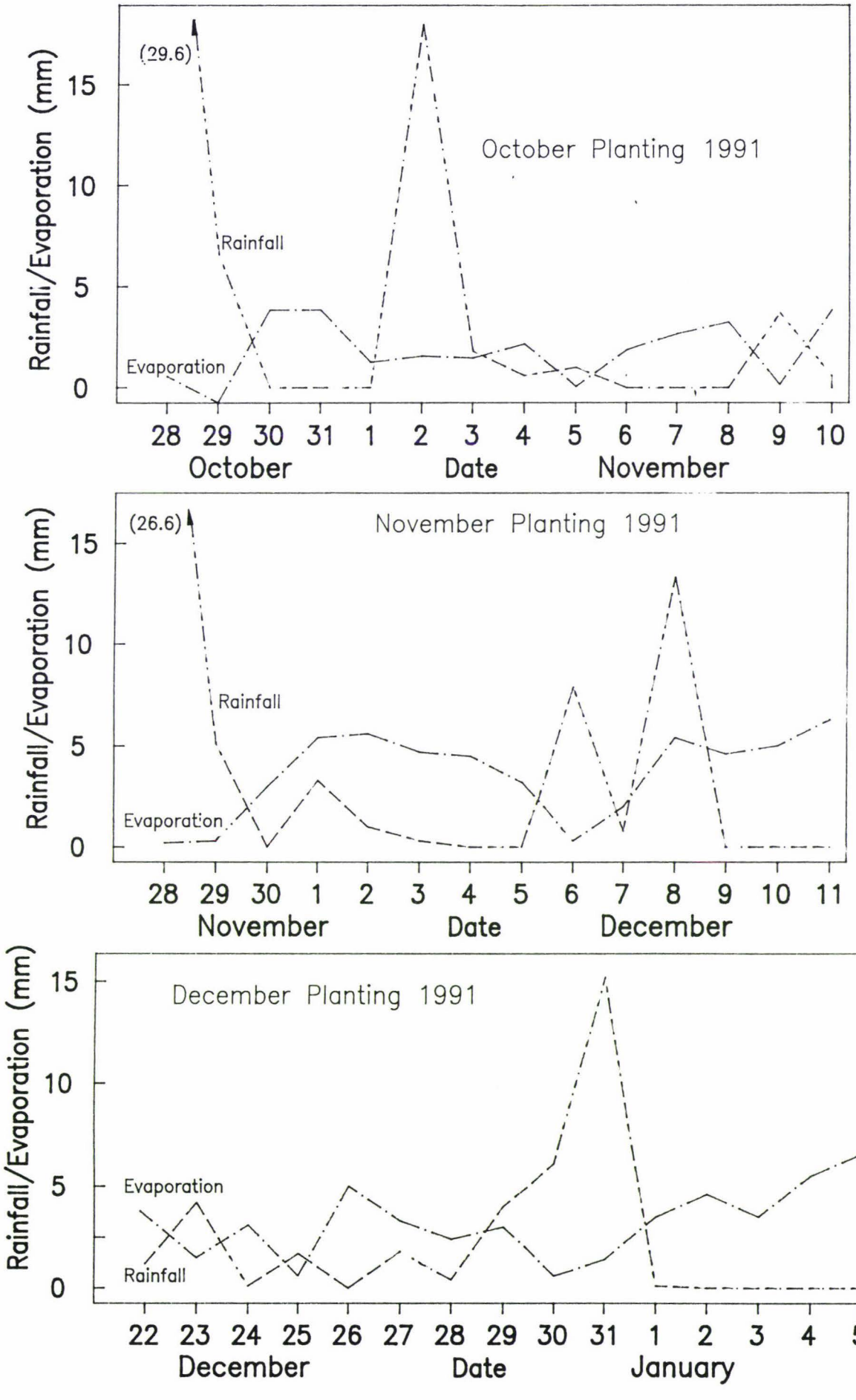

Figure 5.2 **Daily rainfall and evaporation data from planting to maximum emergence for October, November and December plantings.**

In the October planting, field emergence for seed lots HV_1, HV_2, LV_1 and LV_2 ranged from 63 to 64%, and was significantly less ($P < 0.05$) than that from the November planting, which ranged from 71 to 75%. The December planting had the highest field emergence (76 to 81%) (Figure 5.3a). Even the severely aged seed lots (LV_3 and LV_4) did not differ significantly ($P < 0.05$) in field emergence with seed lots HV_1, HV_2, LV_1 and LV_2 (77-82% - Appendix 5.2a). Field emergence rate in the October planting (Figure 5.3b) was significantly ($P < 0.05$) slower (13.15 to 14.28 days) than either in the November or December plantings, with the December planting having a significantly faster ($P < 0.05$) emergence rate (6.90 - 7.29 days) than the November planting (11.10 - 11.40 days). As in percent field emergence for the December planting, seed lots LV_3 and LV_4 did not differ significantly in emergence rate with that of seed lots HV_1, HV_2, LV_1 and LV_2 (6.90 -7.28 days - Appendix 5.2b).

The relationship between seed quality tests and field emergence obtained from the different planting dates using all seed lots are shown in Appendix 5.2X. Surprisingly, there was no significant correlation between seed quality characters and field emergence from any of the planting dates. However, the complex stress vigour test showed a significant relationship with field emergence from the November planting. Other quality characters such as cold germination, soak germination and electroconductivity also did not correlate significantly with the final stand in the field from the November planting (data not shown).

5.1.3 Losses of plants, stunted plants and final stand in the field

Loss of plants due to post-emergence damping-off and seedling blight (Plate 5.1) when recorded at the 5th leaf stage for the October, November and December plantings showed a highly significant difference ($P < 0.001$) among vigour levels and planting dates. The combined effect of these two factors was also significant ($P < 0.05$) (Appendix 5.3).

Post-emergence damping-off (shown as percentage loss of plants in Figure 5.4) in the October planting was significantly ($P < 0.05$) higher than in the November planting. The December planting had greater losses than the other two plantings (Figure 5.4a). Low vigour (LV_1 and LV_2) seed lots in the October planting had a significantly higher (about 27%) loss of plants than the high vigour (HV_1 and HV_2) seed lots (12-15%). In the November planting, loss of plants was minimal (7-8%) and no significant differences ($P < 0.05$) were recorded among seed lots. In the December planting, low vigour seed lots showed a significantly higher ($P < 0.05$) loss of plants (39-40%) than the high vigour seed lots (22-28%). Loss of plants in seed lots LV_3 and LV_4 was similar to those of seed lots LV_1 and LV_2 in the December planting (data not shown).

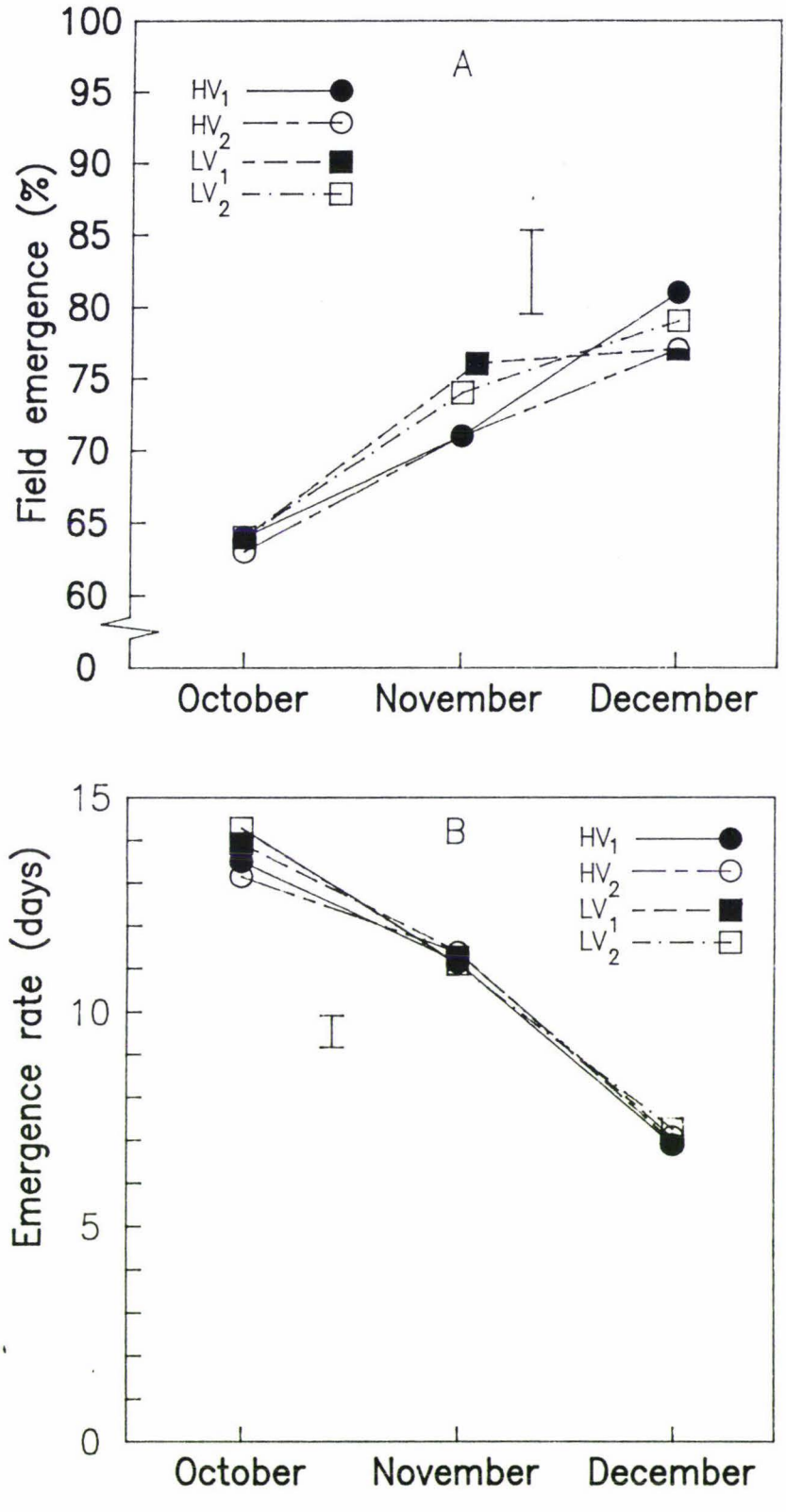

Figure 5.3 Percent field emergence (a) and emergence rate (b) of super sweet corn (cv Illini Gold) as affected by vigour and planting date. Data are means of four replicates. Bar represent a least significant difference at $P < 0.05$.

Plate 5.1 A photograph showing post-emergence damping-off (x) and seedling blight (xx).

Plate 5.2 A photograph showing plants damaged by fungi as in Plate 5.1. The plant on the left is a healthy plant.

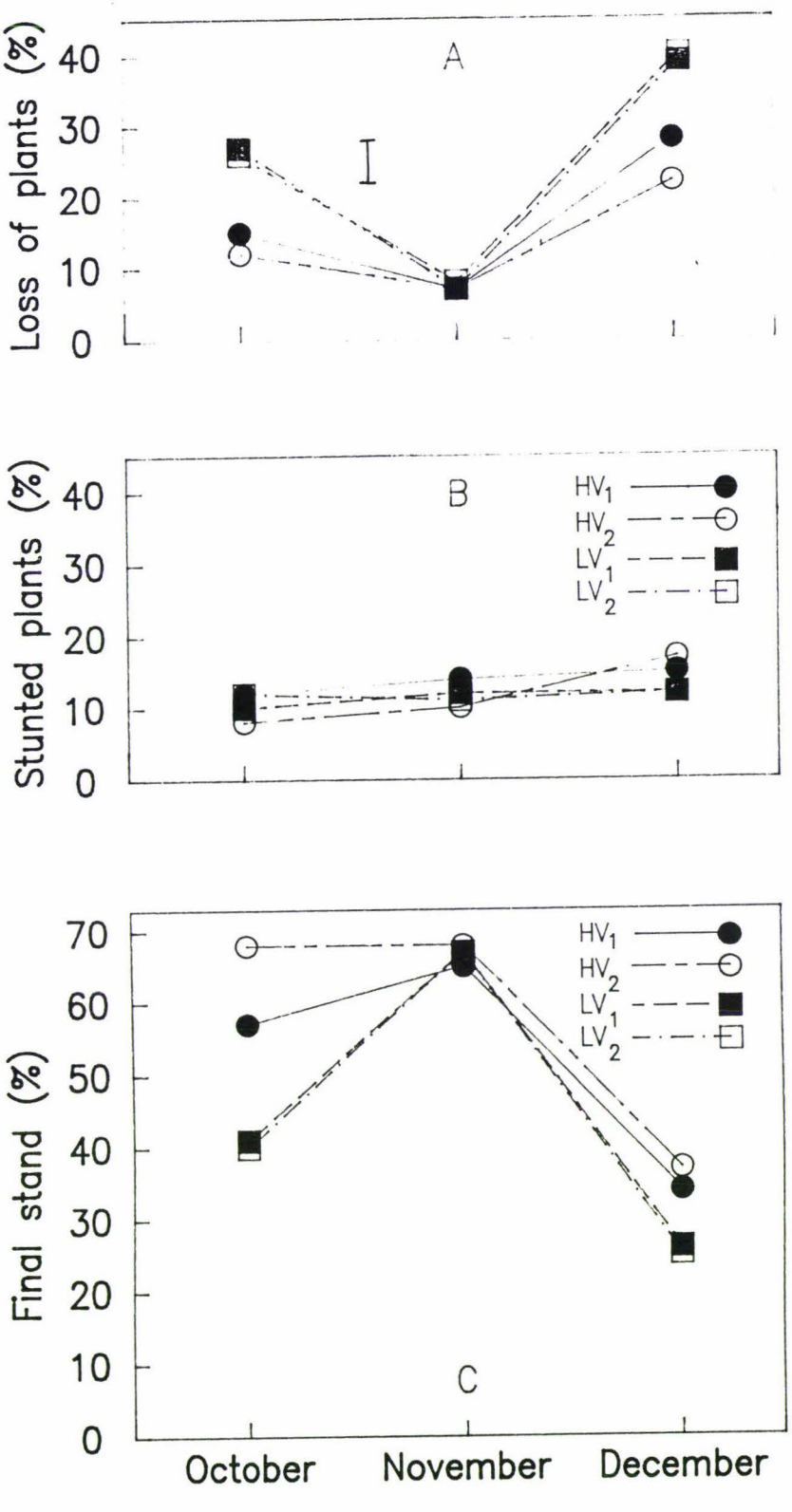

Figure 5.4 **Percent loss of plants from damping off and seedling blight (a), stunted plants (b) and final stand (c) in the field from super sweet corn (cv Illini Gold) as affected by vigour level and planting date. Data are means of four replicates. Bar represent a least significant difference at $P < 0.05$.**

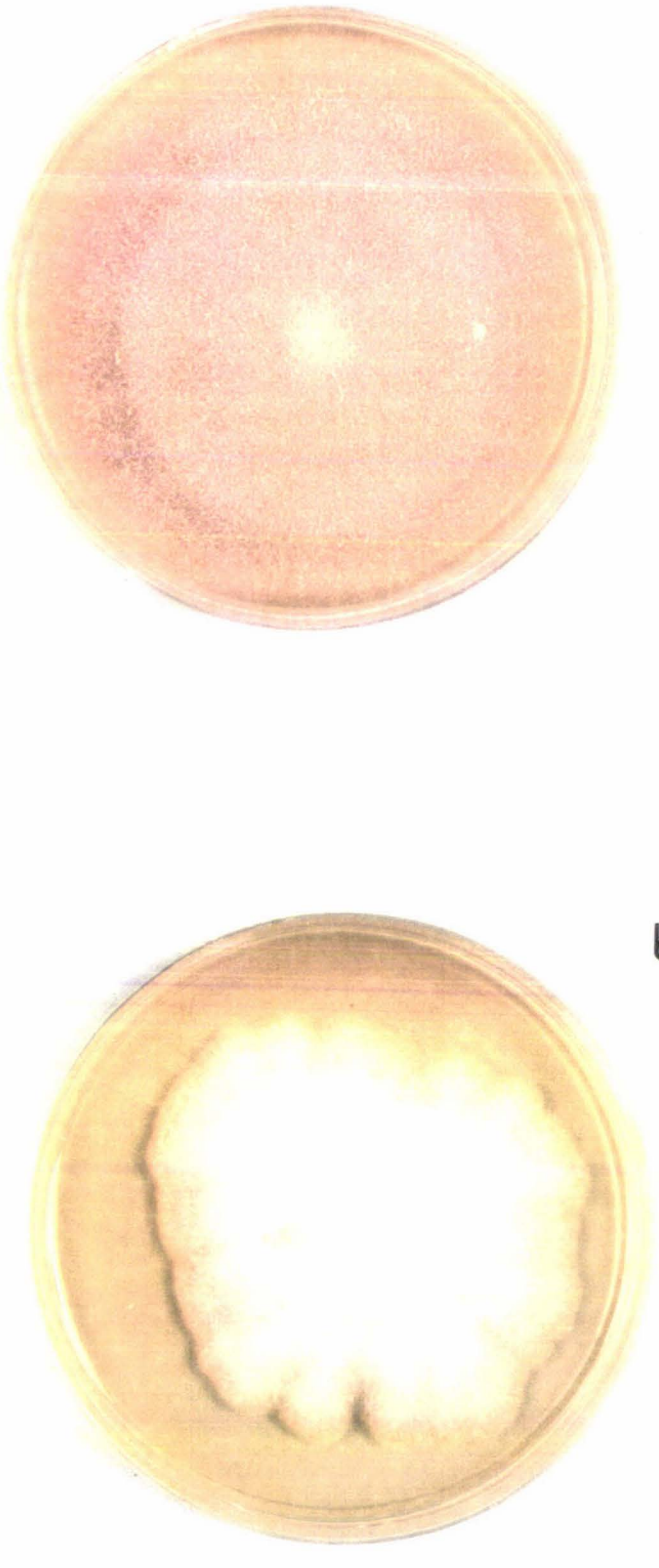

Plate 5.3 Pure cultures of *Fusarium subglutinans* (a) and *Fusarium poae* (b) cultured and isolated from field infected plant tissues.

Examination of the damaged plants indicated the damage was mostly localised on the mesocotyl areas near the ground level (Plate 5.2), and on the root system in some plants. Pathological investigation of pure cultures of inoculum isolated from the damaged parts of the plant revealed an infection of plant tissue by *Fusarium subglutinans* and *Fusarium poae* (Plate 5.3a,b) and *Penicillium* species.

Percentages of stunted plants recorded at anthesis are shown in Figure 5.4b. Neither the vigour levels nor the planting dates differed significantly. Similarly, the interaction between the two factors was not significant. In the October planting, stunted plants ranged from 8-12%, while in the November and December plantings around 10% and 15% stunted plants were recorded, respectively. Final stand in the field at silking stage showed a significant difference for vigour levels ($P < 0.001$) and planting date ($P < 0.0001$) but the combined effect of vigour levels and planting dates was not significant (Appendix 5.3b). Due to elevated losses of plants in low viguor seed lots in the October planting, high vigour (HV_1 and HV_2) seed lots had a significantly ($P < 0.05$) higher (57-68%) final stand in the field than low vigour (LV_1 and LV_2) seed lots (40%) (Figure 5.4c). There was no significant difference ($P < 0.05$) among seed lots (65-67%) for the November planting. At the December planting the lowest final stand was recorded, but because of higher losses of plants in the low vigour seed lots, high vigour lots (HV_1 and HV_2) showed a significantly ($P < 0.05$) higher (34-37%) final stand than the low vigour (LV_1 and LV_2) seed lots (24-26%). Seed lots LV_3 and LV_4 had a similar final stand as seed lots LV_1 and LV_2 in the December planting (data not shown).

5.1.4 Dry weight accumulation during vegetative growth

During vegetative growth and development, leaf, stem and total plant dry weight were determined and recorded at the 3rd, 5th and 7th leaf stages. The number of days from planting to harvesting for every leaf stage x planting date are shown in Table 5.1. Weather data are presented in Appendix 5.4. Only results from high vigour (HV_1), low vigour (LV_1) and low vigour (LV_1) planted at double rate and thinned to standard (LV_{11}) are presented. Seed lots HV_2, LV_2 and LV_{22} showed similar results.

5.1.4.1 Leaf dry weight

At the 3rd leaf stage, there were no significant differences for leaf dry weights among vigour levels for any planting. However, there was a highly significant difference in leaf dry weights among plants harvested from the different planting dates ($P < 0.0001$), but no interaction effect was observed. Leaf dry weights in plants obtained from the October

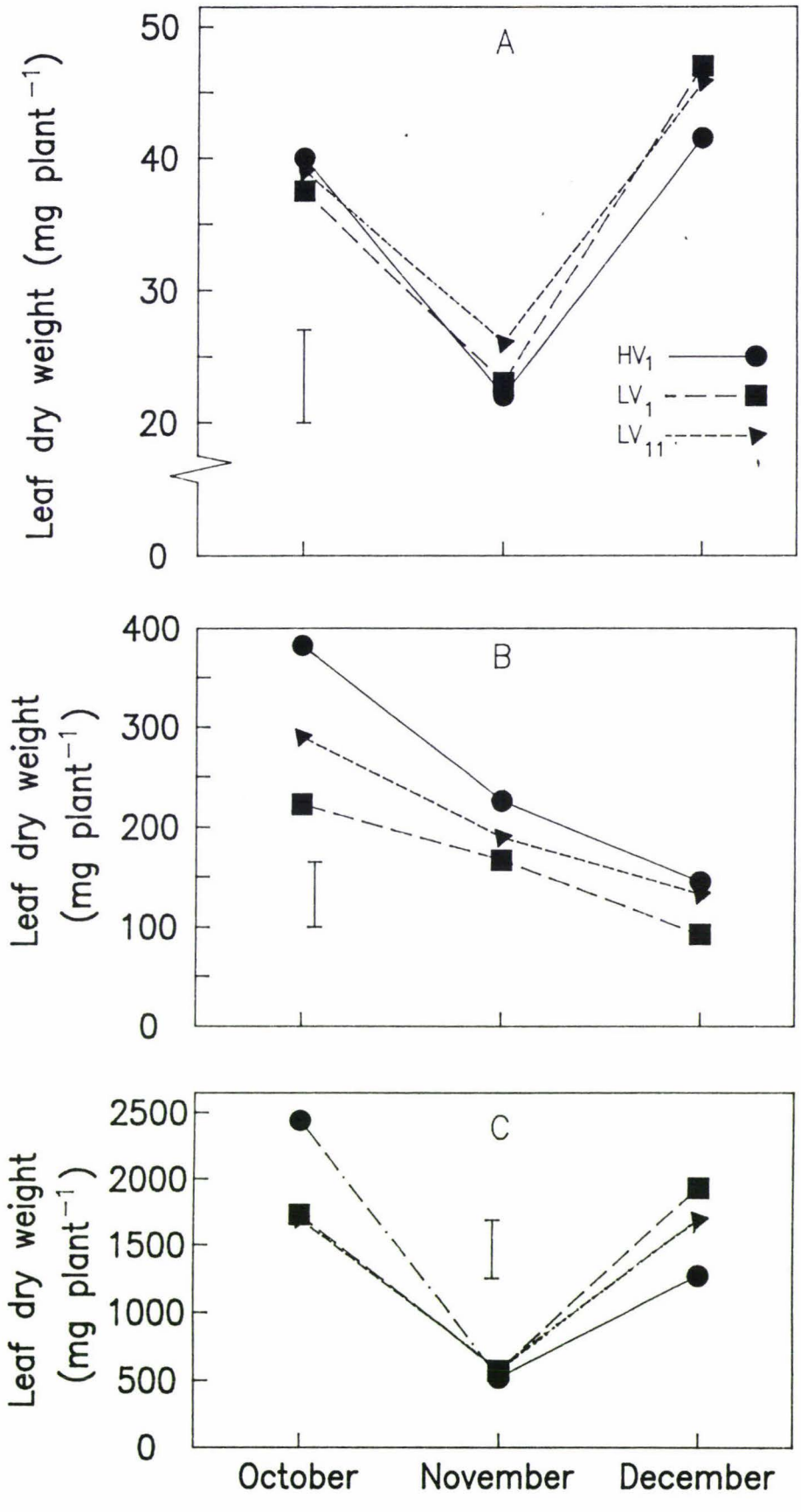

Figure 5.5 **Leaf dry weights at 3rd (a), 5th (b), and 7th (c) leaf stages from super sweet corn (cv Illini Gold) as affected by vigour and planting date. Data are means of four replicates. Bar represent a least significant difference at $P < 0.05$.**

planting ranged from 38-40 mg plant^{-1} and were significantly ($P < 0.05$) higher than that of plants from the November planting (22-26 mg plant^{-1}) (Figure 5.5a). Leaf dry weights of 42-47 mg plant^{-1} were recorded from plants harvested from the December planting; these were similar to those produced from the October planting but significantly higher ($P < 0.05$) than that from the November planting.

Table 5.1 Days from planting to harvesting for leaf stages x planting dates. Figures below days are harvesting dates.

		Days at the nth leaf stage and harvesting date		
Seed lot*	Planting date	3rd	5th	7th
HV_1, LV_1, LV_{11}[1]	October	22	37	60
HV_2, LV_2, LV_{22}		20 Nov 91	5 Dec 91	28 Dec 91
HV_1, LV_1, LV_{11}[1]	November	19	31	42
HV_2, LV_2, LV_{22}		16 Dec 91	28 Dec 91	9 Jan 92
HV_1, LV_1, LV_{11}[1]	December	13	22	31
HV_2, LV_2, LV_{22}		6 Jan 92	15 Jan 92	24 Jan 92

[1] = LV_{11} - low vigour (LV_1) planted at double rate then thinned to stand.
= LV_{22} - low vigour (LV_2) planted at double rate then thinned to stand.
* Other abbreviations refer to Table 3.1.

At the 5th leaf stage significant leaf dry weight differences were recorded for both vigour level ($P < 0.0116$) and planting date ($P < 0.0001$) but the interaction between them remained not significantly different. Plants from the October planting produced significantly higher ($P < 0.05$) leaf dry weights than those from the November planting (Figure 5.5b). Plants from the December planting produced the lowest leaf dry weight. In the October planting, seed lots HV_1 and LV_{11} produced similar leaf dry weight of 290 and 370 mg plant^{-1}, but were significantly higher ($P < 0.05$) than seed lot LV_1 (223 mg plant^{-1}). No significant differences ($P < 0.05$) in leaf dry weight (167-220 mg plant^{-1}) were recorded among seed lots in plants harvested from the November planting. In the December planting, plants from seed lots HV_1 and LV_{11} produced a similar leaf dry weight of 145 and 132 mg plant^{-1}, respectively, which were significantly higher ($P < 0.05$) than that yielded from seed lot LV_1 (92 mg plant^{-1}).

Leaf dry weights measured at the 7th leaf stage showed no significant difference among vigour levels but the planting dates differed significantly ($P < 0.0001$). The vigour level planting date interaction was also significant ($P < 0.05$). Leaf dry weights produced

from seed lots LV_1 (1735 mg plant^{-1}) and LV_{11} (1765 mg plant^{-1}) in the October planting were similar ($P < 0.05$) but significantly lower ($P < 0.05$) than that produced from seed lot HV_1 (2437 mg plant^{-1}). All seed lots (HV_1, LV_1 and LV_{11}) had a significantly higher ($P < 0.05$) leaf dry weight than those produced from the November planting (Figure 5.5c). No significant differences in leaf dry weight were observed among seed lots in the November planting (515-570 mg plant^{-1}). In the plants from the December planting, there was no significant difference ($P < 0.05$) in leaf dry weight from seed lots LV_1 (1932 mg plant^{-1}) and LV_{11} (1520 mg plant^{-1}) but these were significantly ($P < 0.05$) greater than that from seed lot HV_1 (1265 mg plant^{-1}). All seed lots (HV_1, LV_1 and LV_{11}) in the December planting produced a significantly higher ($P < 0.05$) leaf dry weight than those from the November planting. While leaf dry weight from seed lots LV_1 and LV_{11} were not significantly different from those from the October planting, seed lot HV_1 yielded a significantly lower ($P < 0.05$) leaf dry weight than that recorded in the October planting.

5.1.4.2 Stem dry weight

The plants harvested at the 3rd leaf stage demonstrated no significant differences in stem dry weight among the different vigour levels for any planting date. However, the planting date differences were significantly ($P < 0.0001$) different, but the interaction between the two factors was not significant. Plants from the October and December plantings had a similar stem dry weight (80-88 mg plant^{-1}) and were significantly greater ($P < 0.05$) than those produced from the November planting (38-45 mg plant^{-1}) (Figure 5.6a). At the 5th leaf stage, the main factors of vigour levels and planting dates differed significantly ($P < 0.0087$ and $P < 0.0001$, respectively), but the combined effect of vigour and planting dates was not significant. In the October planting, seed lots HV_1 and LV_{11} produced a similar stem dry weight (685 vs 605 mg plant^{-1}), but were both significantly greater ($P < 0.05$) than that produced from seed lot LV_1 (448 mg plant^{-1}) (Figure 5.6b). No significant differences ($P < 0.05$) in stem dry weight were recorded among seed lots for either the November (222-340 mg plant^{-1}) or December (197-295 mg plant^{-1}) plantings. The October planting yielded a significantly ($P < 0.05$) greater stem dry weight than the November or December planting.

The analysis of stem dry weight recorded at the 7th leaf stage indicated no significant differences among the different vigour levels. However, the factor of planting dates was very significant ($P < 0.0001$), while the interaction between vigour levels and planting dates also differed significantly ($P < 0.03$). Stem dry weight from seed lots LV_1 (3212 mg plant^{-1}) and LV_{11} (2855 mg plant^{-1}) recorded from the October planting were the same, but seed lot LV_{11} had a significantly lower ($P < 0.05$) stem dry weight than that

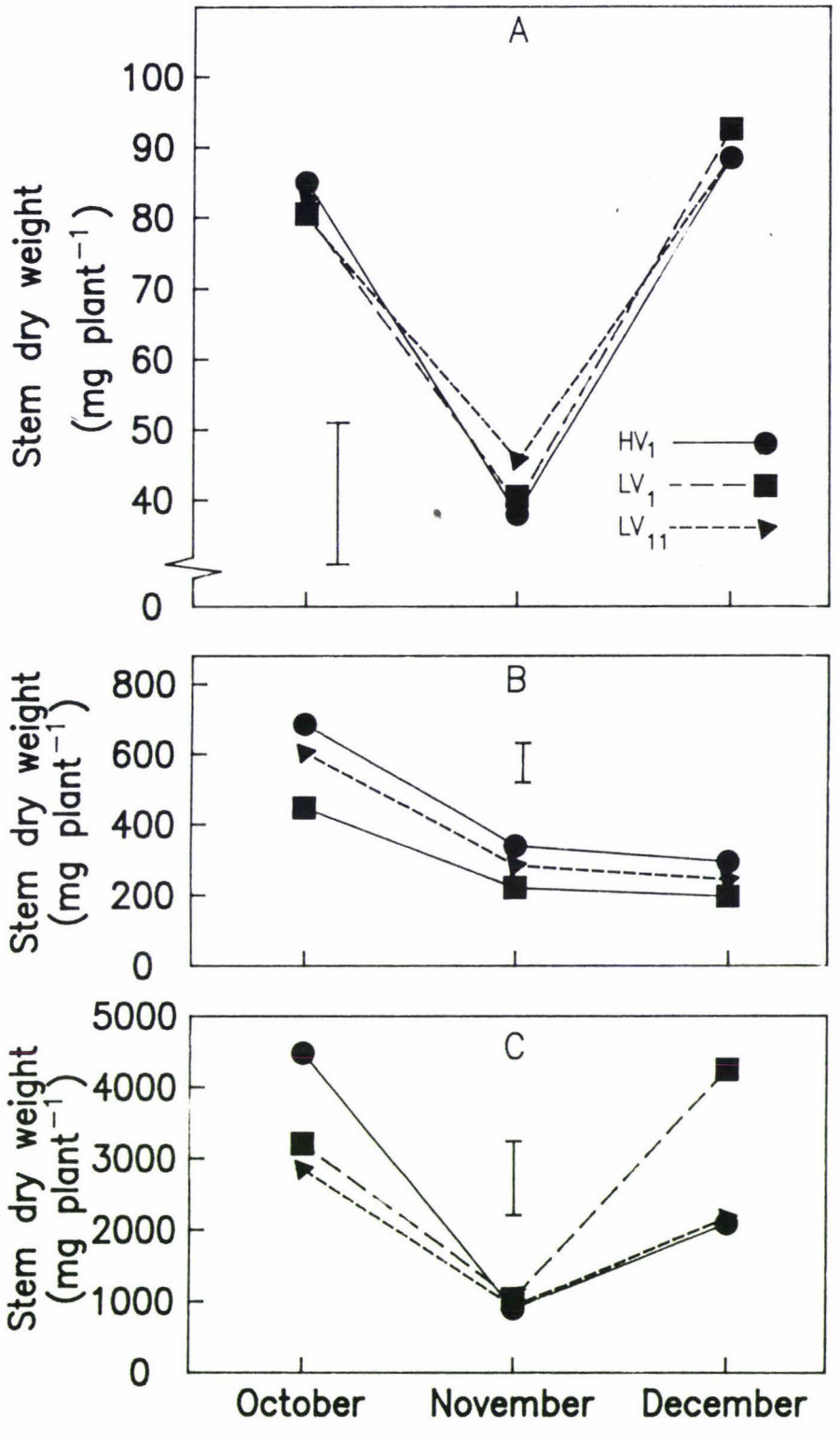

Figure 5.6 Stem dry weights at 3rd (a), 5th (b), and 7th (c) leaf stage from super sweet corn (cv Illini Gold) as affected by vigour and planting date. Data are means of four replicates. Bars represent a least significant difference at $P < 0.05$.

produced from seed lot HV_1 (4482 mg plant^{-1}) (Figure 5.6c). There was no significant difference in stem dry weight among the different seed lots for the plants harvested from the November planting (895-1040 mg plant^{-1}). In the December planting, all seed lots (HV_1, LV_1 and LV_{11}) produced a significantly higher ($P < 0.05$) stem dry weight (2087-4252 mg plant^{-1}) than those produced from the November planting, but seed lots LV_1 and LV_{11} had a similar stem dry weight, while seed lot HV_1 had a significantly lower ($P < 0.05$) stem dry weight compared to those produced in the October planting (Figure 5.6c). Seed lot LV_1 had a significantly greater stem dry weight than seed lot HV_1 or LV_{11} in the December planting.

5.1.4.3 Total plant dry weight

Total plant dry weight at the 3rd leaf stage did not differ for vigour levels, planting date or the interaction between them. Total plant dry weight in the plants from the October planting ranged from 106-131 mg plant^{-1}. In plants from the November and December plantings, total plant dry weight ranged from 88-113 and 99-120 mg plant^{-1}, respectively (Figure 5.7a).

At the 5th leaf stage, significant differences were observed for vigour levels ($P < 0.0015$) and planting dates ($P < 0.0001$), although the combined influence of these two factors was not significant. Plants harvested from the November (340-587 mg plant^{-1}) and December (290-440 mg plant^{-1}) plantings had similar total plant dry weight but were significantly lower ($P < 0.05$) than those produced from the October planting (652-1055 mg plant^{-1}). In the October planting, total plant dry weight from seed lots HV^1 and LV_{11} did not differ significantly ($P < 0.05$) but was significantly greater ($P < 0.05$) than that produced from seed lot LV_1 (Figure 5.7b). Differences among seed lots were not significant for the later two plantings.

No significant differences existed among the different vigour levels for plant dry weight at the 7th leaf stage. While the factor of planting date was highly significant ($P < 0.0001$), the interaction effect was also significant ($P < 0.03$). In the October planting all seed lots (HV_1, LV_1 and LV_{11}) produced a significantly greater ($P < 0.05$) total plant dry weight (4600-6900 mg plant^{-1}) than those from the November planting (1400-1600 mg plant^{-1}) but seed lot LV_1 (5480 mg plant^{-1}) and LV_{11} (4600 mg plant^{-1}) had a similar dry weight to that recorded in the December planting (6200 vs 4000 mg plant^{-1}) (Figure 5.7c). Seed lot HV_1 in the December planting produced a significantly (3300 mg plant^{-1}) lower ($P < 0.05$) dry weight than in the October planting (6900 mg plant^{-1}). Seed lots LV_1 (5500 mg plant^{-1}) and LV_{11} (4600 mg plant^{-1}) in the October planting did not differ although seed lot LV_1 did not differ in total plant dry weight from that of seed lot HV_1 (6900 mg plant^{-1}). In the

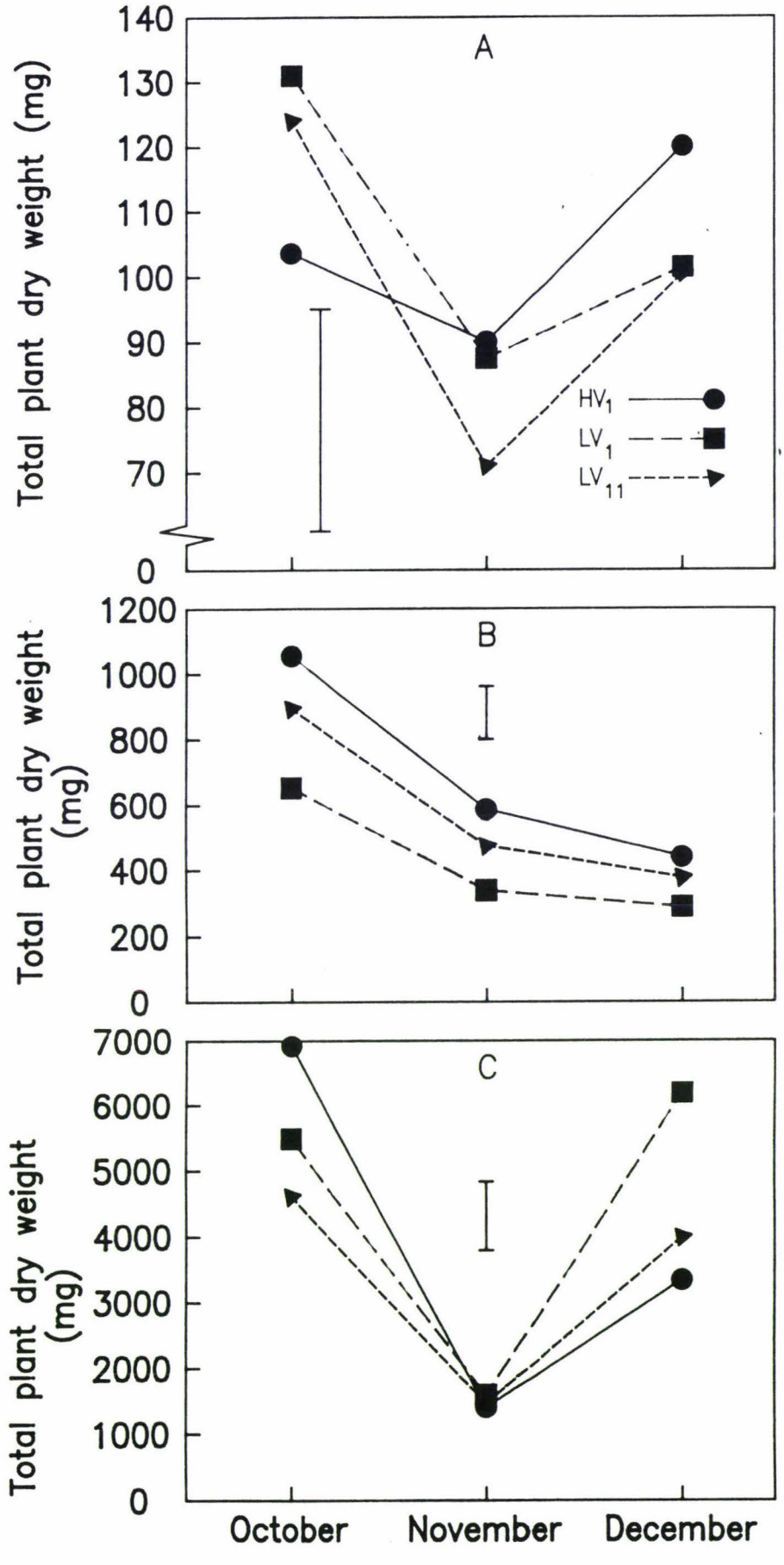

Figure 5.7 **Total plant dry weights at 3rd (a), 5th (b), and 7th (c) leaf stage from super sweet corn (cv Illini Gold) as affected by vigour and planting date. Data are means of four replicates. Bar represent a least significant difference at $P < 0.05$.**

December planting, seed lot LV_1 had a significantly greater dry weight (6200 mg plant^{-1}) than seed lot HV_1 (3300 mg plant^{-1}) or LV_{11} (4000 mg plant^{-1}).

5.1.5 Seed formation and development of super sweet corn

Days to 50% silking tended to decrease from the October planting to December planting, although the days to 50% silking in the November (78) and December (76) plantings were similar and significantly lower ($P < 0.05$) than that recorded in the October planting (87-88) (Appendix 5.5). There was no difference in days to 50% silking between high vigour (HV_1 and HV_2) and low vigour (LV_1 and LV_2) seed lots for all planting dates.

Events occurring during seed development recorded as changes in fresh and dry weights and SMC for seeds from the November planting, are presented in Figure 5.8 and the weather data during this period are shown in Appendix 5.6. There were no significant differences in fresh weight, dry weight and SMC between high (HV_1) and low (LV_1) vigour seed lots throughout the seed development period. At the early stage of seed development (27 DAP), SMC was very high (over 82%) and as the rate of moisture loss was very low, (approximately 2.9% per week). At harvest (134 DAP), SMC was still high (50.8 -51.8%). 100 seed fresh weight (FWT) was low at the 27th day following pollination (31.7 to 32.7 g). Fresh weight increased with time, and reached its maximum (about 48 g) between 41 and 48 DAP, before declining slowly to 22.9-23.8 g at 134 DAP. 100 seed dry weight (dwt) at 27 DAP was also low (5.3-5.6 g), but increased as seeds developed until 41 DAP when seed dry weight started to become stable, ranging from 10.2 to 12.6 g.

5.1.6 Seed yield and yield components

5.1.6.1 Seed yield components

The number of plants per hectare differed significantly among the different vigour levels ($P < 0.0076$) and planting dates ($P < 0.0001$). The vigour level planting date interaction was also significant ($P < 0.0087$) (Appendix 5.7a). For the October planting the number of plants in lot LV_1 (18,518 plants ha^{-1}) and LV_{11} (17,037 plants ha^{-1}) did not differ significantly, but were significantly lower ($P < 0.05$) than lot HV_1 (31,111 plants ha^{-1}) (Table 5.2a). There were no significant differences ($P < 0.05$) in the number of plants among the different seed lots in the November planting (34, 568-37,530 plants ha^{-1}). In the December planting, lot HV_1 and LV_{11} did not differ in the number of plants per hectare (17,037 versus 15,802), but had significantly more ($P < 0.05$) than that observed from lot LV_1 (12,347 plants ha^{-1}). Across planting dates, lot LV_1 and LV_{11} in the October (17,037-18,518) and December

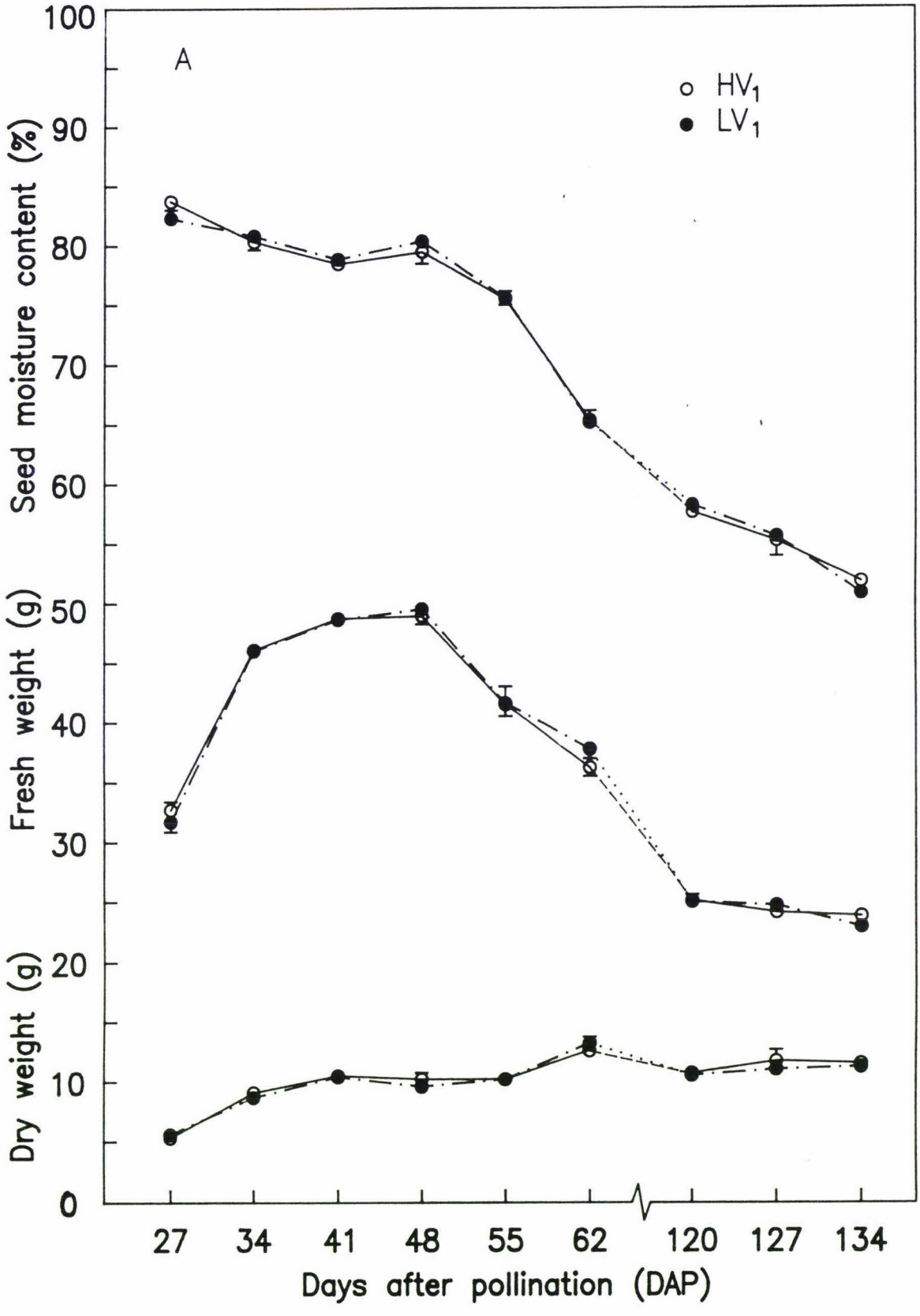

Figure 5.8 Changes in fresh and dry weights and SMC in seed lot HV_1 and LV_1 during seed development. Data are means of three replicates. Vertical bars are standard errors of individual means.

Table 5.2a **The number of plants per hectare of super sweet corn (cv Illini Gold) as affected by vigour levels and planting dates. Data are means of three replicates.**

	Planting date		
Seed lot	October	November	December
HV_1	31,111	34,568	17,037
LV_1	18,518	34,568	12,347
LV_{11}	17,037	37,530	15,802
P-level = **	lsd (P < 0.05) = 4,404		

Table 5.2b **The number of tillers per plants of super sweet corn (cv Illini Gold) as affected by vigour levels and planting dates. Data are means of three replicates.**

	Planting date		
Seed lot	October	November	December
HV_1	2	1	2
LV_1	3	1	3
LV_{11}	3	1	3
P-level = **	lsd (P < 0.05) = 0.48		

Table 5.2c **The number of cobs per tiller of super sweet corn (cv Illini Gold) as affected by vigour levels and planting dates. Data are means of three replicates.**

	Planting date		
Seed lot	October	November	December
HV_1	1	1	1
LV_1	2	1	1
LV_{11}	2	1	1
P-level = **	lsd (P < 0.05) = 0.23		

** significant at P < 0.05

Table 5.2d **The number of seeds per cob of super sweet corn (cv Illini Gold) as affected by vigour levels and planting dates. Data are means of three replicates.**

	Planting date		
Seed lot	October	November	December
HV_1	460	383	490
LV_1	425	441	404
LV_{11}	438	457	397
P-level = ns	lsd (P < 0.05) = 69.43		

Table 5.2e **Seed yield per plant (g) of super sweet corn (cv Illini Gold) as affected by vigour levels and planting dates. Data are means of three replicates.**

	Planting date		
Seed lot	October	November	December
HV_1	131.6	45.5	59.6
LV_1	255.9	42.0	64.4
LV_{11}	324.5	43.5	64.3
P-level = **	lsd (P < 0.05) = 36.9		

Table 5.2f **Seed yield per hectare (t ha^{-1}) of super sweet corn (cv Illini Gold) as affected by vigour levels and planting dates. Data are means of three replicates.**

	Planting date		
Seed lot	October	November	December
HV_1	4.0	1.6	1.0
LV_1	4.6	1.5	0.8
LV_{11}	5.5	1.6	1.0
P-level = **	lsd (P < 0.05) = 0.84		

** significant at P < 0.05
ns = not significant

(12,347-15,802) plantings had a similar number of plants per hectare, but significantly lower than that recorded in the November planting (34,568-37,530) (Table 5.2a). However, lot HV_1 in the October planting (31,111 plants ha^{-1}) and November planting (34,568 plants ha^{-1}) were greater than that observed in the December planting (17,037 plants ha^{-1}). In the December planting alone (Table 5.3), seed lots LV_1 (12,347) and LV_3 (10,370) had significantly lower plants per hectare than lots HV_1 (17,037), LV_{11} (15,802), although lot LV_1 did not differ from LV_{33} (14,074).

There was no significant difference in the number of tillers per plant among vigour levels. The planting dates differed significantly ($P < 0.0001$) but the interaction between vigour levels and planting dates was not significant (Appendix 5.7b). In the October and December plantings, plants from high population densities (eg HV_1) produced 2 tillers per plant, while those from low plant densities (eg LV_1 and LV_{11}) produced 3 tillers per plant, and had more tillers ($P < 0.05$) than those from the November planting (1 tiller $plant^{-1}$) (Table 5.2b). Tillers per plant in lot LV_3 and LV_{33} did not differ from lots HV_1, LV_1 and LV_{11} in the December planting (Table 5.3). The number of cobs per tiller differed significantly among the vigour levels ($P < 0.0065$) and planting dates ($P < 0.0001$). The vigour level planting date interaction was also significant ($P < 0.0019$) (Appendix 5.7c). Plants from the November and December plantings had an average of 1 cob per tiller compared to 1-2 cobs in the October planting (Table 5.2c). In this planting, however, plants from high plant density plots (eg HV_1) had one cob per tiller, while those from low population densities (eg LV_1 and LV_{11}) produced an average of 2 cobs per tiller (Table 5.2c). In the former lot, although some tillers had more than 2 cobs (eg Plate 5.4a), some had barren tillers (eg Plate 5.4b). The latter had an average of 2 cobs per tiller (Plate 5.4c,d). There were no significant differences in the number of seeds per cob among the planting dates or vigour levels (Appendix 5.7d). Seeds per cob ranged from 425-460 in the October planting, and 383-457 or 397-490 in the November and December plantings, respectively (Table 5.2d). In the December planting alone, seed lot HV_1 had a significantly greater number of seeds per cob (490) than lot LV_1, LV_3, LV_{11} or LV_{33} (343-405 seeds cob^{-1}) (Table 5.3).

5.1.6.2 Seed yield

Seed yield per plant or per hectare was adjusted to 15% SMC. Seeds from the October planting had the highest 100 seed dry weight (12.35 g), followed by seeds from the November planting (9.51 g), and seeds from the December planting had the lowest 100 seed dry weight of 6.08 g (data not shown).

Plate 5.4 Photographs showing the number of tillers and cobs in the high (HV_1) population density (a,b) and low population (LV_1 or LV_{11}) densities (c,d).

Table 5.3 **The number of plants per hectare, tillers per plant, cobs per tiller, seeds per cob, seed yield per plant and seed yield per hectare of different seed lots from the December planting. Data are means of four replicates.**

Seed lot	Plants ha^{-1}	Tillers $plant^{-1}$	Cobs $tiller^{-1}$	Seeds cob^{-1}	Yield $plant^{-1}$ (g)*	Yield ha^{-1} (t ha^{-1})
HV_1	17,037^{a}	2^{b}	1^{a}	490^{a}	59.6^{a}	1.0^{a}
LV_1	12,347^{cd}	3^{a}	1^{a}	405^{b}	64.4^{a}	0.8^{ab}
LV_3	10,370^{d}	2^{b}	1^{a}	343^{b}	48.1^{a}	0.5^{b}
LV_{11}	15,802^{ab}	3^{a}	1^{a}	397^{b}	64.3^{a}	1.0^{a}
LV_{33}	14,074^{bc}	3^{a}	1^{a}	350^{b}	63.8^{a}	0.9^{a}
lsd(0.05)	2,335	0.81	0	81.17	17.19	0.35

Mean values in the same coloumn followed by the same letter superscript are not significantly different at $P < 0.05$.

* 100 seed dry weight = 6.08 g.

There was a significant difference in seed yield per plant among the different vigour levels ($P < 0.0011$). The factor of planting date was highly significant ($P < 0.0001$), and the combined effect of vigour levels and planting dates was also significant ($P < 0.0002$) (Appendix 5.7e). Seed yields per plant in the November (42.0-45.5 g) and December plantings (59.6-64.4 g) did not differ significantly, but were lower ($P < 0.05$) than that found in the October planting (131-324 g) (Table 5.2e). In the October planting, seed lot LV_{11} showed a significantly greater ($P < 0.05$) seed yield of 324 g than lot HV_1 (131.6 g) and LV_1 (255.9 g), but lot HV_1 had a significantly ($P < 0.05$) lower seed yield than lot LV_1. There were no significant differences among seed lots in either the November or December plantings. Similarly, seed year per plant in lots HV_1, LV_1, LV_3, LV_{11} and LV_{33} in the December planting did not differ significantly (Table 5.3).

No significant differences in seed yield per hectare among the different vigour levels occurred, but the planting dates differed significantly ($P < 0.0001$). The interaction between these two factors was not significant (Appendix 5.7f). The seed yields per hectare in the November (1.5-1.6 t ha^{-1}) and December (0.8-1.0 t ha^{-1}) plantings were similar but lower ($P < 0.05$) than that recorded in the October planting (4-5.5 t ha^{-1}) (Table 5.2f). Interestingly,

lot LV_3 in the December planting, had a significantly lower yield than all the rest, with the exception of lot LV_1 (Table 5.3).

5.1.7 Seed harvesting and drying

The cobs from the October planting were hand harvested 145 days after silking, when seed moisture content was 30.9%. Exposing the seeds to ambient temperature of 6-16°C and RH of 69-98% for 32 days, allowed the seeds to reduce their moisture to 15.5%. The cobs from the November and December plantings were harvested 134 and 119 days after silking, with seed moisture contents of 50.8% and 63.9%, respectively. Machine drying of wet cobs harvested from these plantings showed a varying drying rate among the cob populations.

Artificial drying of wet cobs (50.8%) harvested from the November planting at 20°C for 86 hours at ambient temperature of 14-28°C and RH of 54-81% showed that 22-32% of cobs resisted drying (Figure 5.9a), as indicated by very low rates of moisture loss (0.1% per hour), and high seed moisture content (40.5%) at the end of the drying period. The remaining cobs (68-78%) dried to a seed moisture content of 15.2% at the rate of 0.4% moisture loss per hour. However, some cobs (approximately 12%) had seeds which had sprouted on the cob (Plate 5.5 - Data not shown). Similar results were observed during artificial drying of cobs (63.9% SMC) harvested from the December planting. Although the drying temperature was raised to 25°C and drying period prolonged to 126 hours, uneven drying and germination on the cob were still evident. Only 54-71% of the cobs were able to dry to seed moisture content of 14.8% at the drying rate of 0.4% per hour. The rest of the cobs (21-46%) defied drying (Figure 5.9b) dissipating moisture at the rate of 0.2% per hour and therefore had very high seed moisture content (44%) at the end of the drying period.

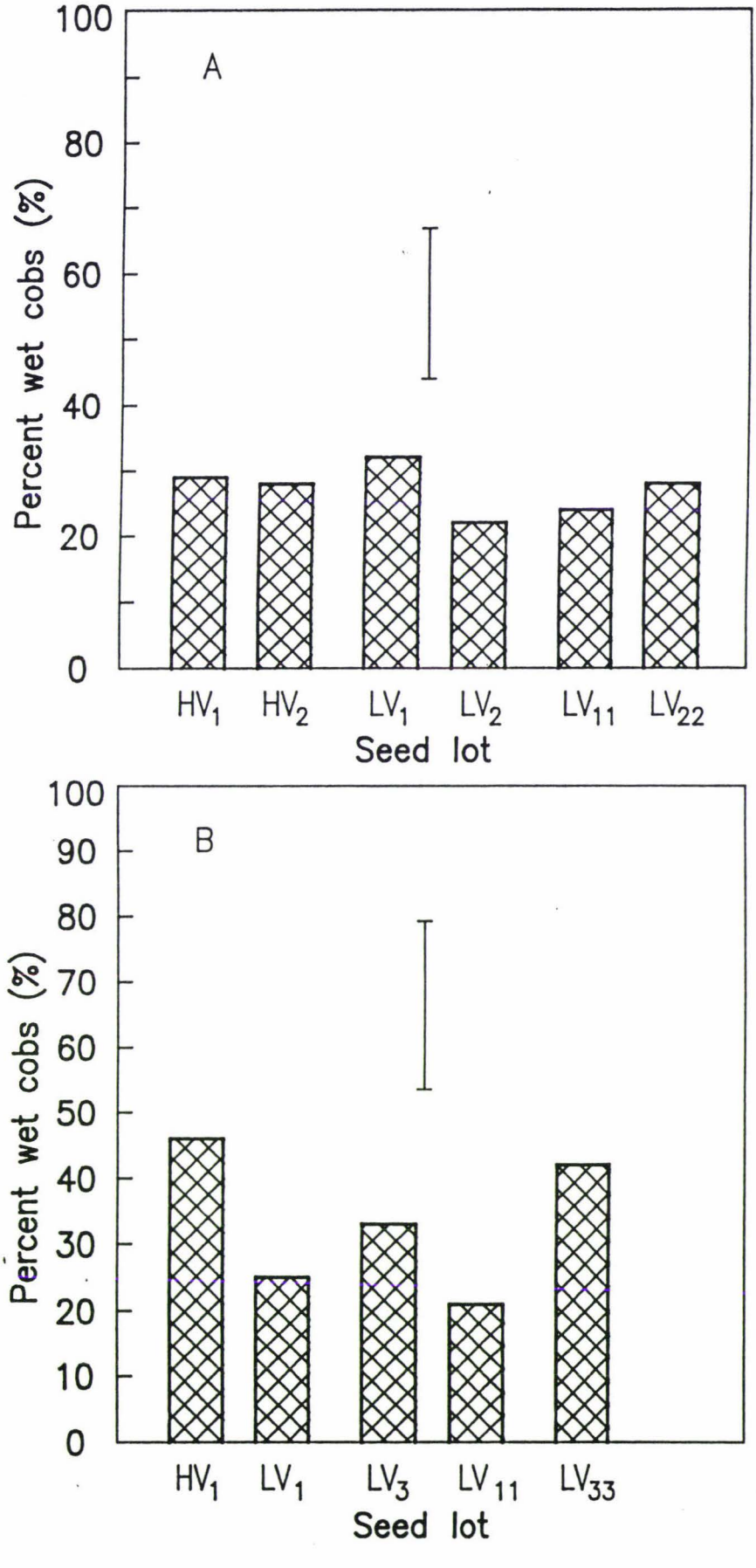

Figure 5.9 Percentage of cobs resisted drying from the November (a) and December (b) plantings. Data are means of three or four replicates. Bar represents a least significance difference at $P < 0.05$.

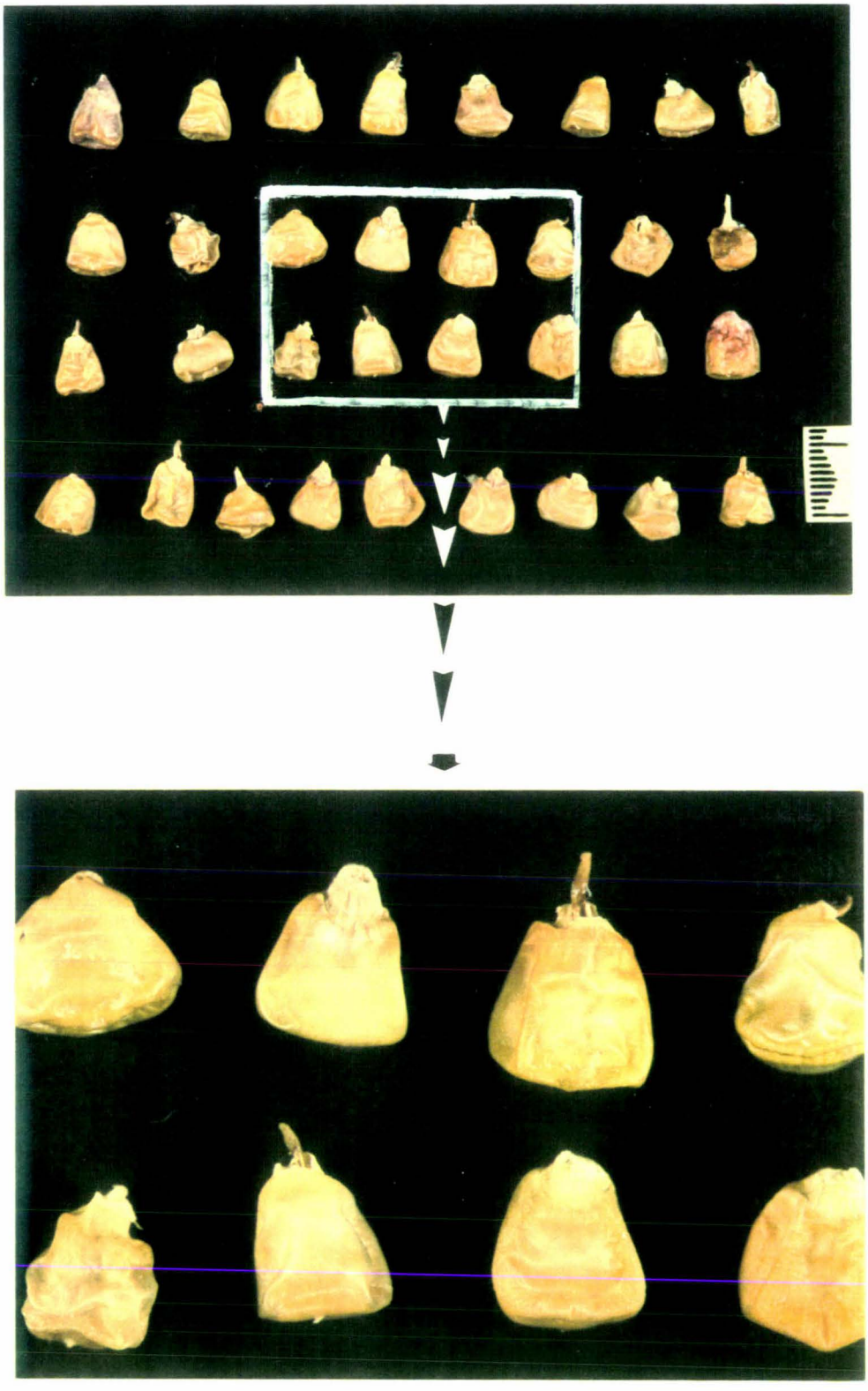

Plate 5.5 A photograph showing seeds germinated on the cob during drying of seeds harvested from the November and December plantings.

5.2 DISCUSSION

5.2.1 Weather at sowing

Failure in field germination or emergence can be caused by a number of stress factors, including availability of moisture, oxygen, temperature, planting depth, seed bed preparation, and pathogens (Herner, 1986; Matthews and Powell, 1986). Moisture is important as a solvent for biochemical reactions in seeds (Leopold and Vertucci, 1989). Dry spells resulting in low soil moisture levels can cause poor germination (Chopra and Chaudhary, 1981). Conversely, heavy rainfall and/or poor drainage results in excessive soil moisture content and this can cause a too rapid uptake of water which can disrupt the cytoplasm materials of the cells resulting in high solute leakage and/or reduce the availability of oxygen necessary for good germination. Soil temperature below 10°C can inhibit maize germination (Eagles, 1982) presumably due to chilling injury. Compact soils can delay or prevent emergence. Soils should be harrowed to produce a friable tilth with good crumb structure. A combination of lower soil temperatures and soil moisture content and an increasing planting depth, can be critical to successful seedling emergence (Perry, 1976). Infection of seeds by either seed-borne or soil-borne pathogens can damage the seeds resulting in lowered field emergence (Kruger, 1989).

The land preparation (section 3.5.3) and field environment during this study (Figure 5.1 and 5.2) did not appear to cause any stress to the seeds during planting and emergence period. This is because the seeds were planted at a depth of 2-3 cm. The soil has a good structure (Appendix 3.1) and was harrowed sufficiently to produce a friable tilth and cleft profile to allow a free root and coleoptile run. Further than that, good rain and fairly favorable soil and air temperatures provided requirements for good germination. Rain was sufficient and well distributed throughout the emergence period and the nature of the soil and environmental conditions did not allow higher and faster loss of moisture.

5.2.2 Percentage field emergence and emergence rate

Field emergence records indicated no significant differences in either percentage emergence or emergence rates between high vigour and low vigour seed lots at all planting dates (Figure 5.3 a,b). This similarity could be related to the suppressive nature or biocontrol of seed-borne pathogens by soil-borne micro-organism antagonists, or the low temperature inhibitory effect on *Fusarium subglutinans* activity, previously demonstrated in section 4.3.1.4, since the soil used for the soil cold test was drawn from the same field.

Alternatively, it may be related to a stimulatory effect of low temperature incubation on germination performance of cold resistant cultivars, as demonstrated in sections 4.3.1.4, 4.3.2.2 and 4.3.2.7. Although soil temperature (10 cm deep) for all plantings ranged from 11.9-20°C, these data were recorded at 9 am. Thus, it is most likely that soil temperature would be lower than this during the night. However, it is most probable that the environment did not exert any major stresses, so that vigour was not a factor in seedling emergence level and rate.

Both percentage field emergence and emergence rate increased from the October planting to the December planting (Figure 5.3a,b). This is interesting because it demonstrates the importance of temperature on percentage and rates of seed germination or emergence (cf Eagles and Hardacre, 1979a,b). As air and soil temperature increased, field emergence and emergence rate increased. Low soil and air temperature at the time of sowing can result in delayed and reduced seed germination and emergence (Groot, 1976). Under such conditions, seeds or seedlings are also more liable to damage by soil-borne and seed-borne fungi which cause seed rot, pre-emergence damping off and consequent reduced emergence (Iseley, 1950; Schultz and Bateman, 1968; Kruger, 1989). Presence of pericarp injuries may permit the seed-rotting micro-organisms to invade the seed and cause poor emergence. Although the application of seed dressing fungicides has greatly reduced these problems, total protection may be impossible (Eagles and Brooking, 1981; Menkir and Larter, 1987), particularly at lower soil temperatures where the seeds may be exposed to these soilborne pathogens for extended periods due to slower germination and emergence rates. Warm temperature allows germination/emergence to start early and proceed faster. The Miedama *et al.* (1982) study, for example, showed that seedling elongation rate (measured as time to emergence of seeds sown at a depth of 4 cm) was recorded as 23 days at 10°C, 8 days at 15°C, 4 days at 21°C and 2 days at 32°C. Rapid emergence has been associated with high percentage emergence (Eagles and Hardacre, 1979a; Eagles and Brooking, 1981).

5.2.3 Relationships between seed quality characters and field emergence

Relationship between seed quality characters and field emergence in any of the planting dates was poor (Appendix 5.2X). Probably these relationships were confounded by infection, antagonism or the adaptive protective mechanisms at low temperatures (see other sections). The complex stress vigour test (CSVT), however, showed a significant relationship with field emergence from the November planting, presumably because the test involves the use of sodium hyperchlorite disinfectant that probably suppressed the

pathogens. This is in agreement with Barla-Szabo and Dolinka (1984, 1989) who suggested that the application of the complex stressing vigour test and a new estimation model load is a more accurate and reliable prediction of field emergence than other tests. Isely (1957) proposed a new model based on division of the seed lot into several parts, according to the results of germination and vigour tests, after which the field behaviour of each part of the lot was determined. By uniting Isely's model with the CSVT method, a more accurate and reliable system was devised for the prediction of field emergence. The test has been used successfully for about 200,000 hectares of maize fields in Hungary (Barla-Szabo *et al.*, 1989). However, the reason(s) for the failure of CSVT to predict field emergence in the October and December planting (Appendix 5.2) is not known exactly, but mayhave been related to both the seed factors, eg susceptibility to seed-borne pathogens, adaptive protective reactions, etc, and environmental factors, eg soil suppressiveness to seed-borne pathogens, temperature, etc, which might have confounded the relationship between CSVT and field emergence.

5.2.4 Losses of plants, stunted plants and final stand in the field

Loss of plants due to post-emergence damping-off and seedling blight (Plates 5.1 and 5.2) were significantly higher in the October planting than in the November planting, while the December planting had the greatest losses (Figure 5.4a). It appears that losses due to diseases were related to the influence of weather on host predisposition, especially low moisture stress immediately after maximum emergence. In the October planting, maximum emergence was reached around 11 November 1991, and was followed by some dry spells before plant loss was recorded on 5 December 1991 (Appendix 5.4). Maximum field emergence in the November planting was recorded on 7 December 1991, and the rain distribution following emergence was fairly good until when plant loss was counted on 28 December 1991. In the December planting, severe moisture stress, coupled with elevated temperatures, persisted from immediately after maximum emergence (31 December 1991) until when plant loss was recorded on 15 January 1992. Many workers (eg Christensen and Wilcoxson, 1966; Kommedahl and Windels, 1981; Burgess *et al.*, 1981; Gilbertson *et al.*, 1985) have observed that *Fusarium* stalk rot caused by *Fusarium subglutinans* and *Fusarium moniliforme* predominates in warm and dry regions rather than in cold and wet environments. The dry conditions that prevailed following maximum emergence in the October and December plantings probably predisposed the plants to post emergence damping-off. Water stresses are known to limit many normal and necessary physiological processes within higher plants. Specifically, inadequate water supply may cause strain or tension on the plant, or weakening of it (Cook, 1973). If the particular low water potential

is adverse to the host but does not impose comparable constraints on the pathogen, then disease development is favoured (Cook, 1973). It appears that the plant water potentials in the October and December plantings were harmfully low to the host physiology, but were nearly ideal for the pathogens which were able to attack the weakened plants and increase plant loss levels. In the November planting, presumably because of the high water potential, losses due to post-emergence damping-off was minimal. Cook (1968, 1973) suggested that under such conditions the pathogens (including *Fusarium* species) establish themselves in the plant but the disease remains mild, often inconspicuous, until water stress begins.

Deteriorated seed lots in the October and December plantings had a significantly higher loss of plants than unaged seed lots, while the November planting showed no significant differences among seed lots (Figure 5.4a). These data are of particular interest because they have revealed vigour differences which were not apparent during emergence. From these results, it appears that the effect of seed vigour may not always necessarily manifest itself in the percentage field emergence or emergence rate, but may become obvious during the subsequent growth and development of the seedling/plant, particularly when the climatic, abiotic or edaphic factors are harmful to deteriorated seed lots.

Examination of the damaged plants showed the damage was mostly localised on the mesocotyl areas near ground level (Plate 5.2). This is typical of *Fusarium* and/or *Penicillium* diseases (Johann and Holbert, 1931; Shurtleff, 1980; Kommedahl and Windels, 1981; Halfon-Meiri and Solel, 1990). Pathological investigation of pure cultures of inoculum isolated from the damaged plant parts indicated an infection of plant tissue by *Fusarium subglutinans*, *Fusarium poae* (Plates 5.3a,b) and some *Penicillium* species. These results suggests the loss of plants post emergence was not only due to seed-borne pathogens, but also soilborne pathogens (eg *Fusarium poae*). Incidences of *Fusarium* diseases in the field used for this study has been noted previously (Hampton, 1993, pers. comm.[1]). Apart from seed-borne pathogens, germinating seeds may be attacked by a number of soilborne fungi that cause seed rot and seedling blights. The massive leakage of solute (section 4.2.4) probably stimulated spore germination and mycelial growth in the soil and thus aided the infection process (cf Short and Lacy, 1976; Agarwal and Sinclair, 1987). Severe infection may kill the embryo before germination (seed rot) or cause pre- or post-emergence damping-off. The severity of the disease is affected by mechanical injury to the pericarp, abiotic factors and genetic resistance to infection - sweet corn is more susceptible than dent maize (Shurtleff, 1980).

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The percentage of stunted plants recorded at anthesis showed no significant difference for either the different vigour levels or planting dates (Figure 5.4b). However, final stand in the field showed a significant difference among seed lots and plantings dates. Due to an increased loss of plants in low vigour seed lots from the October and December plantings, high vigour seed lots had a higher final stand in the field than low vigour seed lots (Figure 5.4c). The December planting had the lowest final stand in the field because it had the highest loss of plants post-emergence, intensified by the severe water deficiency and increased air and soil temperatures than the other treatments. These results disagree with Perry (1980) and TeKrony and Egli (1991) who suggested that total emergence determines plant establishment or plant density; and attribute final stand to many adverse factors during growth and development of the crop. The effects of these adverse conditions can be severe when low vigour seeds with severe pericarp damage or which are heavily contaminated with pathogen are planted (Heydecker, 1977; Shurtleff, 1980; ISTA, 1987; Powell, 1988).

5.2.5 Dry mass accumulation during vegetative development

Although the factors determining leaf dry weight such as leaf initiation and appearance rates, leaf number, leaf elongation, leaf expansion and leaf area are under genetic control, environmental variables such as rainfall, temperature, etc, can influence one or all of these factors and therefore leaf dry weight (Warrington and Kanemasu, 1983a,b,c; Hay and Walker, 1989; Kuipa, 1964; Boyer, 1970). Leaf initiation rates and leaf appearance rate are influenced by temperature, and increase with increases in temperature (Beauchamp and Lathwell, 1966; Warrington and Kanemasu, 1983b). Leaf expansion and elongation are also largely controlled by temperature and the rate of expansion increases with temperatures between 10°C and 30°C (Beauchamp and Lathwell, 1966), while leaf elongation decreases from 28°C to 10°C (Barlows and Boersma, 1972). Water stress can also cause a decrease in leaf elongation and expansion (Kuiper, 1964; Boyer, 1970).

In this study, the analysis of leaf dry weight at the 3rd leaf stage showed no significant differences among seed lots (Figure 5.5a). However, although the plants harvested from the October planting were older (22 days) than those from the December planting (13 days - Table 5.1), they had a significantly similar and greater leaf dry weight than that recorded from the November planting (19 days old) (Figure 5.5a). This is probably because of differences in both emergence date and temperatures. In the October planting temperature was lower than for later plantings, but leaf dry weight was higher than that of plants from the November planting because of a longer vegetative period (Table 5.1) which probably allowed for greater leaf expansion or leaf area development. Plants from the

December planting had the shortest vegetative period but had leaf dry weight similar to that recorded in the October planting, presumably because of increased temperatures (Appendix 5.4) that might have stimulated faster leaf growth, and hence increased leaf dry weight. Plants harvested from the November planting had the lowest leaf dry weight, presumably because the low temperatures (Appendix 5.4) may have an inhibitory effect on leaf initiation and appearance rate, leaf elongation or leaf expansion, and lowered dry weight as a result. Cal and Obendorf (1972) demonstrated that seedlings at cold temperatures resulted not only in delayed leaf elongation and leaf area expansion, but also in delayed leaf dry matter accumulation. This gained support of Struik *et al.* (1982) who reported that high dry matter yield depended on high temperatures during early plant growth.

At the 5th leaf stage, plants from the October planting had a significantly higher leaf dry weight than that of plants from the November plantings, but the November planting produced more leaf dry weight than that of plants from the December planting (Figure 5.5b). This disparity could be in part due to differences in vegetative growth duration. The time (days) to 5th leaf appearance decreased from the October to the December plantings (Table 5.1). Alternatively, the high plant density in the November planting probably created interplant competition that could reduce leaf size and be reflected in reduced leaf dry weight. The severe reduction in leaf dry weight in the December planting may have been due to the persistent severe water stress during vegetative growth and up to the 5th leaf stage (Appendix 5.4) which caused a decrease in water uptake by the plant and affected plant growth, resulting in lowered leaf dry weights. Kuiper (1964) reported that water stress caused a decrease in leaf expansion and growth, and Boyer (1970) reported similar findings.

An interesting observation from this measurement was that, although there was no significant difference in percent emergence/rate among seed lots in all planting dates, there was a significant difference in subsequent growth between high vigour and low vigour seed lots in all plantings but the November planting, as demonstrated by differences in early leaf dry weight accumulation (Figure 5.5b). Many workers (eg Anderson, 1970; Heydecker, 1977; Dickinson, 1980; Ven de Venter, 1990) have suggested that germination/emergence and seedling/plant growth, although closely related, are regulated by different mechanisms which seem to operate independently of deterioration or are limited by different conditions during germination and growth.

Leaf dry weight recorded at the 7th leaf stage showed that plants from the November planting had lower leaf dry weight than the October and December plantings (Figure 5.5c). This is probably the effect of plant density. The higher population density in

the November planting is likely to have caused higher inter-plant competition, resulting in smaller sized leaves of similar dry matter. The high vigour (HV_1) seed lot in the October planting had a significantly higher leaf dry weight than low vigour (LV_1, LV_{11}) seed lots. While no differences in leaf dry weight among seed lots in the November planting, low vigour (LV_1, LV_{11}) seed lots showed significantly higher leaf dry weights than the high vigour (HV_1) seed lot in the December planting (Figure 5.5c). Once again this may be in part due to plant population differences. Low vigour seed lots in the December planting had a higher loss of plants post emergence than the same seed lot in the October planting (Figure 5.4a). The reactions of the survived plants to increased spacing resulting from losses, was a greater production of tillers; and although both the October and November plantings produced about the same number of tillers (Table 5.2b), those in the December planting produced thicker tillers than the October planting, which might have increased leaf dry weight in low vigour seed lots compared to high vigour seed lots (Figure 5.5c).

Stem dry weights at the 3rd leaf stage from the October and December plantings were similar and were significantly greater than those produced from the November planting (Figure 5.6a). Stem dry weight is an expression for the number and length of stalk nodes. Although the maximum number and length of nodes is genetically controlled, environmental variables such as temperature may be important. Blacklow (1972) and Miedama (1982) studied the response of young plants to temperature and found a pronounced effect on shoot and root growth. Shoot growth increased with increasing temperature. This was interpreted as being due to a proportionally higher extension rate of the mesocotyl. The mesocotyl length at 12°C was 54% of total shoot length, at 18°C was 64%, and at 24°C was 70%. This may explain the lowered stem dry weight at low temperature in the November (Appendix 5.4) planting, and increased stem dry weight at high temperature (Appendix 5.4) in the December planting (Figure 5.6a). High temperatures increase the rate of elongation of the nodes, thus increasing plant height and hence stem dry weight (Cal and Obendorf, 1972; Badu-Apraku *et al.*, 1983). The cause of increased stem dry weight in the October planting was probably because, although the elongation of stalk nodes may be low due to lower temperatures, the growth duration in this treatment was the longest (Table 5.1) which enabled the plants to slowly grow and increase their nodes number or length.

At the 5th leaf stage, both vigour and planting date differences were evident. As for leaf dry weight, seed lots HV_1 and LV_{11} in the October planting produced a significantly greater stem dry weight than the LV_1 seed lot, and as expected, the November planting showed no significant difference between aged and unaged seed lots (Figure 5.6b). The observed vigour differences in stem dry weight may further confirm the idea presented

earlier that the effects of the ageing treatment were targeted at the growth mechanisms rather than germination mechanisms (cf Anderson, 1970; Van de Venter, 1990). Evidence of a positive association of seed vigour with early plant growth has been previously reported in maize (eg Glenn *et al.*, 1974; Burris, 1975). On the other hand, infection of seeds by seed- or soil-borne pathogens has been reported to stunt plant growth or induce wilting (Neergaard, 1977; Agarwal and Sinclair, 1987), and thus reduce leaf and/or stem dry weights. In the December planting, however, while leaf dry weights differed between high and low vigour seed lots, and were the lowest of all planting dates (Figure 5.5b), stem dry weights showed no significant difference among seed lots and were similar to that observed from the November planting (Figure 5.6b). Once again this may be associated with the severe water stress (Appendix 5.4). An interesting observation from these data was that stems were much less sensitive to water stress than were leaves, especially for plants from the low vigour seed lots (Figure 5.5b).

The analysis of stem dry weight at the 7th leaf stage indicated that the observed differences in stem dry weight between high vigour (HV_1) and low vigour (LV_1) seed lots at the 5th leaf stage in the October planting (Figure 5.6b), disappeared as growth progressed (Figure 5.6c). Previous work by Akintorin-Adegbuyi (1985) and Adegbuyi and Burris (1989) also showed that vigour effects affected seedling/plant growth only at the early stage of development but that, as the plant grew, the influence of seed vigour decreased, particularly being influenced by vigour levels, plant density and the ever-changing environmental interactions (Egli and TeKrony, 1979). Plants from the November planting produced a consistently lower stem dry weight despite the rise in temperature. Apart from intense inter-plant competition at this stage, earlier studies have indicated that cold soils appear to be most critical for biomass accumulation following emergence. This was illustrated by Mederski and Jones (1963) who observed about 50% more dry matter deposition when soil temperature was increased at seeding than during plant emergence. The increased stem dry weight in the December planting was apparently due to rapid growth of plants at higher temperature accompanied by thicker tillers, because of the lower plant population which resulted from greater plant losses. Total plant dry weight (Figures 5.7a,b,c) showed a response similar to the corresponding stem dry weights at the 3rd, 5th, and 7th leaf stages.

5.2.6 Seed formation and development

The number of days to 50% silking tended to decrease from the October to December planting, although the days to 50% silking in the November and December planting were similar but significantly lower than that recorded from the October planting

(Appendix 5.6). This is in agreement with the work of Cal and Obendorf (1972) and Warrington and Kanemasu (1983a) who have reported that an increase in temperature resulted in the acceleration of developmental rates as evidenced by substantial reduction in days to silking. All seed lots showed no significant differences in days to 50% silking, suggesting that they started the grain filling phase at the same pace.

The physiological changes during seed development are shown in Figure 5.8. Many studies have been done to determine the stage of mass maturity (physiological maturity) and harvest maturity of various crops in order to guide seed growers in deciding when crops reach the right time for harvest. Such studies are important in ensuring harvest of maximum quality.

Changes in seed moisture content (SMC), seed fresh weight (FWt) and seed dry weight (DWt) during seed development (Figure 5.8) indicated stage II and III of seed development of Shrunken-2 cv. Illini Gold (section 2.4.3.1). The food reserve accumulation phase (stage II) of seed development ended at about 41-48 days after pollination (DAP). This stage is usually marked by a continued, often linear, increase in dry weight, lower or no increase in fresh weight and SMC starts to fall (Bryant, 1985; Coolbear, 1990). At the end of this phase (41-48 DAP) seeds reached mass maturity, denoted by attainment of "peak dry weight" (Brooking, 1990). However, the DAP to mass maturity in this experiment was longer than the 34-38 DAP in shrunken-2 cv. "Florida sweet" (Styer and Cantliffe, 1983) but similar to the 43-47 DAP in shrunken-2 cv. "Florida staysweet" (Wilson and Trawatha, 1991). This disparity is possibly related to cultivar differences or environmental conditions during seed development. Sayed and Gadullah (1983), working on several genotypes of cereal crops, suggested that at the end of stage I, individual seed dry weight is a function of the effective grain filling rate (EGFR) and duration of the filling period. The former is always constant and is not sensitive to the changes in environmental conditions, while the latter tends to be affected by environmental factors (cf Badu-Apraku *et al.*, 1983).

The changes in SMC during seed development (Figure 5.8) showed a very slow and linear loss of moisture after mass maturity (cf Wilson and Trawatha, 1991). Whether this was caused by genotypic characteristics of this species or is merely an environmental influence, or both, is not clear, because while moisture loss proceeds at a falling rate following mass maturity (Brooking, 1990), this process can be affected by ambient temperature and relative humidity (Bryant, 1985) - see also section 2.4.3.2. In this study, air temperature and relative humidity after mass maturity ranged from 1°-18.5°C and 69-98% RH, respectively, and may be at least in part been responsible for the observed slower drying rates. On the other hand,

the concentrated sugars in the shrunken-2 endosperms may resist drying (Wilson and Trawatha, 1991) and this too may result in slower drying rates.

5.2.7 Seed yield and seed yield components

Analysis of plants hectare^{-1} showed that HV_1 seed lot in the October planting had a significantly higher number of plants hectare than seed lots LV_1 and LV_{11}, while seed lot LV_1 in the December planting had a significantly lower number of plants hectare^{-1} than seeds lots HV_1 or LV_{11} (Table 5.2a). There were no significant differences in the number of plants hectare^{-1} among seed lots in the November planting. The significant losses of plants due to post-emergence damping-off and seedling blight previously reported in aged seed lots from the October and December plantings (section 5.1.3) must be responsible for the lower number of plants hectare^{-1}. Across the planting dates, although the plant losses in low vigour seed lots were significantly higher in the December planting than in the October planting, when recorded at the 5th leaf stage, seed lots LV_1 and LV_{11} in the October and December plantings showed similar numbers of plants hectare^{-1} at maturity, which were also lower than those recorded from the November planting. These results suggest that aged seed lots in the October planting continued to die after the 5th leaf stage but in December, because of greater stress, from diseases and water deficiency, all seedlings had died at the 5th leaf stage. HV_1 seed lot in the December planting had less plants hectare^{-1} compared to that from the October and November planting (Table 5.2a). This may highlight the severity of the combined effect of diseases and water stresses.

Evaluation of number of tillers plant^{-1} showed that plants from high density (eg HV_1) produced 2 tillers plant^{-1}, while those from the low plant densities (eg LV_1, LV_{11}) produced 3 tillers plant^{-1}. In the highest plant density (November planting) an average of 1 tiller was produced from each plant (Table 5.2b). This is obviously the effect of plant density levels. The higher losses of plants in low vigour seed lots from the October and November plantings lessened the interplant competition, and thus the plants were able to grow and become vigorous and produce more tillers. Assessments of the number of cobs tiller^{-1} indicated that although plants from the October and December plantings had a similar number of tillers, plants from the December planting had 1 cob tiller^{-1}, while those from the October planting produced 1-2 cobs; with plants from high density (eg HV_1) producing 1 cob tiller^{-1} while those from low density (eg LV_1 and LV_{11}) produced 2 cobs tiller^{-1} (Table 5.2c). This is probably because the prolonged drought (April to May - Appendix 5.6) had a greater effect on young plants (December planting) than mature plants (October planting), causing

inadequate soluble carbohydrate supply to sustain tiller reproductive growth, resulting in barren tillers.

Measurements on the number of seed cobs^{-1} showed no significant difference either among the different seed lots or planting dates (Table 5.2d). The number of seeds can be restricted at the cob initiation stage or at pollination. High temperature can affect pollination and kernel set but usually the environmental restriction is moisture stress (Shaw, 1977). Temperature stress may delay tasseling but more so silking, such that silk emergence may occur when most or all the pollen base been shed, resulting in barrenness or poorly filled cobs (Stuik *et al.*, 1985). Alternatively, water stress may cause asynchrony in floral development, egg sac abortion (Moss and Downey, 1971; Herrero and Johnson, 1981) or zygote abortion since water deficiency influences photosynthesis and reduces carbohydrate reserves at pollination (Westgate and Boyer, 1986), resulting in fewer seeds. In this study, pollination and seed set occurred around 27 January 1992 for the October planting, 14 February 1992 for the November planting and 10 March 1992 for the December planting; where the temperatures and moisture levels experienced by plants (Appendix 5.4 and 5.6) did not appear to harmfully affect seed set and hence the similarities in number of seeds cob^{-1}. However, within the December planting, seed lot HV_1 had a significantly greater number of seeds cob^{-1} than the rest (Table 5.3), presumably because of increased temperatures during seed set (Appendix 5.6). An increase in seed number per cob as a result of high temperatures at cob initiation stage has been reported (eg Cooper and Law, 1977), although this was considered to be the effect of temperature on the number of viable seed sites rather than on the total number formed. However, the reason why the number of seeds cob^{-1} were less in seed lots LV_1, LV_3, LV_{11} and LV_{33} than in seed lot HV_1 is not clear. Maybe the combined effects of high temperature, diseases and water stress experienced following seed set had an effect on the number of viable seed sites in the former rather than in the latter, resulting in fewer seeds cob^{-1}.

Another interesting feature of the seed yield components was that seed weight varied greatly among the different planting dates. One hundred seeds from the October planting had the highest dry weight, followed by seeds from the November planting, and the December planting had the lowest dry weight (data not shown). The possible explanation for this is that due to loss of plants post-emergence in the October planting; probably there was competition for nutrients, light, etc, allowing the seeds to deposit reserves during the grain filling period. The lower 100 seed dry weight in the November planting was possibly because of high competition caused by higher plant populations. The reduced seed weight in seeds from the December planting was probably due to the prolonged water stress

experienced during the grain filling period (April to May - Appendix 5.5) rather than the killing frost on 23 April 1992. This is because based on seed development studies from the November planting (sections 5.1.6 and 5.2.5) mass maturity (maximum dry weight) for the December planting would have occurred before or around the killing frost, and would not affect the seed's dry weight to such a great extent. Delouche (1980) suggested that an acute deficiency of water resulting from temporary but severe deficiencies can have disastrous effects because once seeds are set, many crops have little capacity to adjust seed number to the available assimilates, and the result is low weight and small sized seeds. Alternatively, it may be due to differences in the effective grain filling period (EGFP) caused by differences in days to pollination (section 5.1.5). Plants from the October planting had the longest time for accumulation and deposition of assimilates, while those from the December and November plantings had the lowest and intermediate, respectively.

Analysis of seed yield plant^{-1} showed that the November and December plantings had a similar seed yield plant^{-1} but were lower than that found in the October planting (Table 5.2e). Although seeds from the December planting had the lowest dry weight, they had more tillers plant^{-1} with a consequent increase in total number of cobs plant^{-1}, resulting in a seed yield plant^{-1} similar to that recorded in the November planting. The October planting produced the highest seed yield plant^{-1} because it had more cobs plant^{-1} and higher seed weight than that produced from the November and December plantings. While there was no significant differences in seed yield plant^{-1} among seed lots in the November and December plantings, the seed lots in the October planting differed significantly, with seed lot HV_1 having a lower yield/plant than either of the other two seed lots (Table 5.2e). This is apparently because of differences in plant population and tiller numbers. Seed lots LV_1 and LV_{11} had more tillers than seed lot HV_1, which meant an increase in the total number of cobs plant^{-1} and thus the seed yield plant^{-1}.

As for seed yield plant^{-1}, seed yields hectare^{-1} in the November and December plantings were similar (Table 5.2f). These similarities were probably because, although the December planting had initially a lower number of plants hectare^{-1} and seed weight than the November planting, the December planting compensated by producing more tillers and therefore offset the differences in seed yield hectare^{-1}. Plants from the October planting had higher seed yield hectare^{-1} than either the plants from the November or December plantings because they had produced higher numbers of cobs plant^{-1} and also had higher seed weight than the rest. Interestingly, although differences in plants hectare^{-1} and tillers plant^{-1} existed among the different seed lots, there was no significant differences in seed yield hectare^{-1} among the different seed lots because low vigour seed lots counterbalanced by producing

more tillers. However, within the December planting, seed lot LV_3 produced a lower seed yield hectare^{-1} than the rest, because of the very low number of plants hectare^{-1}, and although it produced more tillers, the environmental condition mentioned earlier did not allow the plants to initiate reproductive growth, resulting in barren tillers.

It is clear from these data that vigour status was not an important factor affecting seed yield. Rather, it was the plant density which was more important. These results dispute that of Egli and TeKrony (1979) and Perry (1980) who reported that lower population densities decreased seed yield, and attribute seed yield to the capability of plants to produce tillers and initiate reproductive growth and development.

5.2.8 Seed harvesting and drying

During seed development, abscisic acid (ABA) has long been implicated as an endogenous factor modulating the development of seeds. Specifically, it has been suggested that it acts as an anti-germination agent, preventing germination *in situ* (Walbot, 1978). During maturation of maize grains, endogenous ABA levels are sufficiently high during early and mid-maturation to prevent precocious germination (eg Morris *et al.*, 1991). During the final stage of seed development, embryos become increasingly insensitive to ABA (eg Welbaum *et al.*, 1990; Morris *et al.*, 1991). Presumably, embryos at the latter stage of development, when ABA levels decline and ABA-insensitivity increases, are prevented from germinating by the negative water potential of the seed (eg Morris *et al.*, 1991). Maize seeds must be dried to below 25% SMC for germination to occur (Sprague, 1936; Mashauri, 1990). Drying, whether natural or imposed, elicits changes in gene expression such that subsequent metabolism is germinative rather than developmental (Kermode and Bewley, 1985). Drying may itself promote or lead to a reduction in ABA concentration or decrease the sensitivity of embryos to ABA (Kermode *et al.*, 1989) and enables the aleurone layer to respond to endogenous gibberellic acid (GA) and produce α-amylase (Armstrong *et al.*, 1982; Oishi and Bewley, 1990).

In this study, maturation drying of field-grown super sweet corn was very slow during the available growing season (section 5.1.7). Even as late as 145 DAP for the October planting, 134 DAP for the November planting or 119 DAP for the December planting, the net seed moisture content had not declined substantially and relative SMC remained above 30, 50 and 60% for October, November and December plantings, respectively. Following machine drying of cobs for varying temperatures and times, low drying rates and an uneven drying of cobs were evident. While some cobs dried to about 15% SMC, some cobs resisted

drying (Figure 5.9a,b) and some had seeds which had geminated on the cob (Plate 5.5). Alternatively, the dryers airflow rates and uniformity were altered by position of cobs resulting in an even drying. The higher sugar content in super sweet corn probably created high osmotic potential and lowered the seed's moisture dissipation rates. Sprouting on the cob during drying was surprising. In castor bean, incubation of seeds at high humidity for prolonged periods was sufficient to trigger germination (Kermode and Bewley, 1989). Such seeds incurred only a minimal loss of water, yet exhibited changes in metabolism similar to prematurely dried and mature seeds upon rehydration. The transition of seeds towards a post-germinative metabolism may be associated with reduced sensitivity to ABA (Kermode *et al.*, 1989). Similarly, maize cobs (seeds) held at high relatively humidity did not lose water and embryo ABA remained high; yet the protein synthetic pattern was not characteristic of development but contained post-germinative proteins, suggesting the seed tissue under went a change in ABA sensitivity (Oish and Bewley, 1992). Due to slower drying rates, the super sweet corn cobs were held at 20°C or 25°C for prolonged periods. This was probably sufficient to trigger the metabolic switch-over to a germinative mechanism, or seeds underwent a change (albeit more limited) in ABA sensitivity similar to that which occurs in castor bean and hybrid maize, but did not germinate presumably because SMC was too high. The subsequent dehydration to lower moisture levels, eg below 25% (Prague, 1936; Leopold and Vertucci, 1989) might have increased the responsiveness of seed tissue to gibberellin (germination promotor) (Leopold and Vertucci, 1989). Due to slower drying rates, the seeds were still moist and the temperature used for drying (20°C or 25°C) provided the requirement for cell elongation to proceed before reaching the metabolic quiescence as the tissue dried.

CHAPTER 6

RESULTS

6.1 SEED QUALITY AND VIGOUR ASSESSMENT

6.1.1 Seed quality and vigour testing of freshly harvested seed

6.1.1.1 Thousand seed weight

There were no significant differences in thousand seed weight (TSW) among the different seed lots within planting dates. A significant difference in TSW was observed among the seeds harvested from the different planting dates ($P < 0.0001$), but the interaction between seed lots and harvesting date was not significant (Appendix 6.1a). TSW of seeds from the October planting (144.1-147.3 g) was significantly higher ($P < 0.05$) than that of seeds from the November (112.0-114.7 g) or December (71.3-73.9 g) plantings, while TSW for seeds harvested from the November planting was significantly greater ($P < 0.05$) than that of seeds from the December planting (Figure 6.1a).

6.1.1.2 Standard germination test

Percentage normal seedlings from the standard germination test showed no significant differences among the different seed lots, but the factor of planting dates was significant ($P < 0.0001$). The interaction effect was not significant (Appendix 6.1b). Normal seedlings for seeds harvested from the November (31-40%) and December (30-33%) plantings were not significantly different ($P < 0.05$), but both were significantly less than that of seeds harvested from the October (52-54%) planting (Figure 6.1b). Within the December planting, seeds harvested from lot LV_3 or LV_{33} did not differ significantly ($P < 0.05$) with lot HV_1, LV_1 and LV_{11} (21-33%) (Appendix 6.2a). Abnormal seedlings were similar in all seed lots and planting dates (13-25%), while dead seeds were relatively lower in seeds harvested from the October planting (25-33%) than that from the November (37-45%) or December (44-61%) plantings (data not shown).

6.1.1.3 Electrolyte conductivity

Conductivity test results showed no significant differences in electrolyte leakage among seed lots. A significant difference existed among the seeds harvested from different planting dates ($P < 0.0001$). There was no significant difference for the parent vigour level/planting date interaction (Appendix 6.1c). Seeds harvested from the December planting had a significantly higher conductivity (37.20-43.67 $\mu s\ g^{-1}$ seed), and seeds from the October planting had a significantly lower ($P < 0.05$) conductivity reading than those harvested from the November planting (Figure 6.2). Within the December planting, electrolyte leakage (36.0-43.67 $\mu s\ g^{-1}$ seed) did not differ among seed lots (Appendix 6.2b).

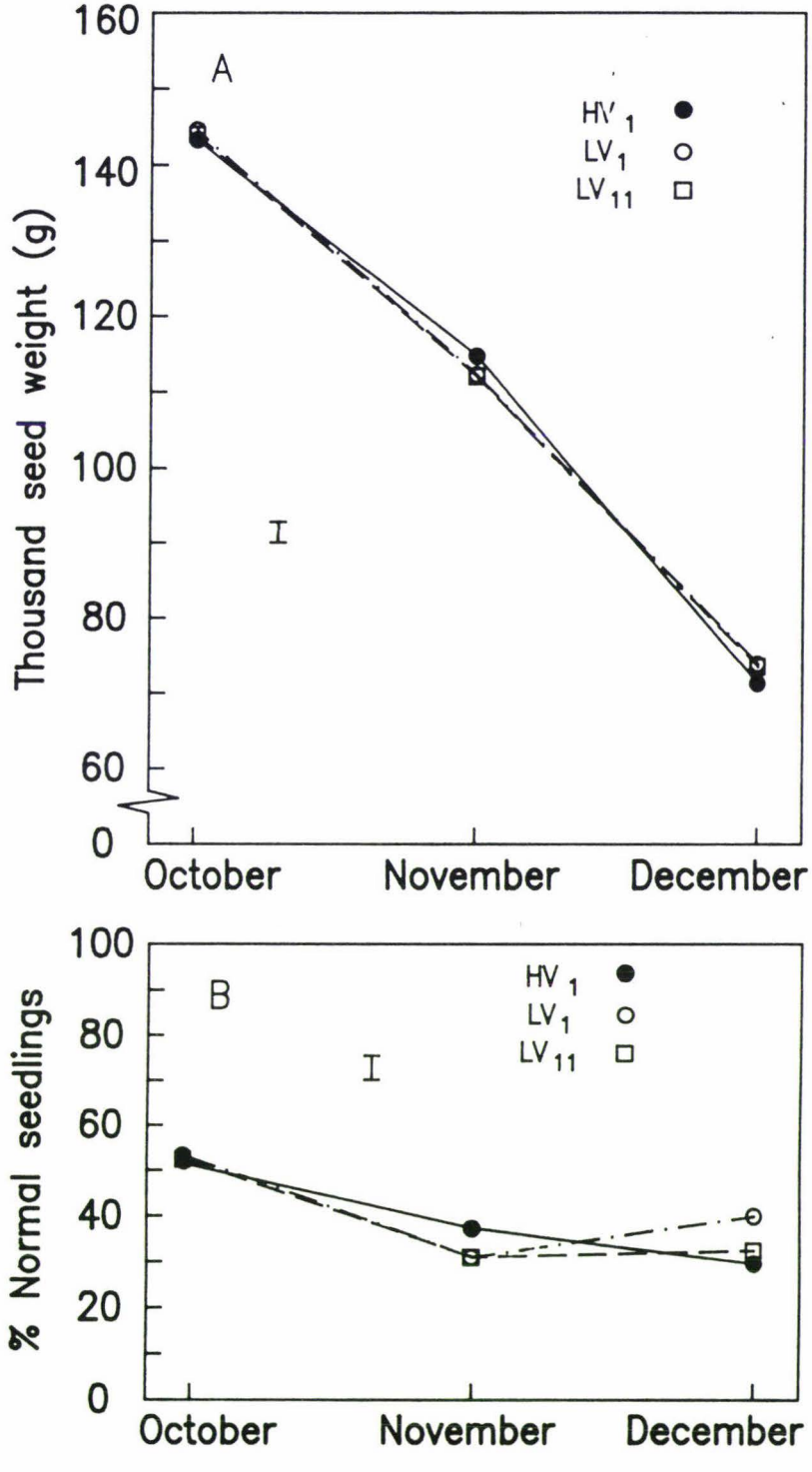

Figure 6.1 Thousand seed weight (a) and percentage normal seedlings (b) of super sweet corn (cv Illini Gold) for seeds harvested from the October, November and December plantings. TSW data are means of eight replicates of 100 seeds. Germination data are means of 3 replicates. Seeds were treated with thiram fungicide. Bars represent a least significant difference at $P < 0.05$.

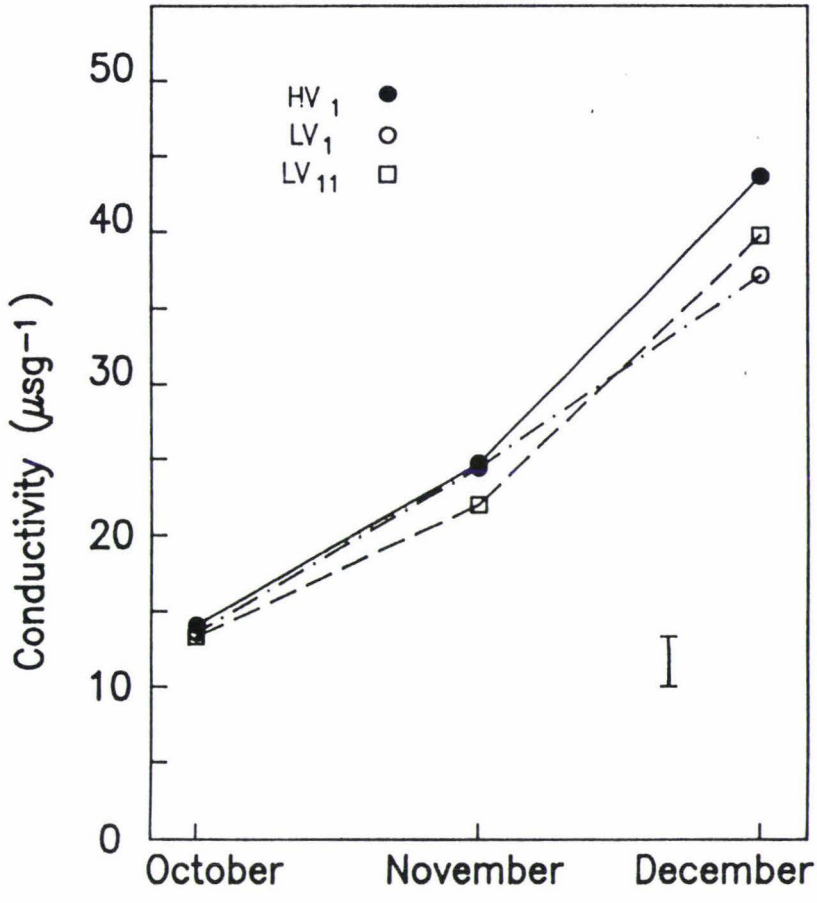

Figure 6.2 Conductivity of super sweet corn (cv Illini Gold) for seeds harvested from the October, November and December plantings. Data are means of three replicates. Bar represent a least significant difference at $P < 0.05$.

6.1.1.4 Cold germination and soil cold tests

Normal seedlings from the cold germination and soil cold test showed no significant differences among the parent vigour levels, but the planting dates differed significantly ($P < 0.0001$). The combined influence between the two factors was not significant (Appendix 6.3a and 6.3b). Normal seedlings from the cold germination test for seeds harvested from the November (16-24%) and December (17-19%) planting did not differ but were lower than those from seeds harvested from the October (45-47%) planting (Figure 6.3a). This cold stress test caused a decrease in normal seedlings compared to that of the standard germination test (Figure 6.1b), particularly in seeds from the November (22-57%) and December (36-45%) plantings. Normal seedlings from the soil cold test for the seeds harvested from the November (39-44%) and December (28-31%) plantings were similar but lower compared to that of seeds harvested from the October (55-66%) planting (Figure 6.3b). Interestingly, this test caused an increase in the number of normal seedlings in the October (17-40%), November (38-61%) and December (35-42%) plantings compared to that recorded in the cold germination test (Figure 6.3a).

6.1.1.5 Health status of seed lots

Visual examination of harvested seeds/cobs showed kernel and cob rots resulting from infection by field fungi (eg Plate 6.1). The pattern of infection was of interest to note. Some cobs (eg Plate 6.2a) showed no visible sign of growth of fungal mycelium or damage on seeds or cobs, although the bottom part of a shank (which connects the cob to the mother plant) showed growth of fungal mycelium. Some cobs had severe damage at both the bottom and top portions of the cobs (eg Plate 6.2b), some showed infection only at the bottom (eg Plate 6.2c) or top of the cobs (eg Plate 6.2d). Some cobs were completely covered by fungal mycelium (eg Plate 6.2e).

Pathological studies indicated that the seeds from the October, November or December plantings harboured various types and magnitudes of internally-borne inoculum (eg Plate 6.3). These pathogens included *Fusarium subglutinans, Fusarium graminearum, Fusarium poae* and 'others' [unidentified *Fusarium* species] (Table 6.1). In this last category, a white thick coarse or fine mycelium, and white thick powdery, pink or pale pink mycelium were observed, possibly some mutants of *Fusarium graminearum* or *Fusarium poae* species. Seeds harvested from the October planting had the highest percentage total infection (90%), followed by seeds harvested from the November planting (79%). Seeds from the December planting had the lowest level (59%) of infected seeds (Table 6.1).

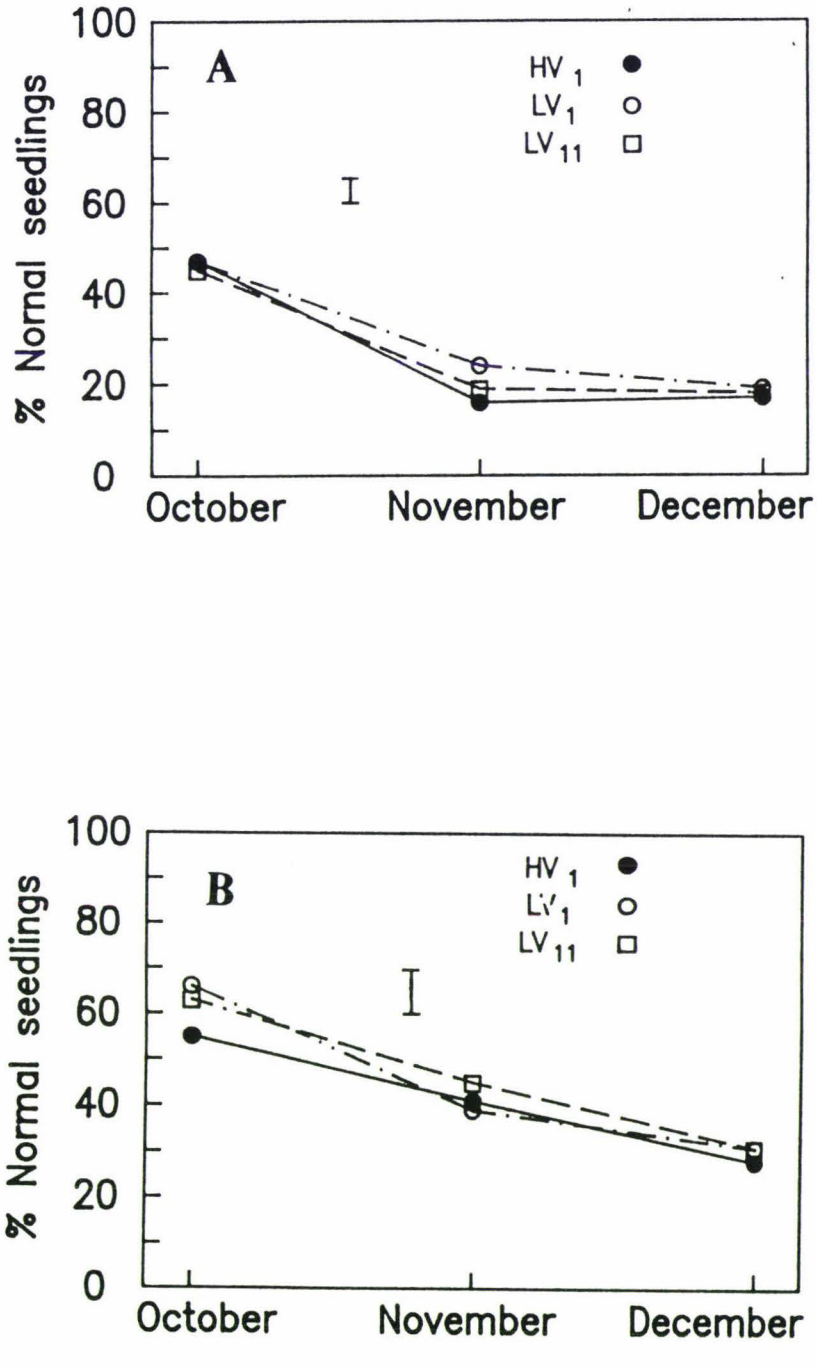

Figure 6.3 Percentage normal seedlings from the cold germination test (a) and soil cold test (b) of super sweet corn (cv Illini Gold) for seeds from the October, November and December plantings. Seeds were treated with thiram fungicide. Data are means of 3 replicates. Bars represent a least significant difference at $P < 0.05$.

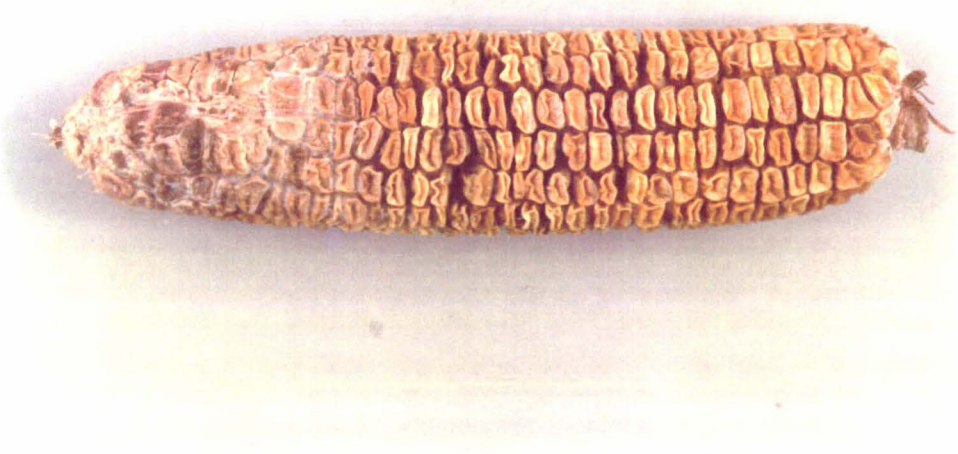

Plate 6.1 A photograph showing kernel and cob rots resulting from fungal infection.

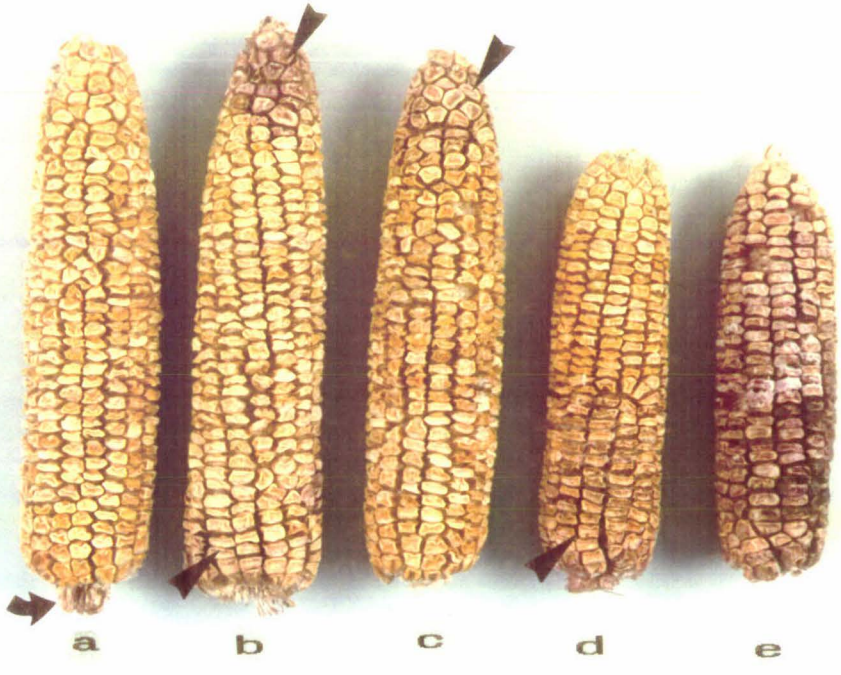

Plate 6.2 A photograph showing a pattern of cob infection by field fungi (see text).

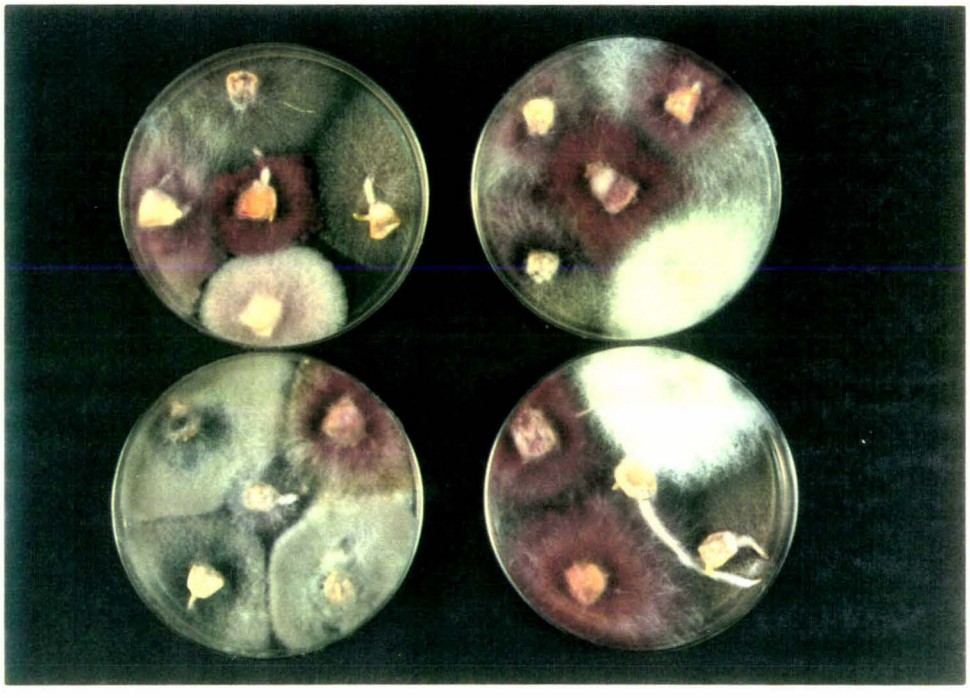

Plate 6.3 A photograph showing various types of pathogens from fresh harvested seed lots.

Table 6.1 **The types and levels of internal infection from freshly harvested seeds from the October, November and December plantings. Data are means of 20 replicates of five seeds drawn from a composite sample of all seed lots in each planting date.**

Planting date	*Fusarium subglutinans* (%)	*Fusarium graminearum* (%)	*Fusarium poae* (%)	Other species (%)	Total (%)
October	24	27	17	22	90
November	18	32	15	14	79
December	14	32	12	9	59

6.1.2 Seed quality and vigour testing of stored original seed lots

The original (F_2) seed lots (HV_1 and LV_1) were stored with varying SMC either at 5°C or 25°C for 14 months. The high vigour seed lot (HV_1) was stored at 5°C, 12.9% SMC for 14 months (lot A-1) or at 25°C, 11.6% SMC for the same time (lot A-2), while the low vigour seed lot (LV_1) was stored at 5°C, 13.7% SMC for 14 months (lot A-3). At the end of the storage period, standard germination, cold germination, conductivity and seed health tests were conducted for all seed lots (A-1, A-2 and A-3) and results collated with the initial (zero storage) potential performance of seed lots (HV_1 and LV_1).

6.1.2.1 Standard germination, cold germination and electroconductivity tests

The standard germination test showed that germinability performance of the high vigour seed lot after storage (A-1) and before storage (HV_1) did not differ significantly (92% and 88%, respectively; Figure 6.4a). Likewise, the low vigour seed lot after storage (A-3) and before storage (LV_1) had a similar ($P < 0.05$) germinability of 77% and 74%, respectively, Seed lot A-2 (82%) and HV_1 (88%) did not differ significantly ($P < 0.05$) in their germinability. Within the stored seed lots, seed lot A-1 had higher germination (92%) than seed lot A-2 (82%) or lot A-3 (77%) (Figure 6.4a).

Cold germination test results showed that seed lot A-1 had a significantly ($P < 0.05$) greater number of normal seedlings (91%) than seed lot HV_1 (78%), but seed lot A-3 (71%) and LV_1 (77%) did not differ (Figure 6.4b). Seed lot A-2 (75%) and HV_1 (72%) also had a similar number of normal seedlings. Within the stored seed lots, seed lot A-1 had significantly more ($P < 0.05$) normal seedlings (91%) than either seed lots A-2 (75%) or A-3 (71%) (Figure 6.4b).

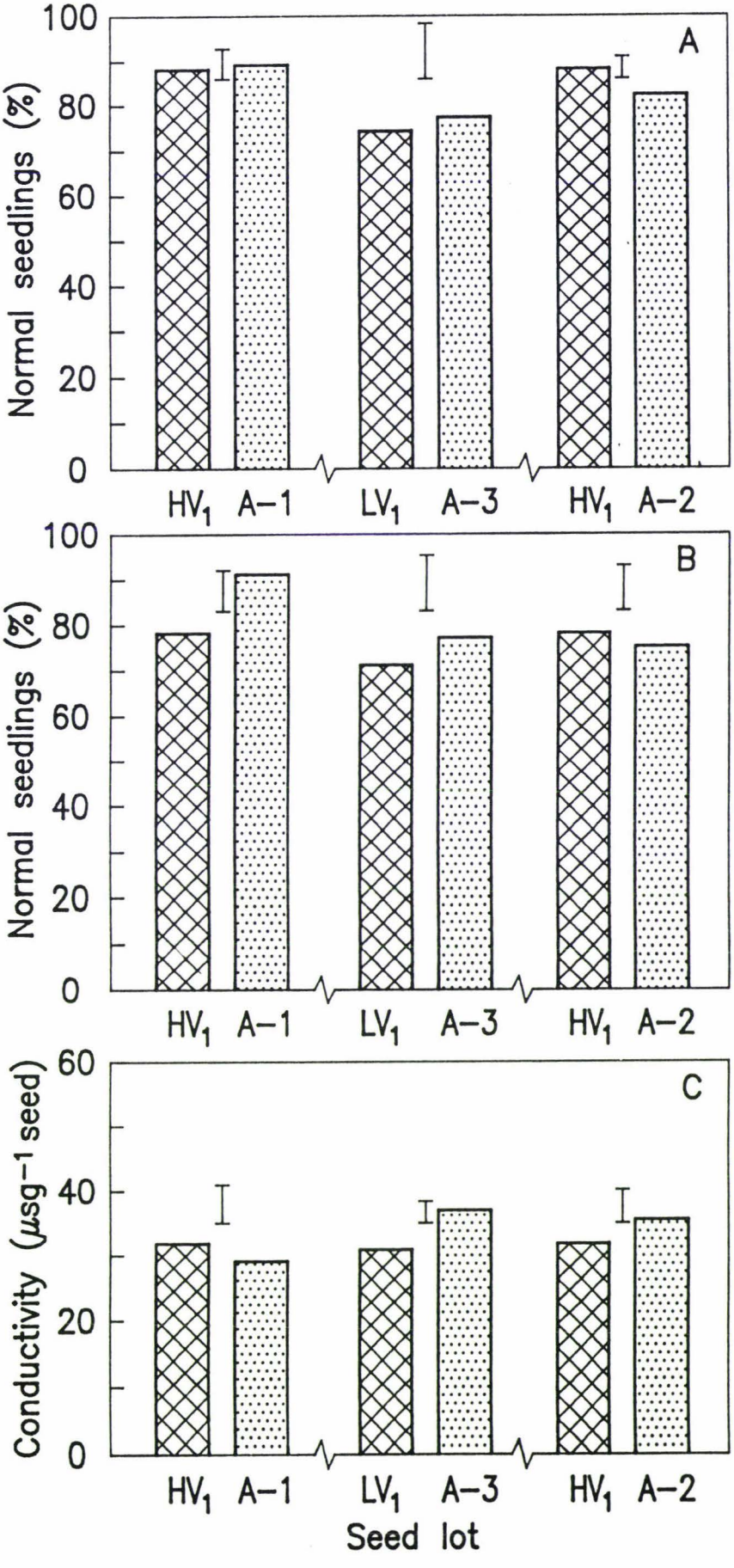

Figure 6.4 Percentage normal seedlings from the standard germination test (a) and cold germination test (b), and conductivity (c) of super sweet corn (cv Illini Gold) for original seed lots unstored/stored at 5°C or 25°C for 14 months. Data of unstored are means of 4 replicates and of stored are means of 3 replicates. Bars represent a least significant difference at $P < 0.05$. Cross hatched and dotted histograms are unstored and stored seeds, respectively.

The amount of electrolyte leakage for seeds soaked for 48 h at 25°C showed that seed lot A-1 (28.98 μs g^{-1} seed) and HV_1 (31.69 μs g^{-1} seed) did not differ significantly ($P < 0.05$), but leakage from seed lot A-3 was significantly greater (36.86 μs^{-1} seed) compared with that leaked from seed lot LV_1 (30.77 μs g^{-1} seed) (Figure 6.4c). No significant differences in electroconductivity were found between seed lot A-2 (35.42 μs g^{-1} seed) and lot HV_1 (31.69 μs g^{-1} seed). Conductivity levels within the stored seed lots indicated seed lots A-2 (35.42 μs g^{-1} seed) and A-3 (36.86 μs g^{-1} seed) had similar conductivity readings, but both were significantly higher than that recorded from seed lot A-1 (28.98 μs g^{-1} seed).

6.1.2.2 Seed health testing

The levels and types of internal infection before and after storage are presented in Table 6.2. Before storage the amount of *Fusarium subglutinans* was high (60-82%). The occurrence of these species decreased considerably following storage at 5°C or 25°C to only 4-19%. Conversely, the isolation of non-pathogenic fungi, such as *Mucor* and *Rhizopus* species increased considerably from 0 and 8-18% before storage to 20-33% and 44-57%, respectively after storage (eg Plate 6.2). The incidences of *Aspergillus* and *Penicillium* species also increased to 2-12% and 12-16%, respectively (eg Plate 6.2).

Table 6.2 Percentage internal infection of original seed lots stored at 5°C (A-1, A-3) or 25°C (A-2) for 14 months. Figures in brackets are fungal infection before storage. Data are means of 20 replicates of five seeds.

Seed lot	*Fusarium subglutinans*	*Penicillium species*	*Aspergillus species*	*Mucor species*	*Rhizopus species*
A-1	19 (60)	16 (8)	2 (0)	20 (0)	44 (18)
A-2	4 (60)	12 (8)	12 (0)	24 (0)	48 (18)
A-3	13 (82)	12 (6)	2 (0)	33 (0)	58 (8)

6.1.3 Field emergence of freshly harvested and stored original seed lots

6.1.3.1 Weather at planting

Daily maximum and minimum air temperatures during the emergence period ranged from 15.3-20.5°C and 7.1-16.1°C (Figure 6.5), with a mean daily temperature of 17.4°C and 10.8°C, respectively. The daily soil temperatures ranged from 14.4-16.9°C. A total rainfall of 21.2 mm was recorded during the entire emergence period. Rain fell (7.2 mm) one day

Plate 6.4 A photograph showing the occurrence of various micro-organisms in stored seed lots.

before the planting day. There was no rain for the first 7 days following planting (Figure 6.5). Rain fell (8.6 mm) on the 8th day after planting and more rainfall (0.4-3.7 mm) was recorded over the next three days. Some rain fell (2.4-2.9 mm) two days before the final emergence count was recorded. Loss of moisture due to evaporation was low, ranging from 0 to 6.0 mm, with a mean daily moisture loss of 3.0 mm.

6.1.3.2 Field emergence

Percentage field emergence for fresh harvested seed stocks showed no significant differences among the different vigour levels, but seeds from the different planting dates differed significantly ($P < 0.0001$). The vigour level planting date interaction was not significant (Appendix 6.3c). Figure 6.6a shows the field emergence for seeds harvested from the October, November and December plantings. Seeds harvested from the October planting had the highest field emergence (58-62%), and those harvested from the December planting had the lowest (9-17%) field emergence. Seeds from the November planting had intermediate field emergence of 27-37%.

Percentage field emergence for original seed lots before and after storage are presented in Figure 6.6b. Field emergence from the stored high vigour lot (lot A-1) was significantly greater (91%) compared to that recorded for the same lot before storage (70%, lot HV_1). The stored low vigour seed lot A-3 produced a significantly lower ($P < 0.05$) field emergence (69%) than unstored (LV_1) seed lot (75%). Seed lot A-2 had a significantly greater field emergence of 85% compared to that recorded from seed lot HV_1 (70%). Within the stored seed lots, seed lot A-1 had the highest (91%) field emergence while A-3 had the lowest (69%). Seed lot A-2 had intermediate field emergence of 85% (Figure 6.6b).

6.1.3.3 Relationship between seed quality characters and field emergence for stored seed lots

The correlation coefficient between the standard germination, cold germination and conductivity test results and field emergence are presented in Table 6.3. The standard germination and cold germination test results were significantly correlated with field emergence, with the latter showing a slightly stronger relationship with field emergence than the former. There was no significant correlation between the conductivity test results and field emergence (Table 6.3).

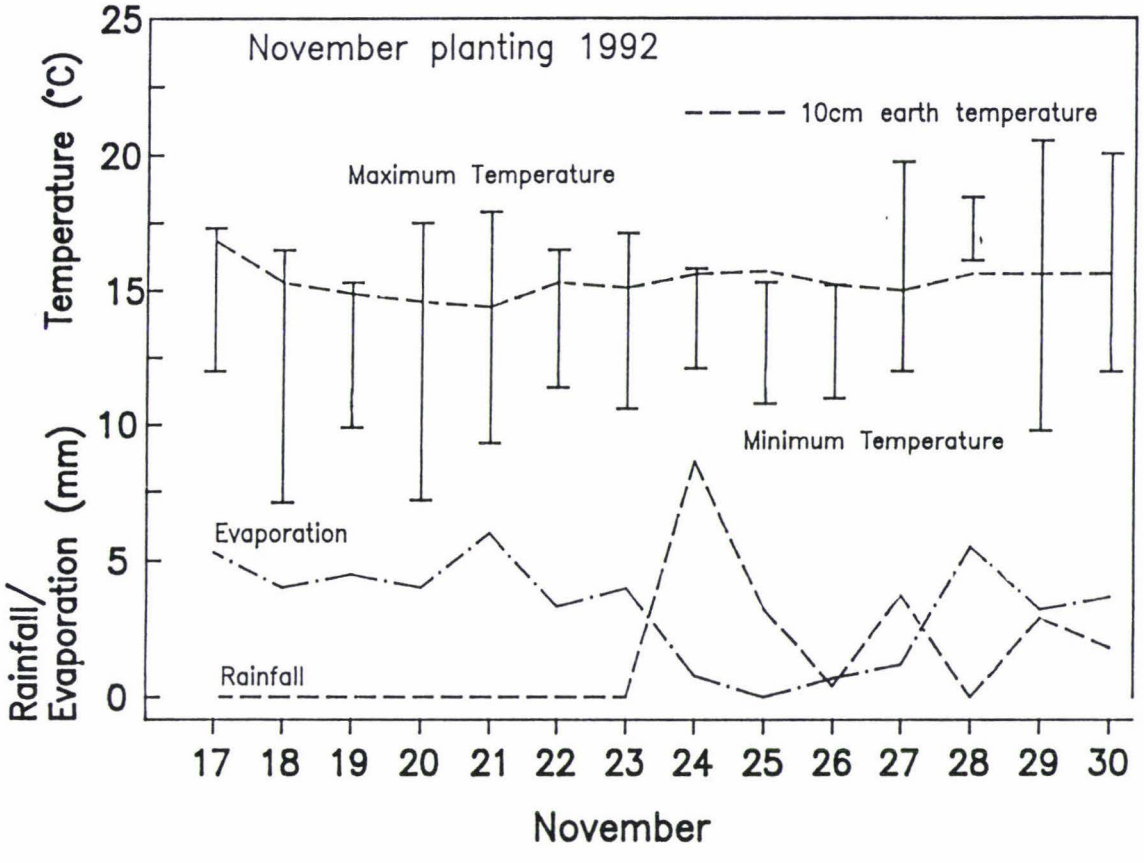

Figure 6.5 **Daily maximum and minimum ambient and soil (10 cm depth) temperature, rainfall and evaporation from planting to maximum emergence for the 1992/93 field emergence trial.**

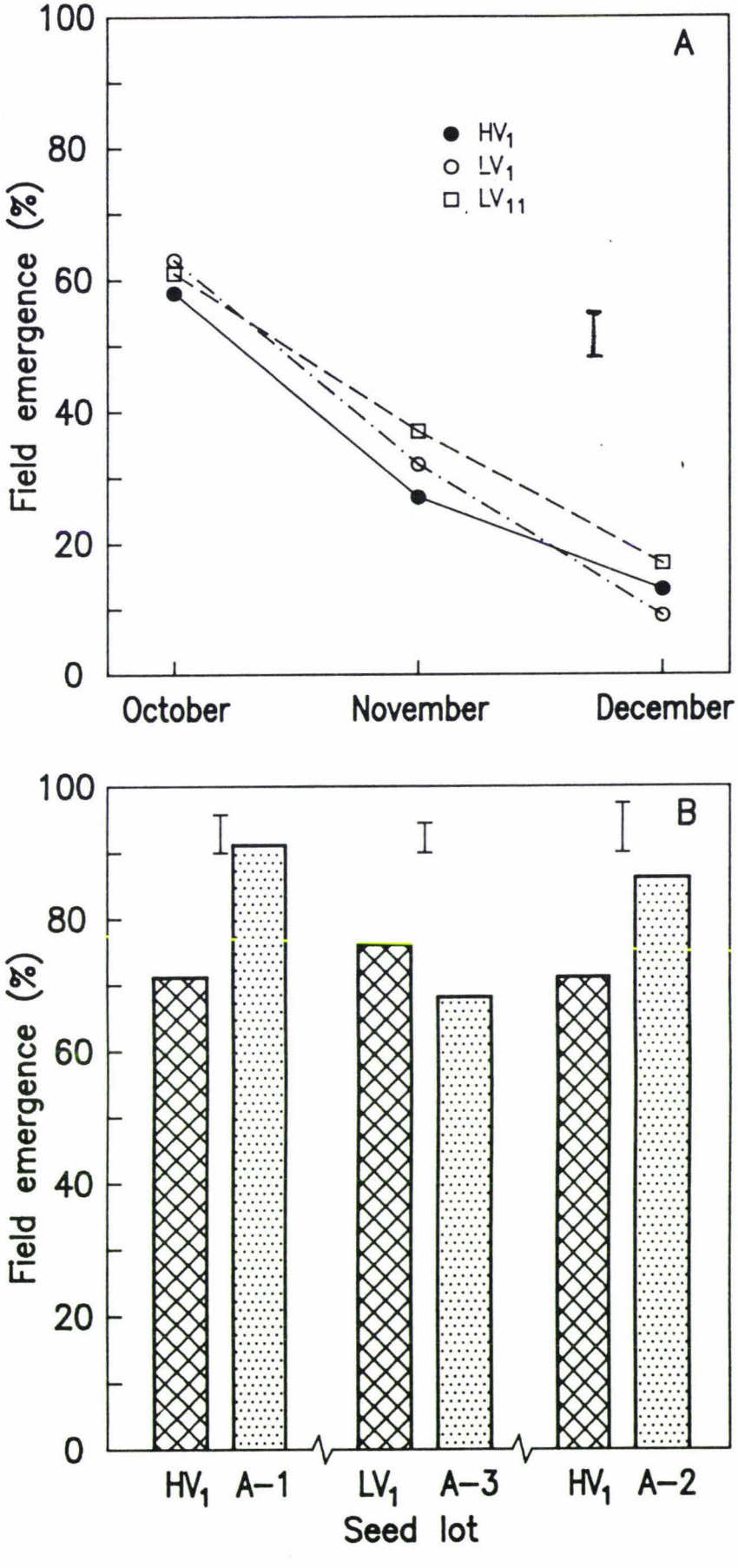

Figure 6.6 Percentage field emergence of fresh harvested (a) super sweet corn (cv Illini Gold) seed lots and original seed lots unstored/stored at 5°C or 25°C for 14 months (b). Data of stored seeds and fresh harvested seeds are means of three replicates. Data of unstored seeds are means of four replicates. Bars represent a least significant difference of any two means at $P < 0.05$.

Table 6.3 Pearsons correlation coefficients for different seed quality characters and field emergence.

Character	Field emergence
Standard germination test	0.80 *
Cold germination test	0.83 **
Conductivity test	0.03

* = significant at $P < 0.05$
** = significant at $P < 0.01$

6.2 DISCUSSION

6.2.1 Seed Vigour and Seed Quality of Freshly Harvested Seeds

Thousand seed weight (TSW) results are shown in Figure 6.1a. TSW of seeds from the October planting was significantly higher than that of seeds from the November and December plantings, while TSW for seeds harvested from the November planting was significantly greater than that of seeds from the December planting. As discussed in section 5.2.6, the lower TSW in seeds from the December planting was probably because of persistent severe water stress which had its effects on seed size and seed weight (cf Delouche, 1980). While TSW in seeds from the November planting was greatly influenced by high inter-planting competition resulting in a reduced seed size and weight, the opposite was true for seeds from the October planting due to decreased plant density caused by post-emergence damping-off and seedling blight.

Although there was a significant difference in TSW between seeds from the November and December plantings, the percentage of normal seedlings did not differ significantly between them (Figure 6.1b). Once again, seed size or weight does not always necessarily relate to seed performance (see also section 4.3.1.2). However, normal seedlings from the November and December planting were significantly lower than that from the October planting (Figure 6.1b). This is obviously the effects of artificial drying. The conditions of artificial drying allowed cell growth in some seeds as illustrated by visible radicle emergence (Plate 5.5). Although the levels of seeds which had sprouted on the cobs was about 12%, it may be possible that more seeds initiated the germination processes at a critical SMC for cell elongation, but because of continued drying, the processes were

prematurely halted causing an irreversible damage that might have upset the subsequent germination.

In general, although seeds harvested from the October sowing were naturally dried, all seed lots from all planting dates were of low vigour, as demonstrated by lower levels (30-54%) of percentage normal seedling (Figure 6.1a). Higher amounts of seeds died, particularly in seeds from the November (37-45%) and December (44-61%) plantings - data not shown. The number one suspect would be the incidences of *Fusarium* microflora in seed tissue (see the following discussion). *Fusarium* mycelium were observed partly or wholly covering cobs (Plates 6.1 and 6.2). On the subsequent germination of seeds, a vigorous growth of pale pink, reddish or whitish mycelium was observed outside of the rolled paper germination (Plate 6.5), highlighting the likely damage to the seeds. Kruger (1986) observed that germination seeds from cobs which were covered partly by *Fusarium* mycelium was poor. Even some distances away from the mycelium, the seeds symptoms and were dead. In his experiments, it was found that if one third of a cob was covered with mycelium, the adjacent one third was also badly damaged. Only the last third showed a slight infection. If the whole cob was infected it was worthless for seed production (Kruger, 1986). Additionally, the high temperature (20-25°C) used during drying of seeds from the November and December plantings might have intensified the activity of *Fusarium* and so done further damage to the seeds as reflected in the increased levels of dead seeds.

When seeds dehydrate during maturation or imposed drying, the cell organelles/membranes undergo dramatic changes as the moisture content falls, and again when the cells rehydrate when the mature dry seeds imbibe water. For successful seed performance, the disorganisation of the organelle/membrane system during maturation and drying must be undergone in an orderly manner so that its reorganisation after imbibition becomes possible in the shortest possible time, and upon rehydration, cell membranes must become fully reorganised before the cells become fully hydrated, otherwise some of the cellular organic compounds and inorganic ions may leak out (Bryant, 1985; Spath, 1987, 1989; Lott *et al.*, 1991).

In this study, seeds harvested from November and December plantings had a significantly higher conductivity than that of seeds from the October planting, but seeds from the December planting had a significantly high conductivity reading than that of seeds from the November planting (Figure 6.2). Some seeds especially from the artificially dried treatments had pericarp crevices, formed as the endosperm collapsed during drying (Styer and Cantliffe, 1983). This may cause an in-rush of water into the seed and may result in

Plate 6.5 A photograph showing *Fusarium* mycelium growth on the rolled germination papers.

increasing leaching of electrolytes (Wann, 1986, Bruggink *et al.*, 1991b). The uneven drying of seeds during artificial drying (section 5.1.7) probably disrupted the usual sequence of disorganisation of organelles and membranes during desiccation, leading to incomplete reformation of the lipid bilayers of the membranes; or incomplete or incorrect re-alignment of proteins in the bilayer state or the bilayer formed might be functionally imperfect resulting in a massive leakage during imbibition (Bryant, 1985; Powell, 1988; Bruggink *et al.*, 1991b; Bochiocchio *et al.*, 1991). Alternatively, as some of the seeds initiated cell elongation on the cob during drying, this process might have reinforced the production of hydrolytic enzymes (eg amylase, protease) that may consequently do extensive damage to the cell membranes. Additionally, as the seeds were heavily infected by internally-borne *Fusarium* pathogens (Table 6.2), their biological activity may have resulted in the production of toxic metabolites (eg mycotoxins, phytotoxins, etc) which may have paralysed the cellular repair metabolism with consequently high solute leakage.

As for the standard germination test, normal seedlings from the cold germination test showed no significant differences between seeds from the November and December planting, but both were significantly lower than that from the October planting (Figure 6.3a). However, this cold stress test caused a decrease in normal seedlings compared to that of the standard germination test (Figure 6.1b), particularly in seeds from the November and December planting. While the degradative activities of *Fusarium subglutinans* are favoured in warm and dry conditions, that of *Fusarium graminearum* is intensified in cold and moist conditions (Shurtleff, 1980; Kommedahl and Windels, 1981; Gilbertson, 1985). The incubation of seeds at 10°C for 7 days during the cold germination test probably exposed the seeds to *Fusarium graminearum* attack. Normal seedlings from the soil cold test followed a similar trend (Figure 6.3b), however, there was an increase in the number of normal seedlings from seed from the November and December plantings compared to the cold germination test (Figure 6.3a). The improvement of performance in the soil cold test was previously observed in the original seed lots (section 4.1.3). This may lend support to the idea presented earlier that the soil medium contains a soil-borne micro-organism antagonist to the seed-borne pathogens.

It is clear from the foregoing discussion that vigour status of the parents (Chapter 4) did not affect vigour status of the progeny as demonstrated by similar performances among seed lots (this section).

6.2.1.1 Seed health status of seed lots

Visual examination of harvested seeds/cobs showed kernel and/or cob rots resulting from fungal infection. The pattern of infection ranged from kernels with slight signs of infection, through to severe damage at both the bottom and top parts of the cobs, cobs infected only at the bottom or top portion, to cobs completely covered by fungal mycelium (Plate 6.2). From this observation, it is difficult to judge which part was attacked by which pathogens, because several species of *Fusarium* can cause kernel and/or cob rots. *Fusarium graminearum*, *Fusarium culmorum* and *Fusarium poae*, for instance, can cover portions or the whole cob. They infect either from the tip or bottom of the cob, growing from the point of entry over the whole surface, forming a red coat, but when *Fusarium poae* is involved, a whitish coat is produced (Kruger, 1986). Others such as *Fusarium subglutinans* and *Fusarium verticillioides* infect single kernels or kernel groups which become pale pink or reddish (Kruger, 1986).

Pathological investigation indicated that the seeds harvested from the October, November or December plantings were internally contaminated by *Fusarium graminearum*, *Fusarium subglutinans* and *Fusarium poae* (Table 6.1). Based on pathological studies for seeds before planting (section 4.2.1) and diseases plants post-emergence (section 5.1.3), the results in this section suggest *Fusarium graminearum* infected the plants or seeds in the seed production field. There are a number of possible pathways for infection. In the seed production field, invasion of vascular parenchyma can occur because airborne fungal spores of *Fusarium* spp frequently lodge between the leaf sheath and the leaf stalk, an area which provides ideal conditions for the spore to germinate (Kingsland and Warnham, 1962) and penetrate into the main stalk (Foly, 1962; Kucharek and Kommedahl, 1966), and from the main stalk to the rudimentary cobs (Kingsland and Warnham, 1962). Pathogens may enter in the region of silks, and then spread to bracts and pedicels through the vascular cylinder, and finally spread into the shank (Koehler, 1936, 1942). Alternatively, any sort of injury during plant growth and development can facilitate additional infection of plant tissue from external sources. Apart from insect-induced damage, plants and/or seeds are also prone to damage by birds, animals, heavy rains, etc (Kucharek and Kommedahl, 1966; Neegaard, 1977; Agarwal and Sinclair, 1987). Seeds harvested from the October planting had the highest percentage total infection, followed by the November planting, and the December planting had the lowest level of infected seeds (Table 6.1). This is probably the effect of harvesting date. Seeds from the October planting were harvested 145 DAP, while that from the November and December plantings were harvested 134 and 119 DAP, respectively. Delayed harvesting allowed a longer time for fungal infection of seeds. The longer the time seeds were left in the field, the higher the infection levels.

6.2.2 Seed vigour and seed quality of stored seeds

During storage, several environmental factors may affect seed longevity. These include both pre-storage factors such as initial quality of the seeds, mechanical damage, etc and post-storage factors such as seed moisture content, temperature, relative humidity, light, oxygen and carbon dioxide. Amongst these, relative humidity (and thus seed moisture content) and temperature are considered to be the major causes of seed deterioration. The importance of these factors is emphasised by Harrington's "Rules of Thumb" which states that, for seeds between 5 and 14% SMC, storage life is doubled for every 1% reduction in SMC, or for every 5°C reduction in temperature.

In this study, storage of seeds at 5°C or 25°C for 14 months did not affect subsequent germination performance of either high or low vigour seed lot (estimated by the standard germination test) as germination post-storage did not differ significantly from that pre-storage (Figure 6.4a). This may be because the storage conditions of low SMC (12.9% or 13.7%), temperature (5°C) in seed lots A-1 and A-3 slowed down the metabolic reactions. Although the storage temperature in seed A-2 was high, the seeds had very low SMC (11.6%) which probably minimised the deteriorative reactions, with a consequent reduction in deterioration rate (Harrington, 1972; Leopold and Vertucci, 1989). Additionally, the incidence of *Fusarium subglutinans* which was rampant in seeds before storage (section 4.3.2.1) had decreased considerably following storage, allowing the seeds to grow normally. However, within the stored seed lots, seed lot A-1 had a significantly higher germination percentage than seed lots A-2 and A-3 (Figure 6.4a). This was expected because, although seed lot A-3 was stored at low temperature, it had previously been physiologically abused during the artificial ageing, while seed lot A-2 was stored at a high temperature. The role of high temperatures in the deterioration of seeds during storage is assumed to be via affecting the rates of biochemical changes in seeds, and Harrington (1972) pointed out that high metabolic rates at high temperatures were related in some ways to rapid losses of germination.

Cold germination test results showed that seed lot A-1 had a significantly higher number of normal seedlings than HV_1, but seed lots A-3 and A-2 did not differ significantly from seeds lots LV_1 and HV_1, respectively (Figure 6.4b). Within the stored seed lots, lot A-1 had more normal seedlings than the other seed lots. These data give support to the suggestion given earlier that the presence of micro-organisms and *Fusarium subglutinans* in particular, was responsible for the poor performance of unstored seeds, because as the incidence of the pathogen declined after storage, there was a substantial improvement in

seed performance as demonstrated by increased germination in seed lot A-1. Although the occurrence of this field fungus also decreased following storage in seed lots A-2 and A-3, physiological deterioration continued because of a high storage temperature in the former and the effects of AA treatment in the latter.

Electroconductivity measurements for seeds soaked at 25°C for 48 hours indicated that seed lots A-1 and HV_1 or A-2 and HV_1 did not differ significantly in solute leakage, while seed lots A-3 and LV_1, although both had the same percentage of normal seedlings (Figure 6.4a), differed significantly in their membrane reorganisation rates, as illustrated by differences in solute leakage (Figure 6.4c). This agrees with the general notion that seed vigour changes before viability begins to decline (Justice and Bass, 1978; Priestley, 1986). Within the stored seed lots, lots A-2 and A-3 had similar levels of electrolyte leakage, which was significantly greater than that leaked from seed lot A-1 (Figure 6.4c). These results demonstrate that the rate of deterioration of different seed lots during storage varies according to storage temperature (eg A-2) and initial seed lot quality (eg A-3). Seed lots A-2 and A-3 leached more solute, showing that damage to membranes or repair metabolisms had occurred (Powell, 1986; Matthews and Powell, 1987). Membrane damage in dry seeds can be related to the formation of radicles by auto-oxidation which in turn may form a free radical chain reaction leading to a whole range of toxic breakdown products which can ruin membrane integrity and other cellular mechanisms. This is regarded as the first sign of seed deterioration (Powell, 1986; Wilson and McDonald, 1986; Benson, 1990).

The occurrence of *Fusarium subglutinans* in all seed lots (A-1, A-2 and A-3) declined considerably following storage (Table 6.2). The storage of seed lot A-2 t high temperature (25°C) and low humidity (11.6% SMC) for 14 months probably suppressed the activity of this field fungus. Christensen and Kaufman (1969, 1974) and Mashauri *et al.* (1992) observed that harvested seeds may be contaminated by field fungi, and during the first few months of storage, the activity of these fungi can continue, but because of lowered SMC and increasing storage time and temperatures, they are unable to perpetuate and eventually die out. Specifically, *Fusarium subglutinans* incidence has been shown to decrease following storage of seeds at low SMC and high temperatures for extended periods (Kabeere, 1993, pers. comm.[1]). A surprising observation was a decrease in the occurrence of *Fusarium subglutinans* after storage at low (5°C) temperature (eg seed lots A-1 and A-3). In dent maize, *Fusarium subglutinans* and *Fusarium* species are known to survive low temperature

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storage at last for 2 years (Kabeere, 1993, pers. comm.). However, low temperatures were unfavourable for *Fusarium subglutinans* growth and development in sweet corn (refer sections 4.1.3 and 6.2.1). Adding to this the likely lower relative humidity created by the seed packaging materials (polyethylene films) during the 14 months of storage, probably shortened the storage life of *Fusarium subglutinans*. Polyethylene films are mostly water resistant, with water vapour transmission rates (WVTR) of around 20 g $m^{-2}d^{-1}$. WVTR is the rate at which water vapour passes through a unit area of material per day at 37.8°C from 100% RH on one side to 0% on the other (Coolbear, 1990). There was also an increasing level of non-pathogenic fungi such as *Rhizopus* and *Mucor* species. It may be possible that these saprophyte fungi contaminated the seeds from the air during planting, and the likely leaching of nutrients through the seed's damaged pericarps provided sufficient substrate for their growth.

6.2.3 Field emergence and its relationships with seed quality characters

The conditions necessary for good seed germination and growth are discussed in detail in section 5.2.1 and will not be discussed further. The field conditions during planting and emergence (Figure 6.5) were not typical of unfavourable conditions. In addition to a well prepared seed bed, the seeds were planted into a moist soil caused by heavy rains on the days before planting. Although there was no rain for 7 days following planting, the soil type (Appendix 3.1) and weather (Figure 6.5) did not allow a high loss of soil moisture and therefore the soil remained most sufficiently to allow germination and growth before rain fell again on the 8th day.

Percentage field emergence for freshly harvested seeds from the October, November and December plantings were poor (Figure 6.6a). Seeds from the November and December plantings in particular, had very low field emergence, as germination was poor presumably due to the combined effects of artificial drying and the heavy infection of seeds by *Fusarium* species. Artificial drying is one of the complex aspects of seed production and one which can cause severe loss of seed quality and vigour if it is not carried out properly (Burris and Navratil, 1980; Hill, 1990). The complexity of this process is because temperature and relative humidity of the ambient air tend to change or fluctuate as they pass through the drier, following a pattern which depends on the design of the drier (Nellist, 1980). In addition to endosperm type, the seeds themselves follow a complicated warming curve which is influenced by the fact that the worming due to exchange of heat between the air and the seeds is partly counteracted by the cooling effect due to latent heat of evaporation (Purdy and Crane, 1967; Nass and Crane, 1970; Roberts, 1981), resulting in poorly dried

seeds of inferior performance. On the other hand, *Fusarium* species are known to greatly affect the seed planting value. Shurtleff (1980) and Kruger (1989) pointed out that poor field emergence usually has its origin from infected seeds. Some seeds may die before germination (seed rot), some may germinate but die before emerging (pre-emergence damping-off), and some seedlings succeed in emerging but they soon wither and die (post-emergence damping-off).

Percentage field emergence of original seed lots showed that stored high vigour seed lots (A-1 and A-2) had a significantly higher field emergence compared to their counterpart unstored high vigour (HV_1) seed lot. Conversely, the stored low vigour (A-3) seed lot produced a significantly lower field emergence in comparison with unstored low vigour (LV_1) seed lot (Figure 6.6b). Within the stored seed lots, seed lot A-1 had the highest field emergence, followed by seed lot A-2 and seed lot A-3 had the lowest percentage field emergence (Figure 6.6b). These results are of particular interest because they clearly show the importance of micro-organisms and storage temperatures on seed performance. The heavy infection of unstored seeds by *Fusarium subglutinans* (section 4.3.2.1) was responsible for lowered field emergence. Elimination of this virulent fungus during storage, improved field emergence substantially in high vigour seed lots. Although the isolation of *Fusarium subglutinans* declined during storage, aged seed lot showed an overriding deterioration. The AA treatment previously imposed on this seed lot and/or phytotoxins resulting from fungal metabolites, probably maimed the cellular systems and functions of the seeds and accelerated deterioration with a consequently poor field performance.

The relationship between seed quality characters and field emergence was also interesting. While the standard germination and cold germination parameters (among others), were not significantly correlated with field emergence of unstored seed materials (section 5.1.2; Appendix 5.2), these tests demonstrated a highly significant correlation with field emergence following storage (Table 6.3). These results lend support to the suggestion presented earlier that poor relationships between seed quality characters and field emergence of unstored seed lots were confounded by several factors, including microflora infection and soil-borne antagonist.

The observed good correlation between the standard germination test results and field emergence is in dispute with the finding of Waters and Blanchette (1983) for sweetcorn. This is because the field environment conditions used in this study were favourable for emergence. There is often a good correlation between the standard germination test results and field emergence under favourable conditions (Roberts, 1984; Bekendam *et al.*, 1987).

However, the good correlation between the cold germination test results and field emergence is in line with that reported by Waters and Blanchette (1983) for sweetcorn and Loeffler *et al.* (1985) for dent maize. Although both the standard germination and cold germination test results were good indicators of field emergence, the latter showed a slightly stronger relationship ($r = 0.83^{**}$) with field emergence than the former ($r = 0.80^{*}$). Similar results were reported by Waters and Blanchette (1983). The conductivity test has been used to predict field emergence of sweetcorn (eg Water and Blanchette, 1983). In this study, however, correlation between the conductivity test results and field emergence was poor, presumably because the field conditions were favourable for good emergence. This conclusion is consistent with other studies, eg Duczmal and Minicka (1989). On the other hand, Herter and Burris (1988) considered that the conductivity test was not sensitive enough for maize, and that cultivar differences strongly influenced the results.

CHAPTER 7

GENERAL DISCUSSION, CONCLUSIONS AND SCOPE FOR FUTURE WORK

7.1 GENERAL DISCUSSION

The seed lots used in this course of study were obtained from different sources (Table 3.1), but were heavily infected with *Fusarium subglutinans* and *Penicillium* species (section 4.2.1). Although these seeds lots, HV_1 and HV_2, were of similar genetic make-up, they differed significantly in both thousand seed weight and mechanical damage (Table 4.2). This may be due to seed factors, eg formation of cracks in the pericarp as the endosperm collapsed during drying; environmental factors during seed development, eg inadequate water supply, temperature; and management and processing technology, eg plant density, untimely and improper harvesting and processing (Hall *et al.*, 1981; Jones *et al.*, 1985; Westgate and Boyer, 1986; Styer and Cantliffe, 1983; Churchill and Andrew, 1987; section 4.3.1.2). Despite the fact that seed lots HV_1 and HV_2 differed in TSW and MD, their germinability, vigour and health performance were the same (sections 4.1.2-4.1.5 and 4.2.1-4.2.3). While seed weight may not always be related to seed performance, the effect of MD depends on the type, position and magnitude of the damage (Delouche, 1980; Escasinas, 1986; TeKrony and Egli, 1991; Mashauri *et al.*, 1992; section 4.3.1.3).

Seed ageing, whether natural or imposed, induces physical and/or physiological changes at the cellular level. Vital systems and functions of cell membranes, organelles, nuclear materials and biochemical mechanisms which control and tone the physiological processes involved in germination and vigour performance are maimed, and the seeds show symptoms of deterioration (AOSA, 1982). In this study, following artificial ageing (section 3.3), aged seed lots (LV_1, LV_2, LV_3 and LV_4) exhibited symptoms of deterioration such as decrease in germination, increase in abnormal seedlings and loss of tolerance to suboptimal conditions (Roberts, 1983; AOSA, 1983; Helm *et al.*, 1989; section 4.1.2 and 4.3.1.3). Internally-borne *Fusarium subglutinans* survived the high temperature during the AA treatment (section 4.3.2.1), and the lack of tolerance to stress in aged seed lots allowed this pathogenic fungus to do extensive damage (section 4.1.2). Other manifestations of deterioration are a slower rate of seedling growth and accumulation of dry weight (Perry, 1980; Priestley, 1986; Sanchez-Nieto *et al.*, 1992; section 4.1.4 and 4.3.1.5), loss of membrane integrity and composition as demonstrated by a massive leakage of electrolytes (Powell and Matthews, 1981; Priestley, 1986; Bruggink *et al.*, 1991; sections 4.2.4 and 4.3.2.4), and loss of mitochondrial integrity and/or its enzyme complex as illustrated by decreased respiratory oxygen uptake and increased respiratory quotients (Anderson, 1970; Abdul-Baki, 1980; Woodstock *et al.* 1984; sections 4.2.6 and 4.3.2.7).

Interestingly, however, the performance of these seed lots in the soil cold test (section 4.1.3), cold germination test (section 4.2.2) and low temperature respiration (section 4.2.6) did not differ significantly between deteriorated and unaged seed lots. This was because, although the seeds were heavily contaminated by *Fusarium subglutinans*, the biological activity of this virulent fungus may have been suppressed by soil-borne antagonist micro-organisms (Baker, 1968; Cook and Baker, 1983; Burpee, 1990; section 4.3.1.4), but more probably lower temperatures (Gonzalez *et al.*, 1988; Gilbertson *et al.*, 1985; sections 4.3.1.4 and 4.3.2.2). At the same time, because the seeds were from a cold tolerant genotype, exposing them at low temperatures in the chilling range (5-15°C) did not cause any physiological disorder apparently due to adaptive protection mechanisms (section 4.3.2.7), and thus they exploited the low temperature incubation period to imbibe slowly and avoid imbibition damage, solute leakage and undertake some cellular repair processes that gave the aged seed lots an advantage on the subsequent performance at favourable temperature and offset the germinability and vigour differences (Van de Venter and Grobbelaar, 1985; Mashauri, 1991; sections 4.3.1.4, 4.3.2.2 and 4.3.2.7).

Field emergence and emergence rates did not differ significantly between high and low vigour seed lots (Figure 5.1.2). This may be related to the suppressive nature or biocontrol of seed-borne pathogens by soil-borne micro-organisms, or the effect of soil-borne *Fusarium* spp subsequently found to be present at the site (F. Kabeere, unpub. data); or simply because the environmental conditions during planting and field germination did not exert major stress (sections 5.1.1 and 5.2.1). Surprisingly, there was poor correlation between seed quality characters and field emergence in most of the characters examined (section 5.1.2). Probably these parameters were confounded by the interaction of infection, soil-borne pathogens and the seed bed environment. Only the CSVT had a strong relationship with field emergence, presumably because the test involved the use of sodium hypochlorite disinfectant, which is likely to have removed some of the fungi from the seed. Higher losses of plants due to post-emergence damping-off and seedling blight caused by seed-borne and soil-borne pathogens were observed in the October and December plantings, with low vigour seed lots having higher losses than higher vigour seed lots (section 5.1.3). This was related to the influence of weather on host predisposition, especially low moisture stress (Cook, 1968, 1973; section 5.2.4). *Fusarium subglutinans* in particular is active in warm and dry conditions (Shurtleff, 1980; Commedahl and Windels, 1981; Gilbertson *et al.*, 1985). Due to this loss of plants post-emergence, high vigour seed lots in these plantings had a significantly higher final stand in the field (sections 5.1.3 and 5.2.3).

During the heterotrophic growth, there was no significant difference in plant growth among the different seed lots, as demonstrated by similarities in leaf, stem and plant dry weights at the 3rd leaf stage, although the planting dates differed significantly (section 5.1.4). These differences were related to differing environments as sowing was delayed into the spring (section 5.2.5). During early autotrophic growth (5th leaf stage), the performance of the high vigour seed lots was superior to that of low vigour seed lots (section 5.1.4). However, these differences decreased as the plants grew (7th leaf stage - sections 5.1.4 and 5.2.5) because low vigour seed lots produced thicker tillers. The seeds from both high and low vigour seed lots started the grain filling phase at the same pace and reached mass maturity (maximum dry weight) at the same time (sections 5.1.5 and 5.2.6). However, the changes in SMC during seed development showed a very slow and linear loss of moisture after mass maturity, presumably because of genotypic and/or environmental effects (sections 5.1.5 and 5.2.6). As expected, the number of plants hectare^{-1} in the October and December plantings were significantly lower than in the November planting. Low vigour seed lots in the former had significantly fewer plants hectare^{-1} than high vigour seed lots (sections 5.1.6.1 and 5.2.7). Interestingly, however, low vigour seed lots produced a significantly grater seed yield hectare^{-1} than high vigour seed lots because low vigour seed lots compensated for lost plants by producing more tillers and hence reproductive parts (section 5.1.6.2 and 5.2.7).

Artificial drying of cobs produced very low drying rates and uneven drying (section 5.1.7). The driers air flow rates and uniformity were probably affected by the position of the cobs (which were stacked in series) or that the high sugar content created high osmotic potential and lowered the seeds moisture loss rates, resulting in uneven drying (section 5.1.7). Surprisingly, some seeds had germinated on the cob during drying. Because of the slower drying rates seeds were held at 20°C or 25°C for extended periods of time. This was probably sufficient to trigger the germination gene expression (Kermode and Bewley, 1989; Oish and Bewley, 1992; section 5.2.8).

Thousand seed weight, standard germination test, cold germination test, soil cold test and conductivity test results of freshly harvested seeds showed no significant differences among seed lots (sections 6.1.1 and 6.2.1), suggesting the vigour status of the parent did not affect the vigour of the progeny. However, these techniques revealed performance differences among the different planting dates with seeds from the November and December plantings showing inferior performance to that from the October planting (albeit also poor - section 6.1.1), presumably because of the heavy infection of seeds by

Fusarium species in the latter and/or drying damage in the former (sections 6.1.1.5 and 6.2.1; Plates 6.1 and 6.2).

The performance of original seed lots after storage for 14 months was of particular interest. High vigour seed lots showed similar and even superior germinability, vigour and field performance in comparison with unstored seed lots (sections 6.2.2 and 6.2.3). This was because the occurrence and activity of *Fusarium subglutinans*, which was rampant in unstored seeds (sections 4.1.2 and 4.3.1.3) decreased considerably following storage (section 6.2.2). The low vigour seed lot, however, demonstrated an overriding deterioration because the AA treatment accelerated deterioration rates (section 6.2.2). Strong relationships existed between field emergence with most of the seed quality characters (section 6.2.3), confirming the suggestion given earlier that pathogens were responsible for poor field emergence and seed character relationships in the original unstored seeds (section 5.1.2).

7.2 CONCLUSIONS

1. It was not possible to determine if seed vigour status affected field emergence or emergence rates at any of the three planting dates because of the influence of pathogens. However, low vigour seeds lots in the October and December plantings lost a greater number of plants due to post-emergence damping-off and seedling blight, than high vigour seed lots.

2. The effects of seed vigour did become evident during the early stage (5th leaf stage) of vegetative growth and development, but as the plants grew, this vigour influence diminished.

3. Final stands in the field for the October and December plantings were higher in high vigour than in low vigour seed lots due to the loss of plants post-emergence.

4. Seed lot quality did not affect days to pollination, seed size or seed weight and seed yield.

5. The vigour status of the parent did not affect the vigour of the progeny.

7.3 SCOPE FOR FUTURE RESEARCH

1. It has been demonstrated that infection of seeds by pathogenic fungi seem to be a serious problem in this crop. Research targeted to control of the pathogens, either chemically, biologically or through plant breeding (disease resistant cultivars) would be a worthwhile area for continued research.

2. In this study, it has been observed that lower plant densities produced higher seed yields than higher plant densities. This is an interesting area for further work, ie to establish the optimum plant population for maximum yield of this cultivar of sweetcorn.

3. In the present study uneven drying and sprouting on the cob during drying were evident. Methods for artificial drying of this high sugar content cultivar would be another rewarding area for future investigation.

4. It was not possible to determine the effect of seed lot vigour status on plant field performance, or to determine whether vigour tests were related to field emergence performance because of the confounding effects of fungi. This work needs to be repeated with a) disease-free seed, or b) seed which has been fungicide treated.

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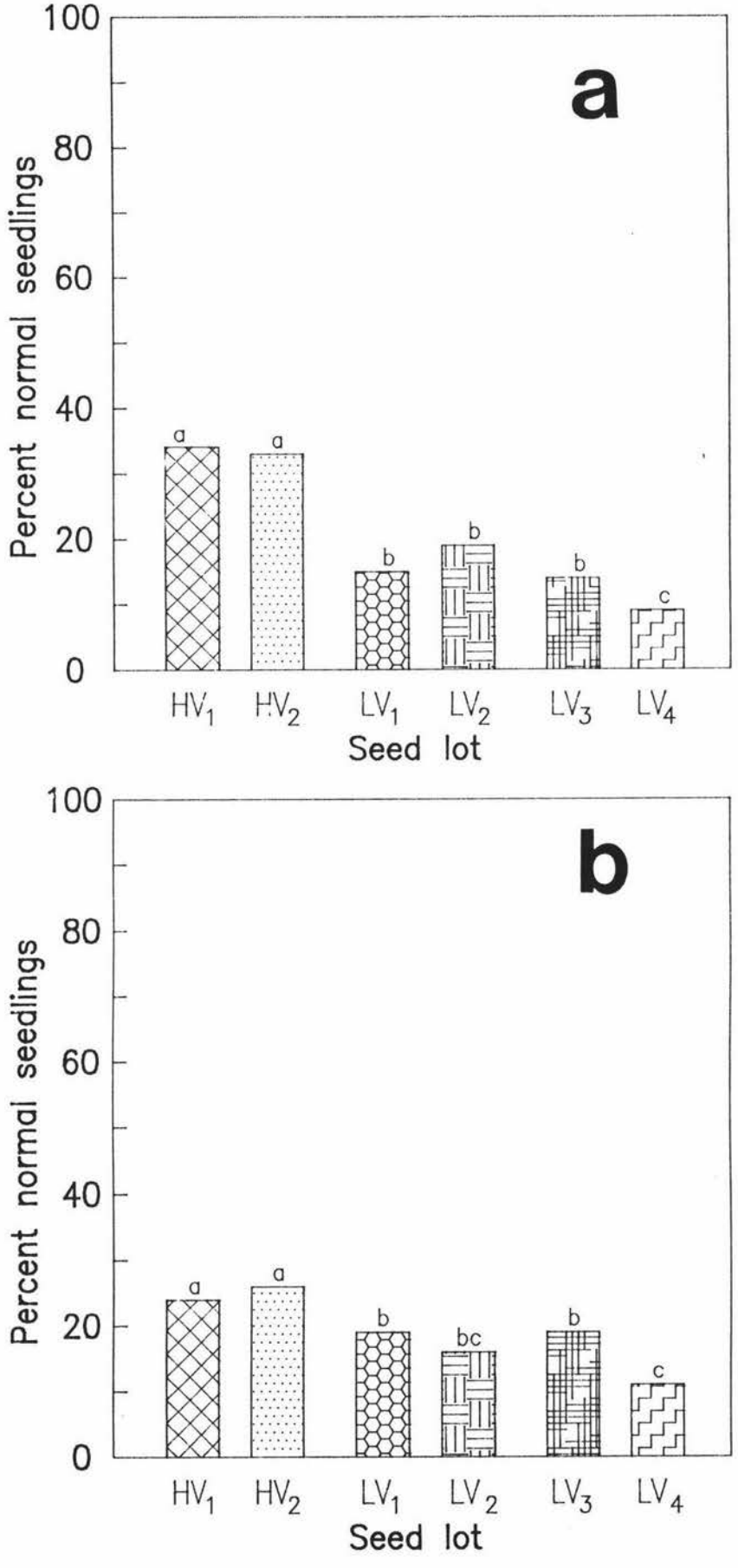
APPENDICES

Soil name	Parent material or rock	Slope: Topographic position	Description of representative soil profile		Distinguishing features of soil and environment	Nature of underlaying subsurface	Drainage	
							Overall	Internal
Ohakea silt loam	Colluvium	Flat: old fans over-lying low terraces	A_1	0-23 cm dark brown silt loam; few reddisk brown mottles; friable; moderate nut structure	Occurs on low terrace from old colluvium overlying stony alluvium. Characterised by compact light grey heavy silt loam to clay loam subsoil with yellowish brown mottles and black concretions, overlying gravels and stones below about 1 m from the surface	Underlain by 2 to 5 m of weakly cemented gravels and stones with sand. In areas north-east of Aokautere is underlain by a layer of peat over plastic clay	Imperfectly to poorly drained	Slow
			B_1gc	23-41 cm greyish brown heavy silt loam; few to many yellowish brown mottles; abundant black concretions; friable; moderate nut structure				
			B_2g	41-71 cm light grey clay loam; abundant yellowish brown mottles; firm; weak blocky structure				
			B_3g	71-99 cm mottled light grey and yellowish brown heavy silt loam; few light grey vertical veins; very firm; massive				
			D	on iron-stained gravels and stones				

Appendix 3.1 Soil description for the trial area used in this study.



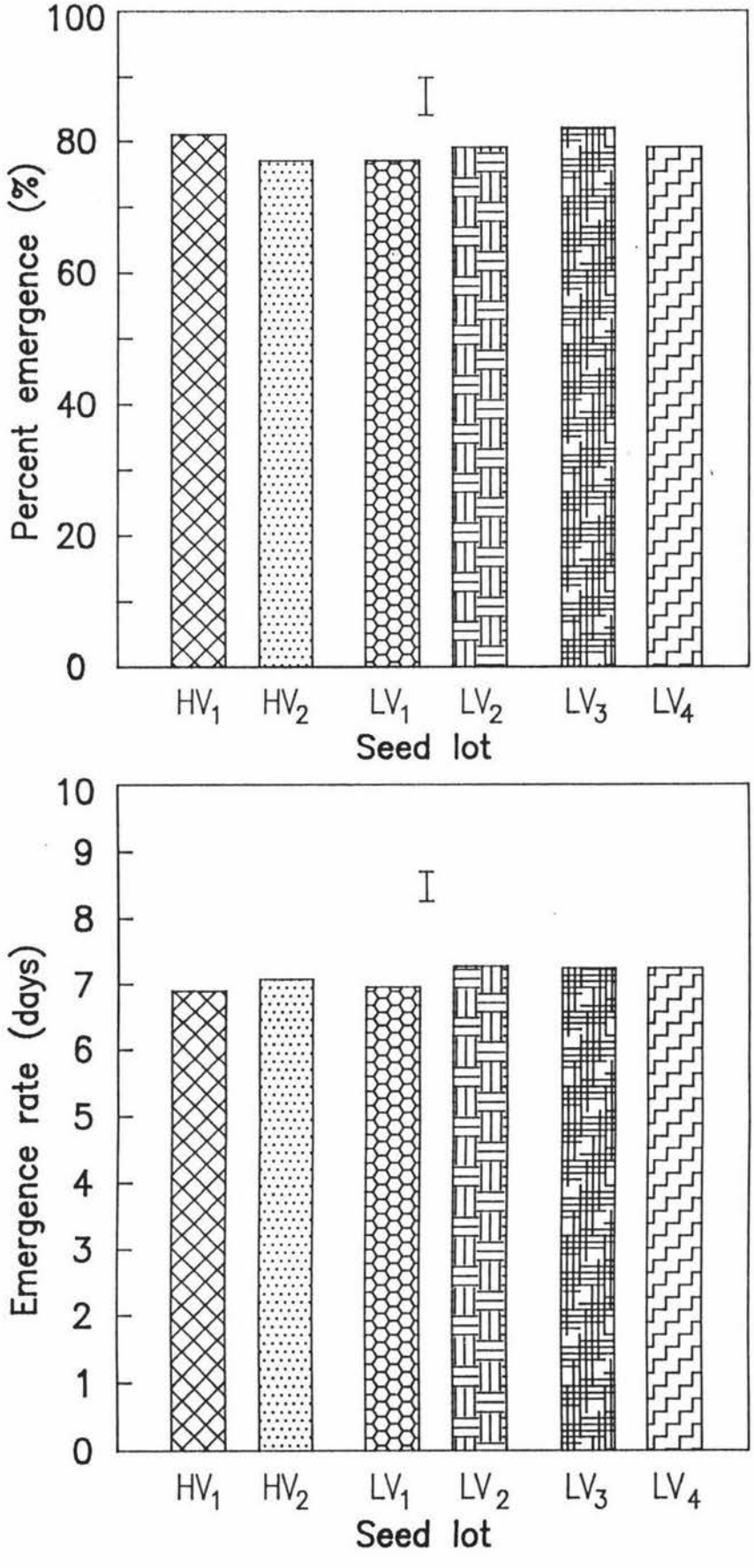
Appendix 4.1 Percentage normal seedlings obtained from different seed lots of super sweet corn (cv Illini Gold) soaked at 10°C for 24 hours (A) or 48 hours (B). Mean values with the same letter are not significantly different at $P < 0.05$, based on Duncan's multiple range test.

Appendix 5.1a **Analysis of variance (ANOVA) table for field emergence in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	14	1923.458333	137.389881	4.31	0.0003
Error	33	1052.354167	31.889520		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	101.395833	33.798611	1.06	0.3794
Vigour	3	32.895833	10.965278	0.34	0.7938
Date	2	1708.875000	854.437500	26.79	0.0001
Vigour*Date	6	80.291667	13.381944	0.42	0.8606

Appendix 5.1b **Analysis of variance (ANOVA) table for emergence rate in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	14	370.0566667	26.4326190	48.59	0.0001
Error	33	17.9533333	0.5440404		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	4.0116667	1.3372222	2.46	0.0802
Vigour	3	1.1633333	0.3877778	0.71	0.5514
Date	2	362.5537500	181.2768750	333.20	0.0001
Vigour*Date	6	2.3279167	0.3879861	0.71	0.6415



Appendix 5.2 Percent field emergence (a) and emergence rate (b) of super sweet corn (cv Illini Gold) for different seed lots in the December sowing. Data are means of four replicates. Bar represent a least significant difference at $P < 0.05$.

Appendix 5.2x **Pearsons correlation coefficients between seed quality characters and field emergence for the October, November and December plantings.**

Character	October planting	November planting	December planting
Standard germination test: NS	0.02	0.47	0.19
Soil cold test: NS	0.20	0.12	0.22
Soil cost test: HV	0.21	0.29	0.23
Soil cold test: SL	0.35	0.27	0.16
Soil cold test: SDWT	0.17	0.04	0.14
Seedling growth test: SL	0.14	0.33	0.12
Seedling growth test: RDWT	0.31	0.05	0.03
Seedling growth test: SDWT	0.20	0.34	0.03
Complex stress vigour test: NS	0.20	0.60*	0.04
Complex stress vigour test: HV	0.07	0.64**	0.11

NS = Normal seedlings
HV = High vigour
SL = Shoot length
RDWT = Root dry weight
SDWT = Shoot dry weight

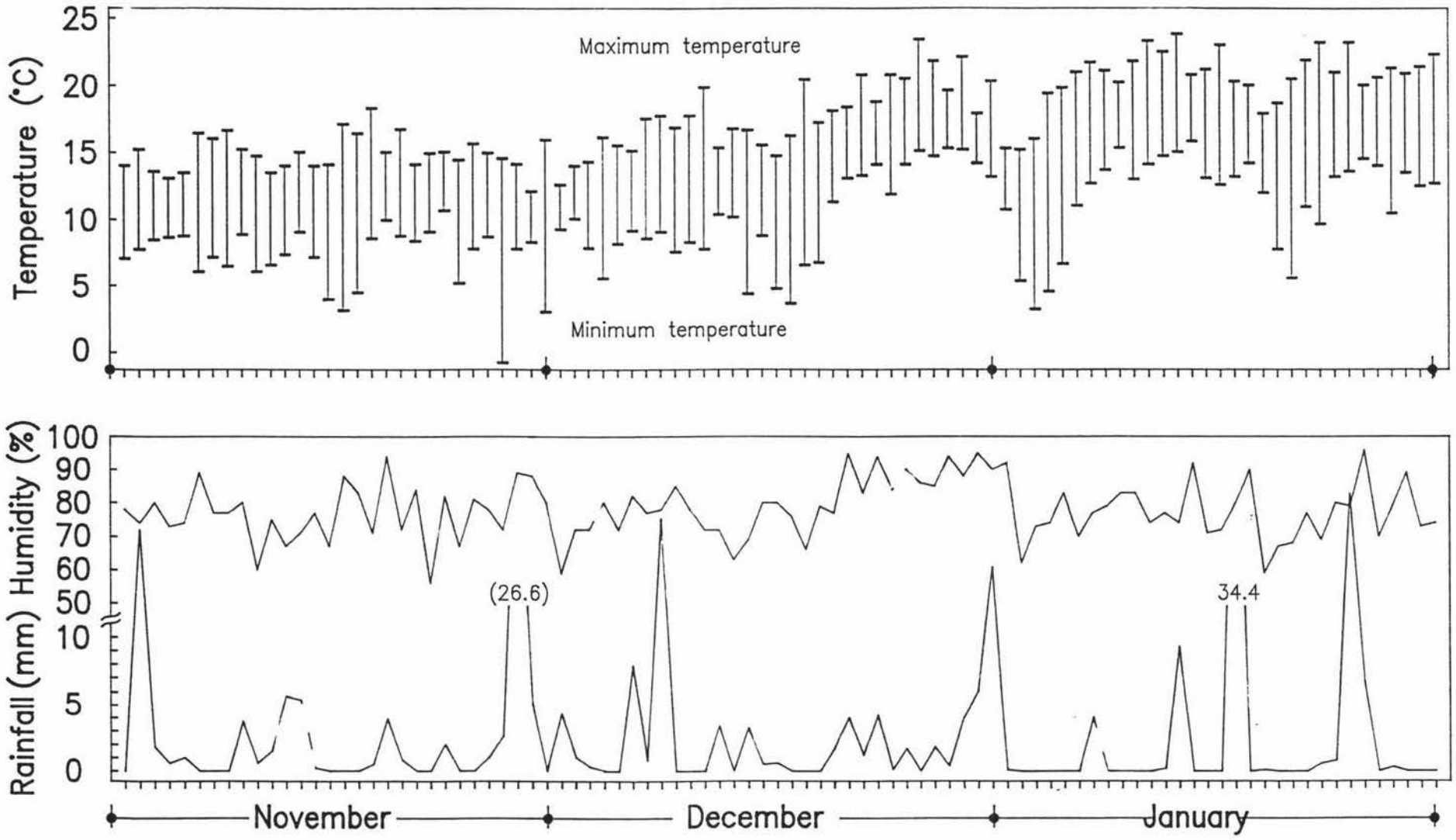
* = significant at $P < 0.05$
** = significant at $P < 0.01$

Appendix 5.3a **Analysis of variance (ANOVA) for losses of plants in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	14	6670.000000	476.428571	13.01	0.0001
Error	33	1208.666667	36.626263		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	66.833333	22.277778	0.61	0.6144
Vigour	3	1088.333333	366.444444	10.00	0.0001
Date	2	4976.791667	2488.395833	67.94	0.0001
Vigour*Date	6	527.041667	87.840278	2.40	0.0493

Appendix 5.3b **Analysis of variance (ANOVA) for final stand in the field in a 4 x 3 factorial experiment.**

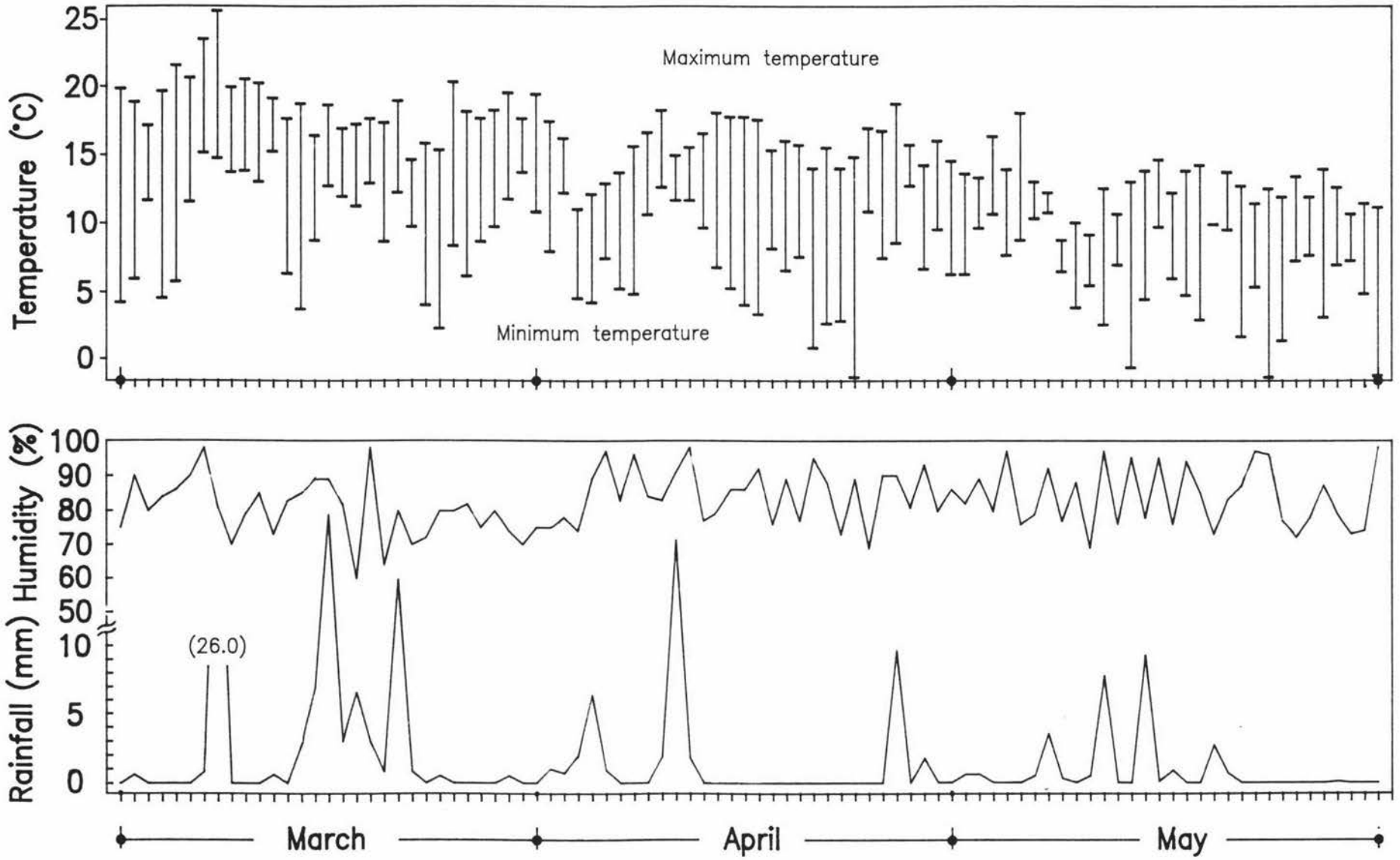
Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	14	13501.62500	964.40179	12.46	0.0001
Error	33	2554.18750	77.39962		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	3	74.06250	24.68750	0.32	0.8116
Vigour	3	1560.89583	520.29861	6.72	0.0012
Date	2	10762.87500	5381.43750	69.53	0.0001
Vigour*Date	6	1103.79167	183.96528	2.38	0.0510



Appendix 5.4 Weather data during vegetative development.

Appendix 5.5 Number of days to 50% silking of super sweet corn (cv. Illini Gold) as affected by vigour levels and planting dates. Data are means of four replicates.

Seed lot	October	November	December
HV_1	88	78	76
HV_2	88	78	76
LV_1	87	78	76
LV_2	88	78	76



Appendix 5.6 Weather data during seed development.

Appendix 5.7a **Analysis of variance (ANOVA) table for the number of plants per hectare in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	2460720506	246072051	19.01	0.0001
Error	16	207140193	12946262		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	27515835	13757918	1.06	0.3687
Vigour	2	173969161	86984580	6.72	0.0076
Date	2	2003052480	1001526240	77.36	0.0001
Vigour*Date	4	256183031	64045758	4.95	0.0087

Appendix 5.7b **Analysis of variance (ANOVA) table for number of tillers per plant in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	16.22222222	1.62222222	10.62	0.0001
Error	16	2.44444444	0.15277778		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	0.22222222	0.11111111	0.73	0.4985
Vigour	2	0.66666667	0.33333333	2.18	0.1453
Date	2	14.00000000	7.00000000	45.82	0.0001
Vigour*Date	4	1.33333333	0.33333333	2.18	0.1175

Appendix 5.7c **Analysis of variance (ANOVA) table for number of cobs per tiller in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	3.48148148	0.34814815	9.40	0.0001
Error	16	0.59259259	0.03703704		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	0.07407407	0.03703704	1.00	0.3897
Vigour	2	0.51851852	0.25925926	7.00	0.0065
Date	2	0.85185185	0.92592593	25.00	0.0001
Vigour*Date	4	0.03703704	0.25925926	7.00	0.0019

Appendix 5.7d **Analysis of variance (ANOVA) table for number of seeds per cob in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	28121.25926	2812.12593	0.87	0.5737
Error	16	51475.70370	3217.23148		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	134.29630	67.14815	0.02	0.9794
Vigour	2	2039.407412	1019.70370	0.32	0.7238
Date	2	957.85185	478.92593	0.15	0.8629
Vigour*Date	4	24989.70370	6247.42593	1.94	0.1525

Appendix 5.7e **Analysis of variance (ANOVA) table for seed yield per plant in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	265159.5489	26515.9549	29.18	0.0001
Error	16	14540.5978	908.7874		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	2658.4289	1329.2144	1.46	0.2610
Vigour	2	19641.2067	9820.6033	10.81	0.0011
Date	2	205035.3267	102517.6633	112.81	0.0001
Vigour*Date	4	37824.5867	9456.1467	10.41	0.0002

Appendix 5.7f **Analysis of variance (ANOVA) table for seed yield per hectare in a 4 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	76.46222222	7.64622222	16.32	0.0001
Error	16	7.49777778	0.46861111		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	0.46222222	0.23111111	0.49	0.6197
Vigour	2	1.26000000	0.63000000	1.34	0.2886
Date	2	72.46888889	36.23444444	77.32	0.0001
Vigour*Date	4	2.27111111	0.56777778	1.21	0.3444

Appendix 6.1a **Analysis of variance (ANOVA) for thousand seed weight of fresh harvested seed lots in a 3 x 3 factorial experiment.**

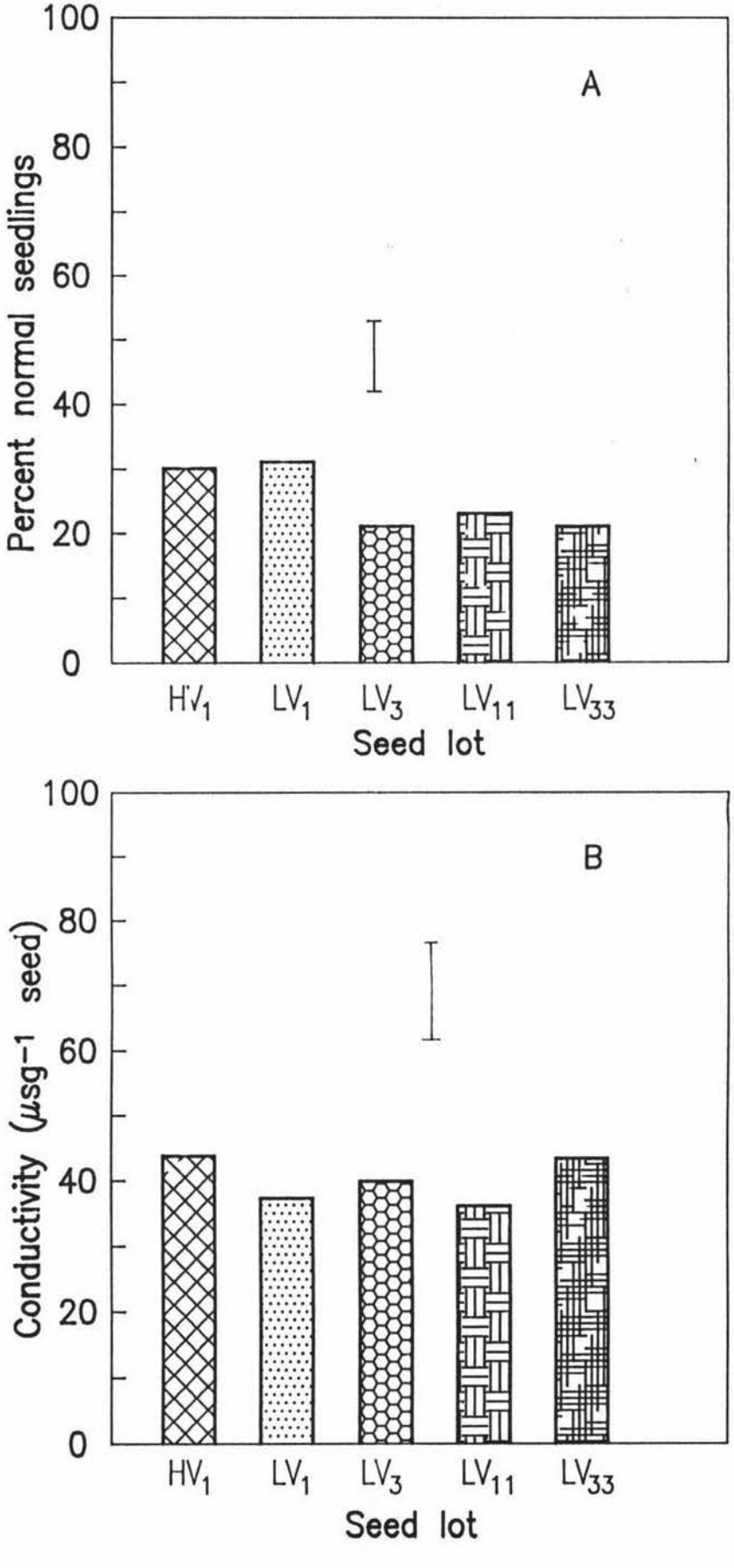
Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	15	63320.37639	4221.35843	286.94	0.0001
Error	56	823.85472	14.71169		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	7	88.99778	12.71397	0.86	0.5403
Vigour	2	19.36861	9.68431	0.66	0.5217
Date	2	63115.28028	31557.64014	2145.07	0.0001
Vigour*Date	4	96.72972	24.18243	1.64	0.1761

Appendix 6.1b **Analysis of variance (ANOVA) for standard germination of fresh harvested seed lots in a 3 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	3528.888889	352.888889	19.13	0.0001
Error	16	295.111111	18.444444		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	134.222222	67.111111	3.64	0.0498
Vigour	2	14.222222	7.111111	0.39	0.6862
Date	2	3086.222222	1543.111111	83.66	0.0001
Vigour*Date	4	294.222222	73.555556	3.99	0.0197

Appendix 6.1c **Analysis of variance (ANOVA) for electrical conductivity of fresh harvested seed lots in a 3 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	3371.655556	337.165556	45.31	0.0001
Error	16	119.051111	7.440694		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	52.148889	26.074444	3.50	0.0547
Vigour	2	36.028889	18.014444	2.42	0.1206
Date	2	3241.428889	1620.714444	217.82	0.0001
Vigour*Date	4	42.048889	10.512222	1.41	0.2747



Appendix 6.2 Percentage normal seedlings (a) and electrical conductivity (b) for all seed lots harvested from the December planting. Data are means of three replicates. Bar represent a lsd at $P < 0.05$.

Appendix 6.3a **Analysis of variance (ANOVA) for cold germination test of fresh harvested seed lots in a 3 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	4779.555556	477.955556	24.79	0.0001
Error	16	308.444444	19.277778		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	59.555556	29.777778	1.54	0.2436
Vigour	2	59.555556	29.777778	1.54	0.2436
Date	2	4610.666667	2305.333333	119.59	0.0001
Vigour*Date	4	49.777778	12.444444	0.65	0.6380

Appendix 6.3b **Analysis of variance (ANOVA) for soil cold test of fresh harvested seed lots in a 3 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	4910.888889	491.088889	7.91	0.0002
Error	16	993.777778	62.111111		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	94.888889	47.444444	0.76	0.4821
Vigour	2	138.666667	69.333333	1.12	0.3517
Date	2	4530.666667	2265.333333	36.47	0.0001
Vigour*Date	4	146.666667	36.666667	0.59	0.6745

Appendix 6.3c **Analysis of variance (ANOVA) for field emergence of fresh harvested seed lots in a 3 x 3 factorial experiment.**

Source	DF	Sum of Squares	Mean Square	F value	Pr > F
Model	10	10541.03704	1054.13070	16.26	0.0001
Error	16	1037.03704	64.81481		
Source	DF	Type I SS	Mean Square	F Value	Pr > F
Block	2	21.62963	10.81481	0.17	0.8478
Vigour	2	165.62963	82.81481	1.28	0.3056
Date	2	10218.96296	5109.48148	78.83	0.0001
Vigour*Date	4	134.81481	33.70370	0.52	0.7224