MASSEY UNIVERSITY

1. * (a) I give permission for my thesis, entitled

Vigour assessment in Pinus radiata D. Don seeds

.....

.....

to be made available to readers in the Library under the conditions determined by the Librarian.

(b) I agree to my thesis, if asked for by another institution, being sent away on temporary loan under conditions determined by the Librarian.

(c) I also agree that my thesis may be copied for Library use.

2. * I do not wish my thesis, entitled

.....

.....

.....

to be made available to readers or to be sent to other institutions without my written consent within the next two years.

Signed .....

Date 3 - 8 - 1990

* Strike out the sentence or phrase which does not apply.

The Library
Massey University
Palmerston North, N.Z.

The copyright of this thesis belongs to the author. Readers must sign their name in the space below to show that they recognise this. They are asked to add their permanent address.

Name and Address Date

.....

.....

.....

.....

.....

VIGOUR ASSESSMENT IN *Pinus radiata* D. DON SEEDS

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

HERO DIEN P. KARTIKO

1990

ABSTRACT

Kartiko, H.D.P.: M.Agr.Sc. (Seed Technology)

Title of Thesis: Vigour Assessment in *Pinus radiata* D. Don Seeds

Supervisors: [1]Dr P. Coolbear and [2]Mr A. Firth

[1]Seed Technology Centre, Massey University, Palmerston North

[2]Forest Research Institute, Rotorua

The sensitivity and/or predictivity of various vigour test methods (which include conductivity, tetrazolium, x-ray contrast, seedling growth, controlled deterioration, complex stressing vigour, and low temperature/osmotic stress tests) for prepared lots of Pinus radiata seeds were investigated in this study. The best tests were the controlled deterioration test with two days aging treatment (CD2d) test), the prechilled seedling growth test (SG+pr test), and the complex stressing vigour test (CSV test). These were then further investigated to evaluate their ability to predict the performance of different seed lots at the Forest Research Institute (FRI) nursery, Rotorua.

The CD2d, SG+pr and CSV tests showed good correlation, especially with percentage of plantable seedlings at the FRI nursery. In addition, these tests seem to have met most of the AOSA's (1983) criteria for a practical vigour testing, as they are simple and can be done in a relatively short period of time. For application purposes, it is suggested that the test parameters which gave the highest correlation coefficient value with percentage of plantable seedlings in the nursery should be used as a reliable measurement. Therefore,

percentage normal seedlings should be used in either the CD2d or the CSV test, whereas T_{50} radicle emergence seems more predictive in the SG+pr test.

For application in other nurseries, these tests may still be valid, especially if pre-sowing treatment and nursery conditions are about the same as in the FRI nursery. If conditions do differ, however, the CD2d and SG+pr tests are more likely to be useful than the CSV test. This hypothesis is based on the fact that the CD2d and SG+pr tests also gave good correlations with the glasshouse (optimum conditions) and winter field tests (sub-optimum conditions). In contrast, there was no significant correlation given by the CSV test in relation to the glasshouse and winter field tests.

Seed weight had a significant effect on seedling dry weight and T_{50} radicle emergence if there was a large seed weight variation between seed lots. In this case, generally heavier seeds had better performance than the lighter ones. If there was only small variation in overall seed weight among seed lots, however, the important effects of individual differences in seed weights were masked.

The direction of further studies would seem to be to evaluate the reproducibility of correlation coefficient values and regression equations by the CD2d, SG+pr and CSV tests in the same nursery site over several sowings. Additionally, vigour test evaluation using seed lots from individual clones would also seem to be important.

ACKNOWLEDGEMENTS

In the name of Allah most gracious, most merciful. Praise be to Almighty Allah, the Lord of Universe, and may peace be upon prophet Muhammad s.a.w. and his family.

I wish to express my appreciation and sincere gratitude to my parents, my parents-in-law, my wife, my son, and my brothers and sisters for all their support and sacrifices.

Then I also extend my thanks and gratitude to Dr P. Coolbear for his constructive suggestions and supervision in the conduct of this study, and to Dr M.J. Hill, Mr C.R. McGill and all of the staff of the Seed Technology Centre for all their help and support. I also gratefully acknowledge the help and support of Mr A. Firth, Dr M.I. Menzies, and Mr Mike Dibley of the New Zealand Forest Research Institute, Rotorua.

I also extend my thanks to the Indonesian and New Zealand Governments which have contributed greatly in various phases of this study, and also to the Director and staff of the Forestry Seed Technology Centre, Bogor, Indonesia for all their help and support.

Finally, I also express my gratitude to all of my friends and other people not already mentioned for all of their cooperation and good relationships.

TABLE OF CONTENTS

Page

ABSTRACT i
ACKNOWLEDGEMENTS iii
TABLE OF CONTENTS iv
LIST OF TABLES viii
LIST OF FIGURES ix
LIST OF APPENDICES xii

I. INTRODUCTION 1

II. LITERATURE REVIEW 4
2.1. *Pinus radiata*: the need for high vigour seed 4
2.1.1. The role of seed vigour in *P. radiata* forest establishment 4
2.1.2. The problem of low vigour 4
2.1.2.1. The condition of the mother trees 5
2.1.2.2. Collection date 7

2.2. Seed deterioration 8
2.2.1. Factors affecting seed deterioration 8
2.2.1.1. Effect of genotype 8
2.2.1.2. Effect of storage environment 10
2.2.1.3. Artificial aging conditions 11
2.2.2 Mechanism of seed deterioration 12

2.3 Seed vigour testing 14
2.3.1. The importance of seed vigour testing 14
2.3.2. Seed vigour testing methods 15
2.3.2.1. Tests not involving germination 16
2.3.2.2. Tests involving germination 20
2.3.2.3. Stress tests 22
2.3.3 Scope for further investigation 23

III. MATERIALS AND METHODS 35
3.1. Deterioration in *P. radiata* seeds 35
3.2. Vigour tests evaluation in *P. radiata* seeds using 5 seed lots which varied according to seed size and age 37
3.2.1. Seed lot preparation 37
3.2.2. Vigour testing 38
3.2.2.1. Seedling growth tests 38
3.2.2.2. Tests not involving germination 38
3.2.2.3. Stress tests 41
3.2.3. Glasshouse test 43
3.2.4. Winter field test 44
3.3. Vigour tests evaluation in *P. radiata* seeds using 16 seed lots which varied according to type of mother tree and collection date 45
3.3.1. Seed weight and vigour tests 47
3.3.1.1. Seed weight test 47
3.3.1.2. Vigour tests 47
3.3.2. Nursery and standard germination tests carried out by FRI (the New Zealand Forest Research Institute) 47

IV. RESULTS 48
4.1. Deterioration in *P. radiata* 48
4.2. Vigour tests evaluation in *P. radiata* seeds using 5 seed lots which varied according to seed size and age 54
4.2.1. Seed lot performance in the glasshouse test 54
4.2.2. Conditions and seed lots performance in the winter field tests 54
4.2.3. Seed lots performance in seedling growth test 62
4.2.4. Seed lot performance in radiographic test 68
4.2.5. Seed lot performance in topographical tetrazolium test 68
4.2.6. Seed lot performance in the conductivity test 69
4.2.7. Seed lot performance in the controlled deterioration (CD) test 69
4.2.8. Seed lot performance in the complex stressing vigour test 69
4.2.9. Seed lot performance in low temperature/osmotic stress test 72
4.2.10. Correlation between vigour tests and glasshouse/winter field test 72

4.3. Vigour tests evaluation in *P. radiata* seeds using 16 seed lots which varied according to type of mother tree and collection date 87
4.3.1. Seed lot performance in the standard germination and nursery tests carried out by FRI 87
4.3.2. Seed weight 88
4.3.3. Seed lot performance in the vigour tests done at Seed Technology Centre 88
4.3.4. Correlation of seed weight, standard germination, and vigour tests with FRI nursery test 94
4.3.4.1. Correlation between seed weight and nursery test 94
4.3.4.2. Correlation between standard germination test by (FRI) and the nursery test 97
4.3.4.3 Correlation between vigour tests and nursery test 97

V. DISCUSSION 103
5.1. Deterioration in *P. radiata* seeds 103
5.1.1. The pattern of deterioration 103
5.1.2. The role of high temperature and relative humidity 104
5.1.3. Changes which may be associated with the loss of vigour and viability 105
5.1.4. Changes in normal seedling dry weight 106
5.2. Vigour test evaluation 106
5.2.1. Promising suitable vigour tests for *P. radiata* 107
5.2.1.1. The controlled deterioration test with 2 days aging (CD2d test) 108
5.2.1.2. The prechilled seedling growth test (SG + pr test) 110
5.2.1.3. The complex stressing vigour test (CSVT) 113
5.2.1.4. The low temperature germination test at 10°C, 0 bar 114
5.2.2. Suitable vigour tests for assessing seedling establishment at Rotorua (FRI) nursery 115
5.2.3. The effect of seed coat condition on germination performance and the conductivity test result 117
5.2.3.1. The effect of seed coat condition on germination performance 117

5.2.3.2. The effect of the condition of the seed coat on the conductivity test result 120
5.2.4. The relationship between seed weight and seedling performance 122

VI. CONCLUSION 125
6.1. Deterioration in *P. radiata* seeds 125
6.2. Promising suitable vigour tests for *P. radiata* 125
6.3. Suitable vigour tests for assessing seedling establishment of *P. radiata* at Rotorua (FRI) nursery 126
6.4. The relationship between seed weight and seedling performance 128
6.5. Scope for further studies 128

BIBLIOGRAPHY 130
APPENDICES 146

LIST OF TABLES

Table		Page
2.1.	Vigour tests not involving germination	24
2.2.	Vigour tests involving germination	29
2.3.	Stress tests	32
3.1	Planting years and clone number of parent trees of the seed lot used in the aging study of *P. radiata* seeds	35
3.2.	Moisture contents after the controlled deterioration test	42
3.3	Description of *P. radiata* seed lots	46
4.1.	100 seed weight data for the different seed lots	89

LIST OF FIGURES

Figure		Page
4.1.A.	Changes in % radicle emergence, during accelerated aging at 40°C and 45°C with 100% RH up to 14 days	49
4.1.B.	Changes in % normal seedlings during accelerated aging at 40°C and 45°C with 100% RH up to 14 days	50
4.1.C.	Changes in T_{50} radicle emergence and T_{50} normal seedlings during accelerated aging at 40°C with 100% RH up to 14 days	51
4.1.D.	Changes in T_{50} radicle emergence and T_{50} normal seedlings during accelerated aging at 45°C with 100% RH up to 14 days	52
4.1.E.	Changes in normal seedlings dry weight during accelerated aging at 40°C and 45°C with 100% RH up to 14 days	53
4.2.A.	Percentage of normal seedlings in glasshouse test	55
4.2.B.	T_{50} normal seedlings in glasshouse test	56
4.2.C.	Normal seedlings dry weight in glasshouse test	57
4.3.A.	Cumulative emerged and normal seedlings in the winter field test	58
4.3.B.	Surviving emerged and normal seedlings in the winter field test	59
4.3.C.	Time of 50% emergence and 50% establishment of normal seedlings in the winter field test	60
4.3.D.	Dry weight of shoots of normal seedlings in the winter field test	61
4.4.A.	Percentage of radicle emergence in seedling growth test	63
4.4.B.	Percentage of normal seedlings in seedling growth test	64
4.4.C.	T_{50} radicle emergence in seedling growth test	65
4.4.D.	T_{50} normal seedlings in seedling growth test	66
4.4.E.	Normal seedlings dry weight in seedling growth test	67

Figure		Page
4.5.	Seed lot performance in controlled deterioration (CD) test	70
4.6.	Seed lot performance in complex stressing vigour test	71
4.7.A.	Percentage of radicle emergence at 10°C	73
4.7.B.	T_{50} radicle emergence at 10°C	74
4.7.C.	T_{50} radicle emergence at 15°C	75
4.8.	Radicle emergence from seedling growth test + prechilling treatment vs surviving normal seedlings in the field	77
4.9.	Radicle emergence from seedling growth test without prechilling vs surviving normal seedlings in the field	78
4.10.	Normal seedlings from the controlled deterioration test with 2 days aging treatment vs normal seedlings in the glasshouse	79
4.11.	Normal seedlings from the controlled deterioration test with 2 days aging treatment vs T_{50} normal seedlings in the glasshouse	80
4.12.	Normal seedlings from the controlled deterioration test with 2 days aging treatment vs surviving normal seedlings in the field	81
4.13.	Normal seedlings from the controlled deterioration test with 2 days aging treatment vs T_{50} normal seedlings in the field	82
4.14.	Normal seedlings from the complex stressing vigour test vs surviving normal seedlings in the field	83
4.15.	Radicle emergence in 10°C, 0 bar vs surviving normal seedlings in the field	84
4.16.	Radicle emergence in 15°C, 0 bar vs surviving normal seedlings in the field	85
4.17.	Radicle emergence in 20°C, 0 bar vs surviving normal seedlings in the field	86

Figure Page

4.18.A. 100 seed weight of *Pinus radiata* clones on lot 13 at moisture content 7.69% 90

4.18.B. 100 seed weight of *Pinus radiata* clones of lot 14 at moisture content 7.69% 91

4.18.C. 100 seed weight of *Pinus radiata* clones of lot 15 at moisture content 7.69% 92

4.18.D. 100 seed weight of *Pinus radiata* clones of lot 16 at moisture content 7.69% 93

4.19. Seed weight vs seedling height in the nursery 95

4.20. Seed weight vs seedling diameter in the nursery 96

4.21. Radicle emergence from the controlled deterioration test with 2 days aging treatment vs plantable seedlings in the nursery 98

4.22. Normal seedlings from the controlled deterioration test with 2 days aging treatment vs plantable seedlings in the nursery 99

4.23. T_{50} radicle emergence in the controlled deterioration test with 2 days aging treatment vs T_{50} germination in the nursery 101

4.24. T_{50} normal seedlings in the controlled deterioration test with 2 days aging treatment vs T_{50} germination in the nursery 102

LIST OF APPENDICES

Appendix		Page
1.	Quantitative tetrazolium test results	146
2.	Nursery and standard germination test procedures (carried out by FRI)	148
3.	Temperature in glasshouse test	150
4.A.	Temperature in the winter field test	151
4.B.	Rainfall in the winter field test	152
5.	Seed lot performance in glasshouse and winter field tests	153
6.	Seed lot performance in vigour tests	154
7.	Correlation (r) between vigour tests and glasshouse/winter field test	157
8.	Seed lot performance in standard germination and nursery tests (carried out by FRI)	161
9.	Seed lot ranking in standard germination and nursery tests according to relative vigour score	162
10.	Seed lot ranking in the overall seed lots in standard germination and nursery tests according to relative vigour score	163
11.	Calculation of mean relative vigour score in nursery test	164
12.	Seed lot performance in vigour tests done at Seed Technology Centre	165
13.	Seed lot ranking in vigour tests according to relative vigour score	166
14.	Seed lot ranking (within groups) in vigour test according to mean relative vigour score	167
15.	Seed lot ranking in vigour tests according to mean rvs	168
16.	Calculation to obtain mean relative vigour score (rvs) in vigour tests	169

Appendix Page

17. Correlation of seed weight, standard germination, and vigour tests with nursery test 170

18. Summary of weather during seedling growth of *P. radiata* in the nursery at the FRI, Rotorua in 1987/88 171

I. INTRODUCTION

Pinus radiata which originally might have come from Ano Nuevo Point, on the south-western part of the North American coast (Bannister, 1973) nowadays covers over one million hectares of plantation forests in New Zealand (FRI, 1987) and produces a very useful and versatile wood, e.g. logs, solid timber, wood chips, pulp and paper. These account for ten percent of New Zealand's overseas earnings (Clifton, 1985). It has a medium density soft wood with an even texture. In addition, the physical structure of the wood permits ready preservative treatment. Therefore, the end products are stable, strong, resistant to insects and fungi, and easily finished with a variety of stains, clear finishes, paint and overlays (FRI, 1987).

For planting purposes, a large number of genetically improved seed is needed, and for 1984-1985 season, for example, the seed demand was about 3500 kg. To fulfil this demand, almost all of the current seed production is from 850 series clones and half of the total quantity is collected from Gwavas orchard. By 1990, production from 850 series clones is planned to be reduced and almost completely replaced by seed from 268 and 875 series clones collected mainly from Kaingaroa orchard (Vincent, 1986). Despite the fact that these seeds are genetically improved, the vigour of the seeds at present seems to be quite low, even though laboratory germination tests show that at least 90% of seeds are viable (see section 2.1.2.). Therefore, it is important to select the best seed production methods, and the best clones which can produce high vigorous seeds. This requires the identification of suitable vigour tests for this species.

It appears that there are not many reports concerning vigour tests in *P. radiata* or other tree species. In the few studies which have been conducted there has been little attempt to

correlate results with field performance. This analysis is very crucial, as high vigour seeds according to a vigour test do not always produce good performance in the field (see section 2.3.).

In some agricultural seeds, some vigour tests gave high and significant correlation with field performance in certain stations. However, they may give poor correlations with field performance in other stations (see section 2.3.). Therefore, an investigation to look for a general vigour test with suitability for all kind of field conditions seems to be over ambitious (see Hampton and Coolbear, 1990).

Based on these reasons, vigour test evaluation in *P. radiata* was conducted in this study with objectives as follows:

(i) to characterise the seed deterioration pattern in *P. radiata*, in order to determine suitable aging treatments for creating seed lots which have different vigour levels,

(ii) to investigate promising suitable vigour tests for *P. radiata*, and

(iii) to investigate these tests for their suitability for predicting seedling establishment in the nursery at the Forest Research Institute (FRI), Rotorua.

To fulfil these objectives, three stages of experimentation were conducted in this study. The first stage was a study using accelerated aging techniques to determine the best methods of preparing deteriorated seed lots. The second stage was evaluation of various vigour test methods using 5 prepared seed lots which varied according to seed weight and age. The third stage was evaluation of the best test methods (i.e. the controlled deterioration test with 2 days aging treatment, the prechilled seedling growth test, and the

complex stressing vigour test) to predict seedling performance at the FRI nursery using 16 mixed seed lots which varied according to type of mother tree and collection date.

II. LITERATURE REVIEW

2.1. *Pinus radiata*: the need for high vigour seed

2.1.1. The role of seed vigour in *P. radiata* forest establishment

Seed of high vigour is important to New Zealand forestry as there is a shortage of adequate numbers of plantable seedlings which can easily and rapidly establish contact with water and nutrients in new soil when transplanted from the nursery and can also survive under drought or frost conditions. This capability is very significant since transplantation from the nursery bed to planting sites may cause damage to fine roots and reduce or stop photosynthesis temporarily. Desirable characteristics are seedlings with adequate food and water reserves and high root growth potential. Drought or frost tolerance is also very important as some sites have severe stress conditions such as sandy areas like the Canterbury plains, and Central Otago, and cold, high altitude sites in Central North Island and Southland (FRI, 1988).

2.1.2. The problem of low vigour

Despite its importance, the general level of seed vigour in *P. radiata* seems to be quite low at present as the number of plantable seedlings raised in New Zealand nurseries is only about 50% of the number of seeds sown, even though laboratory tests show that at least 90% of seeds are viable (FRI, 1985). Reasons for this condition may involve mother tree condition and collection dates.

2.1.2.1. The condition of the mother trees

Factors which contribute to the effect of mother trees on seed vigour probably include clonal series, propagation system, pollination system and age. These will be discussed below:

a. Clonal series

Clonal series is most likely to have a great effect on seed vigour as different genotypes will usually produce different vigour levels. The effect of genotype on seed vigour can be seen, for example, in Norway spruce, where seeds from a higher latitude had faster rates of oxygen uptake and carbon-dioxide output than seeds from lower latitudes (Bhumibhamon, 1976). Variation in photosynthesis, respiration and photorespiration were also found in this species among seedlings from three different Finnish stands (Pelkonen and Luukkanen, 1974).

b. Propagation system

The propagation system (using seed, grafts or cuttings) may have a significant impact on seed quality as differences in these systems may affect mother tree condition. For example, mother trees from grafting, often suffer from delayed incompatibility between scion and rootstock which restricts the movement of food material from the stem to the roots so that the tree becomes unhealthy and can only produce small cones and seeds (FRI, 1974). Mother trees from cuttings were also reported to suffer some mortalities associated with bark splitting and resin-bleeding (Vincent, 1986). The effect of propagation system on mother tree growth can also be seen in a trial at Kaingaroa, planted 1978, and assessed at 4 years (FRI, 1984). In this trial, seedlings (propagated from seed) and cuttings showed similar growth in height, but those grown from cutting had smaller diameters than those

from seedlings. However, the cuttings had significantly less malformation than seedlings, with straighter stems and fewer stem defects.

c. Pollination system

The effect of the pollination system on seed vigour may depend on the condition of the seed during development and mother tree growth. In controlled pollinated seed orchards, trees are hedged regularly to about 1.5 metres, and the female flowers are covered with cellulose bags before pollen shedding and then hand pollinated with known pollen parents. Cones are harvested at the end of June and artificially cured and extracted for sowing in September. Mother trees are hedged again at harvesting time to stimulate new female flower bearing shoots to develop (Vincent, 1986).

In open pollinated seed orchards, however, trees and flowers are grown normally and wind pollination is allowed to occur. These differences may have an effect on seed vigour. Rimbawanto *et al.* (1988a) reported that there was no significant difference in germinability nor in time to 50% cotyledon emergence between seed (of 268 clonal series) from open or controlled pollinated trees harvested in July 1985. However, no work was done in this study to assess seedling performance in a nursery.

d. Age of mother trees

At an early age (e.g. 6 year old plantation), only a small amount of seed is produced by *P. radiata* trees, the cones tend to be small, the number of seeds per cone low and the percentage of empty seeds high (Fielding, 1964). This condition would seem likely to affect seed vigour. In other tree species like *Pinus merkusii* (Tasimin, 1980), *Abies balsamea*, and *Sequioa sempervirens*

(Kozlowski, 1971, cited in Tasimin, 1980) it was reported that age of mother trees had a significant effect on seed quality. In these species it seems likely that there was an optimum range of mother tree ages for producing good quality seeds. In *Pinus merkusii*, seeds collected from parent trees of 11-15 and 16-20 years old produced better germination and better seedlings in term of dry weight, height, and diameter than those from parent trees of 21-25 and 26-30 years old. In *Abies balsamea*, seeds from 40 year old mother trees had better germination than those from 155 year old (60% compared to 10%), whereas in *Sequioa sempervirens*, seeds from parent trees less than 20 years old had less than 1% viability, while those from more than 1200 years old were either sterile or of not more than 3% viability.

2.1.2.2. Collection date

Collection date has a great effect on seed vigour as different collection dates will produce different maturities which lead to different vigour levels. The more mature a seed is when collected, the higher its vigour (Pollock and Ross, 1972). In New Zealand, under normal practice in open pollinated seed orchards, cones are harvested in November or December when the cones have turned brown. Rimbaranto *et al.* (1988a) found that the seeds were fully germinable and of high vigour by the end of July when the cones are still green, indicating that seed development was independent of cone development (Rimbawanto *et al.*, 1989). Furthermore, Rimbawanto *et al.* (1988b) also found that *P. radiata* cones harvested as early as April ripened successfully in dry storage and produced high germinability and vigour. However, no field trial studies were done in this work to ensure that the ripened seed produced good performance in the field. Additionally, different orchard sites may show different maturating patterns as the maturation process is strongly affected by the surrounding environment.

2.2. Seed deterioration

Seed deterioration plays a significant role on seed vigour as the higher the degree of deterioration, the lower the seed vigour (Delouche and Caldwell, 1960). Information about deterioration is also important in vigour research as it is the simplest way of creating a range of seed lots with different vigour levels.

2.2.1. Factors affecting seed deterioration

In general seed deterioration is affected by genotype and storage environment.

2.2.1.1. Effect of genotype

Genotype has a great role in determining seed longevity. Hence different species, cultivars, or clones may have different longevity. Various species like Borneo camphor, rubber, cocoa, sweet orange, and sessile oak (reviewed by King and Roberts, 1980) have a short longevity, while others like slash pine, ponderosa pine, *Acacia aneura*, *A. glaucescens* and *Cassia suratensis* (reviewed by Harrington, 1972) have good potential storability.

The short longevity group are known as recalcitrant seeds. These seeds cannot be dried below a relatively high critical moisture content (e.g. 30%) and cannot tolerate freezing temperatures (Chin *et al.*, 1989). Seeds in the long longevity group are known as orthodox seeds. Those seeds can be dried to very low moisture content without damage at least to 5% and in many cases down to about 1% (Roberts and King, 1980). The difference of dehydration tolerance between recalcitrant and orthodox seeds is probably associated with the minimum amount of water which is needed for maintaining the stability and integrity of subcellular structure, particularly membranes (Berjak *et al.*, 1984).

Dessication injury occurs in recalcitrant seeds if they are dried below a relatively high moisture content. In *Aviciennia marina* seeds, for example, dehydration from 63 to 52% moisture content causes loss of viability (Berjak *et al.*, 1984), whereas in Borneo camphor (*Dryobalanops aromatica*), seed will be damaged at moisture content below 35% (Tamari, 1976, cited in King and Roberts, 1980). Liability to chilling damage is also a major obstacle to long term storage, as some recalcitrant seeds are killed if they are stored at sub ambient temperatures. Cocoa seeds, for instance, are killed by temperatures of 10°C or below, and mango seeds were damaged at temperatures of 3-6°C. Microbial contamination and germination during storage is also an important constraint to long term storage as high moisture content in recalcitrant seeds will stimulate microbial growth and seed germination (reviewed by King and Roberts, 1980).

In orthodox seeds the importance of genotype in longevity may be associated mostly with seed coat characteristics. This suggestion is supported by the fact that many of the species with long lived seeds have hard seeds (Harrington, 1972). The role of seed coat in deterioration resistance is probably related to its function as an impenetrable physical barrier, as an inhibitor of fungal growth (usually by phenolic compounds), or as a barrier to leaching of nutrients for microbial growth outside the seeds (Halloin, 1983).

There is an indication that *P. radiata* under optimal storage conditions might have a relatively high longevity. Mirov (1947, cited in Schubert, 1952) reported that air dried (6-10% moisture content) *P. radiata* seed still had 86% germination after 21 years storage at 5°C (initial germination 96%).

2.2.1.2. Effect of storage environment

The storage environment also has an important role in seed deterioration. The two most important factors which affect the speed of deterioration are the relative humidity of the air which controls seed moisture and temperature which influences the rates of biochemical processes in seeds (Harrington, 1972).

Orthodox seeds, contrary to recalcitrant ones, require low relative humidity, moisture content, and temperature to maintain their viability. Harrington's rule of thumb (1959, cited in Harrington, 1972) pointed out that for these seeds each 1% reduction in seed moisture doubles the life of the seeds, as does each 5°C reduction in seed temperature. This rule is applied between 5-14% moisture and between at least 0 and 50°C. Thus, under low moisture content or relative humidity and also low temperature, viability of the seeds can be generally maintained for relative long periods. *Pinus ponderosa*, for example, still produced 94% germination capacity after 18 years storage in airtight containers at 41°F with a seed moisture content 6-10% (Mirov 1946, cited in Schubert, 1952). Austrian pine (*Pinus nigra*) also still had 99% germination after 10 years storage at 4°C with 7% seed moisture content (Heit, 1967, cited in Harrington, 1972). *Pinus glabra* seeds with 15% moisture content, for instance, produced 34% germination after 3 years storage at 34°F, whereas those with 6% moisture at 0°F still produced 91% germination after a similar storage time (Barnett, 1979). *Chamaecyparis obtusa* seeds with 4-6% moisture content completely lost germinability after 4 years storage at 2°C, whereas those at -20°C still had 90% (Asakawa, 1976).

2.2.1.3. Artificial aging conditions

Under accelerated aging conditions (conditions of elevated relative humidity or moisture content and temperature), orthodox seeds will lose their viability dramatically. The viability of soybean seeds, for example, were reduced from about 61% to 0% within 2 days under 45°C, 100% relatively humidity (Stewart and Bewley, 1980). The germination of pea seeds dropped from about 98% to 19% within 10 weeks storage at 30°C and 92% relative humidity (Harman and Mattick, 1976). In this case, the fall in germination was initially detected after 8 weeks storage. However, a fall in vigour was detected earlier, i.e. after 6 weeks storage. It appears that there are no reports concerning the deterioration pattern of *P. radiata* or other tree species under accelerated aging conditions. However, it is presumed that the pattern will be similar to other orthodox seeds, although *P. radiata* seeds may have a slightly longer life span as it possesses a hard integument, typical of a gymnosperm species (Baldwin, 1942).

An exception from the general view mentioned above, may happen when elevated relative humidity (or moisture content) and temperature can, to a certain extent, have "an invigoration" effect on seeds. In sorghum, for example, 6 days aged seed (aged at 30°C, with 17% moisture content) produced a better germination rate, field emergence, and yield, than unaged ones. In this case, yield was increased by 20%. Over the next 42 days of aging treatment, however, seed performance fell dramatically (Gelmond *et al.*, 1978).

Another related exception may happen if the seeds are stored in a fully imbibed condition but they are kept in a dormant state. In lettuce, for example, viability of fully imbibed seeds (stored at 30°C in the dark) are not reduced by storage for up to 10 weeks. While other seed lots which have lower moisture contents (i.e. 7, 9.7

and 13.0%) lost viability from about 95% to 80, 26 and 0% respectively (Villiers, 1973). In both these cases it appears that the conditions facilitate the seed's capacity for self-repair.

2.2.2 Mechanism of seed deterioration

The basic causes of seed deterioration may fall into two broad categories:

1. seed tissues may deteriorate due to aging, and

2. seed deterioration may also be caused by invasion of and damage to tissue by micro-organisms, insects or rodents (McGee, 1983).

At present, however, it is quite difficult to identify the sequence of the processes mentioned above and how their interactions occur in seeds. However, there are some changes which can be detected as seed lose their viability and vigour in storage. These include: membrane changes (Priestley, 1986), lipid degradation (St Angelo and Ory, 1983), protein degradation (Cherry, 1983), respiration changes, chromosomal abberations and deterioration of DNA, impaired RNA and protein synthesis, and hormonal changes (Priestley, 1986). Certain events may be more important than others depending on species or cultivar and aging environment. In certain conditions, lipid peroxidation may be the most important, but in other conditions, autolytic degradation of nucleic acids may have the greatest significance (Priestley, 1986).

Variations in the nature of the aging process between species can be seen, for example, in Lin and Pearce's (1990) study which reported that under a range of aging conditions lipid changes seemed unimportant in the aging process of maize

seeds, but occurred in bean seeds in a way which was consistent with both peroxidation and lipid hydrolysis. Similarly, the influence of aging conditions on different mechanisms can be seen in Priestley and Leopold's (1983, cited in Lin and Pearce, 1990) study which reported that polyunsaturation of the fatty acids from total lipids and polar lipids decreased significantly under slow aging conditions (at 4°C over several years), but no change in unsaturation occurred in accelerated aging conditions (at 40°C, relative humidity close to 100%, over a week).

An important point to be noticed is that aging damage is not always irreversible as seeds have capacity for repair and detoxification (see section 2.2.1.3. for example). Repair and detoxification may involve antioxidants [including α-tocopherol (vitamin E), which is membrane associated, vitamin C, and ß-carotene] which function to prevent the onset of free radical formation (Bewley, 1986). This protection may also involve the superoxide dismutase (SOD) enzyme which in soybean seeds may play a key role in restricting peroxidation damage after 90 minutes of imbibition (Stewart and Bewley, 1980).

The role of micro-oragnisms in accelerating seed deterioration is very clear. However, it is not clear whether they initiate deterioration or merely accelerate the inevitable aging process (Coolbear, 1988). There are two major mechanisms by which micro-organisms (primarily field and storage fungi) damage seeds:

1. production of exocellular enzymes like cellulases, pectinases, amylases, lipases, proteases, and nucleases, and

2. production of toxins such as victora toxin, aflatoxin, and compounds like tentoxin.

Some symptoms of exocellular enzymes or toxins attack mimic the symptoms of seed tissue deterioration due to physiological aging such as an increase in the concentration of free fatty acids as a result of lipases attack, and an increase in solute concentration of seed leachates as a result of toxin-induced damage to membranes (reviewed by Halloin, 1986). These conditions increase the difficulty of identifying the nature of the aging process in seeds.

2.3. Seed vigour testing

2.3.1. The importance of seed vigour testing

Seed vigour is a quality factor which determines seed performance under a wide range of environmental conditions and it is defined by AOSA (1983) as follows: "Seed vigour comprises those properties which determine the potential for rapid, uniform emergence, and development of normal seedlings under a wide range of field conditions." Seed vigour has a great effect on field emergence, yield and seed storability (Hampton, 1985). The vigour level of a seed or seed lot is affected by genotype and environment during maturation on the mother plant, during harvest, and during handling and storage (Perry, 1976).

Today seed vigour testing is becoming increasingly important since it is realised that the standard germination testing methods do not have enough capability to estimate seed emergence under a wide range of field environments. There are two reasons for the inadequacy of the standard germination test (McDonald, 1980):

1. The test is conducted under favourable conditions for maximum germination which are rarely encountered in the field. Therefore, field emergence is often less than that predicted by the germination test.

2. Germination testing does not provide a complete evaluation of seed lot deterioration or quality since there is no distinction for strong and weak germinable seed in seedling evaluation.

2.3.2. Seed vigour testing methods

Any evaluation of the events which precede loss of germinability can be used as vigour tests, and (in theory) the farther removed the parameter is from loss of germinability, the more sensitive the assessment of seed vigour (AOSA, 1983).

An important point to be noticed is that the farthest event from the loss of germinability (and the entire sequence of deterioration) may differ depending on species or cultivar and aging conditions (see also section 2.2). Another important point is that for the purpose of vigour assessment the test method is not only required to distinguish seed lots according to deterioration level, but is also challenged to identify quality differences due to other factors, e.g. mother tree conditions and collection dates in *P. radiata* (see also section 2.1).

There are many types of seed vigour testing methods which have been studied on many species and varieties and they are summarised in Tables 2.1, 2.2 and 2.3. The classification on the tables follows that suggested by Stormonth (1978). However, there are other classifications like direct and indirect tests, suggested by Perry (1981) or biochemical, growth rate, stress, and physical measurement tests,

suggested by Delouche and Caldwell (1960). These differences are not crucial, and they do not have a great effect in practice.

From Tables 2.1, 2.2 and 2.3 it can be seen that, in general, seed vigour testing methods can be divided into three groups, i.e.: (1) tests not involving germination, (2) tests involving germination, and (3) stress tests. These will be discussed in turn with specific reference to tree seeds.

2.3.2.1. Tests not involving germination

These tests are relatively quick since they do not involve a germination period. These tests include physiological and biochemical tests (respiration, GADA, ATP, volatile aldehydes, radioactive tracer multiple criteria, starch, quantitative tetrazolium (TZ), topographical TZ, and aleurone TZ), conductivity, transmittance and x-ray contrast tests (Table 2.1).

a. Physiological and biochemical tests

Table 2.1 shows that, for tree seeds, some of the test methods have quite good sensitivity as they could differentiate a specific quality difference among seed lots, e.g. the respiration test in Norway spruce, *P. radiata*, and douglas-fir seeds, the ATP test in douglas-fir seed,and the topographical tetrazolium (TZ) test in cherrybark oak acorns and scots pine seeds. In addition, some tests appear to have quite good correlation with other vigour indices, e.g. the ATP test in douglas-fir seeds, the starch test in pine seeds, and the topographical TZ test in cherrybark oak acorns. However, generally there is no information concerning their correlation with field performance. This information is very crucial to determine the suitability of the tests (for assessment of seed performance in the field) as high

performing seeds in certain physiological and biochemical tests do not, of course, guarantee high performing seeds in the field.

An example of this can be seen in the results of the respiration test. In this test, theoretically, the higher the rate of oxygen uptake, the more and faster are the various metabolic activities (Ching, 1972), therefore the higher is the seed vigour. In *P. radiata* seeds, however, the rate of oxygen uptake appears to be negatively correlated with maturity and germinability (Rimbawanto, 1987). This seemed to be caused by harder seed coat in the more mature seeds which inhibit oxygen penetration into the seeds. Ching and Fang (1963) also reported that oxygen uptake in douglas-fir seeds decreased with increasing maturity. Respiration tests in barley seeds have also been reported to show lack of correlation with age (Anderson, 1970).

It appears that there is no report in tree seeds for GADA, volatile aldehyde, radioactive tracer multiple criteria, quantitative TZ, and aleurone TZ tests, although there have been reports of their successful use in certain agricultural seeds.

b. Conductivity testing

In the early imbibition stage seeds reorganise and repair their membranes which consist of phospholipid and protein. During this reorganisation and repair the membrane is slow to develop its characteristics as a permeability barrier which allows some cellular contents to leach. In vigorous seed, reorganisation and repair is relatively fast, so only a small amount of leachate comes out. In contrast, in non vigorous seed, the reorganisation and repair process is slower, so there is a high amount of leachate. The

level of ionised cellular contents which leach are then measured by conductivity meter, the higher the vigour, the less the conductivity (reviewed by AOSA, 1983 and Bewley, 1986).

This method has received widespread approval for measuring vigour for peas since the seeds are dicotyledonous seeds which have a regular cellular structure throughout and there is no extraneous contamination as the seed develops in pods. On the other hand this method appears not to be suitable for cereal seeds which have a large starchy endosperm where there is little membraneous organisation (Stormonth, 1978).

For pine seeds [which may be subject to extraneous contamination from solutes as the seeds develop from naked ovules without enclosing ovaries (Baldwin, 1942)], it appears that there is no report concerning sensitivity or predictivity of this method. However, there is a report (Vozzo, 1984) concerning some rinsing techniques in order to remove any surface contamination in some pine species. Results of this study indicated that, in slash and scotch pine, a 30 minute rinse (in running water) reduced the conductivity reading compared to the control. In loblolly and virginia pine, however, this rinsing technique significantly increased the conductivity reading, whereas in sand pine there was no effect of the rinsing technique on conductivity. It is clear that the value of conductivity testing for vigour assessment of tree seeds still needs further investigation.

The results of some trials in some agricultural seeds (Table 2.1) indicated that this method has had various degrees of success. Results of the ISTA collaborative test for pea and broad bean, for instance, indicated that only 2

out of 8 stations gave a good correlation coefficient (r) value with field emergence. Whereas in soybean none of 8 stations gave good r values with field emergence. Variation in r value due to cultivar differences were shown by Scott and Close (1976) in pea seeds.

c. Sugar exudate test

The principles of this method are quite similar to the conductivity test, as it measures the leakage level of the seeds except in this case sugar rather than ions are measured. The analysis is conducted using the phenol-sulphuric acid method (Hocking and Etter, 1969; Barnett, 1985).

The result of Barnett's study (1985) indicated that % transmittance of sugar exudates of spruce pine seeds gave a significant correlation (r = 0.981*) with radicle length. In longleaf pine, however, there was a very low correlation (r = 0.064) between those two parameters. In loblolly pine, the method did not work as there was no colorimetric change of leachate following the 24 hours imbibition period. The varying degree of success in this method might be affected by variation in the thickness of the seed coat. In the loblolly pine, for instance, the seed coat consists of nearly 60 percent of total dry weight, whereas in longleaf pine that is less than 30% of total dry weight. However, no correlation analyses with field performance were carried out in these studies.

d. X-ray contrast test

The principle of this test is to evaluate the degree of impregnation of a contrast agent such as $BaCl_2$ on seed tissue through radiographs which are obtained by x-ray exposure on seeds (Simak and Kamra, 1963; Kamra,

1971). The results of some studies (Table 2.1) indicated that this method had ability to identify quality differences due to storage in scots pine seeds, and due to variation in locality and country of origin in Norway spruce seeds. However, there was no attempt at correlation analysis with other vigour indices nor with field performance. In corn (Table 2.1), results of this test gave significant correlation with those of the conductivity test ($r = -0.72^{**}$) and root length ($r = 0.69^{**}$).

2.3.2.2. Tests involving germination

This test is based on the presumption that seed vigour is the total expression of the biochemical and physiological processes, so all of those processes should be evaluated by germinating the seed for an authentic and valid measurement of vigour (Stormonth, 1978).

In general these tests consist of three methods (Table 2.2), i.e.:

1. the seedling growth rate test,
2. seedling evaluation tests, and
3. improved vigour index tests.

The principle of these tests is to measure the growth characteristics of seedlings during or after a certain germination period in optimum conditions.

These methods have some advantages since they can be conducted in conjunction with the standard germination test which can be easily handled by seed analysts. However, they have some weaknesses especially on the conditions used. In these methods all of the factors which are needed for germination are supplied at the level which favours maximum germination and growth, in contrast to the field conditions which are experienced by seed. Furthermore, the moisture and

temperature should be accurately controlled and standardised since slight differences in those factors may cause a great effect on seedling growth. Another important point is that the correct evaluation of germination by seed analysts is difficult to obtain because it is subjectively assessed. Furthermore, seedling appearance varies between species, between varieties, and also within a variety (Stormonth, 1978).

Despite those weaknesses, Wang's (1974) study indicated that the seedling evaluation test in red pine (using six classes of seedling vigour where the lower the class number, the more developed the seedlings) had good sensitivity and was able to predict nursery emergence at 44 days after sowing (Table 2.2). However, there were clear differences between germination tests conducted under different conditions.

Good sensitivity and good correlation with other vigour indices were shown by the result of germination value (using Czabator's formula, 1962) test in cherrybark oak acorns (Bonner, 1974). In this study, the test of germination value could differentiate quality differences among seed lots due to aging treatment. In addition, it gave significant correlations (r values not less than 0.78) with tetrazolium and total seedling fresh weight tests. Good sensitivity was also shown by similar tests for germination value (using Djavanshir and Pourbeiks's formula, 1976) in *Pinus merkusii*, median germination time in *P. sylvestris* and *P. radiata*, seedling evaluation test in *P. merkusii*, and by improved vigour index tests in lodgpole pine and white spruce. Once again, however, no correlation analysis with field performance was conducted in these studies (Table 2.2).

In the seedling evaluation test of pea, broad bean, and soybean seeds, the results of ISTA's collaborative tests (Fiala, 1987) indicated that only a small number of stations (4, 4 and 6 respectively) produced quite high correlations (r values not less than 0.70) with field emergence, whereas some others (12, 11 and 6 respectively) produced quite low correlations (r values less than 0.70). This indicates that this test is not valid for all types of field conditions as variation in climatic and soil conditions seems to have a great effect on seed lot ranking.

2.3.2.3. Stress tests

Field environments usually provide suboptimal conditions for growth. Under those environments vigorous seed have a greater potential to emerge and establish.

Stress tests are principally simulating certain stress conditions which may be encountered by seed in the field. The stress conditions may be applied to the seed prior to imbibition, like an accelerated aging test and controlled deterioration test, or during germination like the cold test, cool germination test, and Hilther test. A summary of stress tests can be seen in Table 2.3.

Except for the osmotic stress test, it appears that there is a lack of reports concerning stress tests in *P. radiata* or other tree species. In *Pinus ponderosa*, an osmotic stress test using polyethylene glycol (PEG) 4000 at -7 bar could differentiate quality differences among seed lots due to pre-sowing treatment (Larson and Schubert, 1969), and that at -4 bar could differentiate quality differences due to variations in seed collection zone (Moore and Kidd, 1982). However, no correlation analysis with field performance was conducted in these studies.

In agricultural seeds, some trials in stress tests (Table 2.3) indicated that some test, e.g. accelerate aging, controlled deterioration, and the complex stressing vigour test could give high and significant correlations with field emergence at certain sites or planting year. But in other site or planting year they gave low correlation with field emergence. This might be caused by many factors. In the accelerated aging test, one of them might be the difference in initial seed moisture contents, as this difference can cause different results in the accelerate aging test (McDonald, 1977). In addition, different sites or sowing years will have different soil or climatic conditions which may cause variation in seed lot ranking.

2.3.3. Scope for further investigation

Based on data in Tables 2.1, 2.2 and 2.3, it is clear that there is no single vigour test which is valid for any species and any field conditions. Therefore, for the purpose of vigour assessment study in *P. radiata* seeds, it is suggested to investigate the sensitivity of some simple vigour tests and their correlations with seed performance under a limited range of field conditions. Investigations attempting to find general vigour tests with suitable predictivity for all field conditions seem to be unrealistic.

Table 2.1 Vigour tests not involving germination

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
A. Physiological and Biochemical tests						
1. Respiration test	- to evaluate metabolic energy by measuring O_2 uptake or CO_2 production during germination	- mechanical injury, which reduces vigour may increase rather than decrease respiration rate	- Norway spruce (Bhumibhamon, 1976)	- genetic variation between seeds from higher and lower latitude	-	-
			- lima bean, (Woodstock & Pollock, 1965, cited in AOSA, 1983)	- could detect chilling injury	-	-
			- cherrybark oak (Bonner, 1974)	- none	- not significant	-
			- barley (Anderson, 1970)	- none	- oxygen uptake did not correlate with age	-
			- soybean (Burris et al, 1969)	- age	high correlation with cold test, coefficient velocity test, 4 & 8 days germination, and growth rate (but no information concerning confidence interval of r)	
			- Pinus radiata (Rimbawanto, 1987)	- respiratory rate ↓ with maturity, but respiratory quotient ↑	-	-
			- douglas fir (Ching & Fang, 1963; Ching & Ching, 1962)			
2. Glutamic acid decarboxylase activity (GADA) test	- to evaluate GADA by measuring the amount of CO_2 produced in the presence of glutamic acid	-	- corn (Grabe, 1965, cited in AOSA, 1983)	- storability	-	-
			- soybean (Burris et al, 1969)	-	- little relation with age	-

Table 2.1 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
3. Adensine triphosphate (ATP test)	- to evaluate biochemical energy by measuring ATP content by a photometer or liquid scintillation counter using a luciferin-luciferase system (reviewed by AOSA, 1983)	-	- douglas-fir (Ching & Ching, 1973)	- variation due to stratification treatment	- seems to be well correlated with seedling dry weight and seedling length	-
			- annual ryegrass, rape and crimson clover (Ching, 1973)	-	- significant by correlated with seedling size or seed weight	-
			- soybean (Yacklitk et al, 1979)	-	-	- correlated with field emergence in a 1976 trial, but not in 1975
			- corn, cucumber, onion and radish (Styer et al, 1980)	-	- not correlated with reduced germination or vigour	
4. Volatile aldehyde test	- to measure the production of volatile aldehydes from seed during early germination (0-24 hours)	-	- soybean (Wilson & Donald, 1986)	-	-	- high correlation with field emergence
5. Radioactive tracer multiple criteria test	- to evaluate metabolic level of seed by measuring the uptake, incorporation, and leaching of radio isotopes	-	- soybean (Yacklik et al, 1979)	- cultivar variation	-	- radioactivity of leachate gave quite high correlation with a 1975 trial but gave low correlation with a 1976 trial. Radioactivity of protein and soluble radio-active fraction gave low correlation with both trials

Table 2.1 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
6. Starch test	- to evaluate the colour of embryos after treating them with iodine and potassium iodide (Kuznetsova, 1939, cited in Baldwin, 1942)	-	- pine (Schmidt, 1940 cited in Baldwin, 1942)	-	- agreed well with germination speed (4 days germination)	-
7. quantitative tetrazolium test	- to evaluate dehydragenase enzyme activity by measuring concentration of formazan resulting from tetrazolium application	-	- pea (Gorecki and Harman, 1987)	- age	-	-
			- wheat (Kittock and Law, 1968)	- age	- high and significant correlation with vigour score[1]	-
			- wheat (Johnston et al., 1986)	- genotype	- good correlation with shoot length and shoot dry weight	-
8. topographical tetrazolium test	- to evaluate dehydrogenase enzyme activity by means evaluating the tissue colour of the seeds which have been treated by tetrazolium solution	- subjectivity of analyst (Perry,	- soybean (Burris et al., 1969) 1981)	- age	- gave good correlation with arasan treated cold test, coefficient velocity test, 4 and 8 days germination tests, and growth rate test (but no information concerning confidence interval of their r values)	-
			- cherrybark oak acorns (Bonner, 1974)	- variation due to aging treatment	- high and significant correlation with peak value[2] and germination value[3]	-
			- scots pine (Simak and Kamra, 1963)	- variation due to differences in storage	-	-

[1] vigour score = reciprocal of days from planting and daily stand in greenhouse
[2] peak value = PV = maximum value of cumulative germination percentage divided by days of the test
[3] germination value = GV = PV x mean daily germination

Table 2.1 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
9. Aleurone tetrazolium test (Fiala, 1981)	- to evaluate dehydrogenase enzyme activity in aleurone layer by evaluating the colour of the tissue after treating it with tetrazolium solution	- difficulty in evaluating the stained aleurone layer (Fiala, 1987)	- maize (Germ and Kitreiber, 1953, 1954 cited in Fiala, 1981)	-	-	- produced a good correlation with field emergence
B. Conductivity test (AOSA, 1983)	- to evaluate deterioration level of seeds by measuring leakage level of the seeds in soaked water by means of conductivity meter	- variation in moisture content, mechanically injured seeds, and chemical seed treatment	- vining pea (Carver and Matthews, 1975, cited in Matthews and Powell, 1981)	-	-	- gave good correlation with field emergence
			- sand, slash, scotch lobbolly, and virginia pine (Vozzon, 1984)	- variation due to differences in species	-	-
			- pea (Fiala, 1987)	-	-	- 2 stations gave good correlation (absolute values of r more than 0.65) but 6 other stations gave quite low correlations (r value < 0.65)
			- broad bean (Fiala, 1987)	-	-	- only 2 out of 8 stations gave good correlation
			- soybean (Fiala, 1987)	-	-	- 8 stations produce low correlations ($r \leq 0.63$)
			- pea cv Dark Skin Perfection (Scott and Close, 1976)	-	-	- high correlation ($r = -0.93^{***}$)
			- pea cv Small Sieve Freezer (Scott and Close, 1976)	-	-	- low correlation ($r = -0.44^{ns}$)

Table 2.1 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
C. Sugar exudate test	- to measure the transmittance of sugar exudates of the seeds by means of spectrophotometer	-	- spruce pine (Barnett, 1985)	-	- high correlation with radicle length	-
			- longleaf pine (Barnett, 1985)	-	- no correlation with radicle length	-
			- white spruce (Hocking and Etter, 1969)	- variation in germinability	-	-
D. X-ray contrast	- to evaluate the degree of impregnation of a contrast agent on a seed tissue through a radiograph which is obtained from x-ray exposure on the seeds	-	- scots pine (Simak and Kamra, 1963)	- variation due to differences in storage	-	-
			- Norway spruce (Kamra, 1971)	- variation due to differences in locality and country or origin	-	-
			- corn (Smith and Grabe, 1985)	-	- showed a good correlation with conductivity and the growth rate test	-

Table 2.2 Vigour tests involving germination

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
A. Seedling Growth test	- to measure the growth characteristics of seedlings during or after a certain germination period in optimum conditions					
	1. total seedling fresh weight	-	- cherrybark oak acorns (Bonner, 1974)	- variation due to aging treatment	- gave good correlation with peak value and germination value	-
	2. mean normal seedling dry weight	- result must be interpreted within genotype (AOSA, 1983)	- corn, soybean (AOSA, 1983)	- capable of identifying slight differences in vigour due to genotypes, seed size, location of production and freeze damage	-	-
	3. plumule length	- genotype variation in plumule length may not relate to emergence - comparison should be made within genotype	- wheat and barley (Perry, 1981b)	- unacceptable vigour quality	-	-
			- wheat (Perry, 1978 cited in Perry, 1981b)	-	-	- large differences among laboratories
		- moist or dry seed should be allowed to equilibrate to a similar moisture content before commencing a test (Perry, 1981b)				

Table 2.2 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
A. Seedling growth test (continued)	4. root length	-	- lettuce (Smith et al. 1973, cited in Perry, 1981)	-	-	- correlated well with head size in the field
	5. speed of germination with indices	- variation in temperature or moisture in the test chamber and substrata (AOSA, 1983)				
	a. first count germination	-	- soybean (Burris et al. 1969)	- variation due to aging	- gave high correlation with glucose, respiration and tetrazolium test (but no information concerning confidence interval of r value)	-
	b. germination value	-	- Cherrybark oak acorns (Bonner, 1974 using Czabator's formula, 1962)	- variation due to aging	- gave high and significant correlation with the result of tetrazolium test and total seedling fresh weight, but did not correlate with oxygen uptake carbohydrate leakage	-
			- Pinus merkusii (Arisman and Powell, 1986, using Djavanshir and Pourbeik's formula, 1976)	- variation due to cone colours and cone treatment	-	-
	c. median germination time	-	Pinus sylvestris (Bergsten, 1988)	- variation due to unvigoration treatment	-	-
			- Pinus radiata (Kimbawanto, 1987)	- variation due to artificial ripening and collection date	-	-

Table 2.2 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
B. Seedling evaluation tests	- to measure the growth characteristics after a certain germination period in optimum conditions by evaluating the number of vigorous seedlings (Perry, 1981)	- subjective evaluation in differentiating vigorous and non-vigorous seedlings (Perry, 1981b)	- pea, broad bean, and soybean (Piala, 1987)	-	-	- only a few stations gave quite high correlations (r not less than 0.70) whereas some others gave quite low correlation (r values less than 0.70)
			- red pine (Wang, 1973)	- variation due to seed source location and year of collection	-	- high and significant correlations with nursery emergence at 44 days after sowing
			- Pinus merkusii (Arisman and Powell, 1986)	- variation due to differences in cone colour	-	-
C. Improved vigour index test	- to measure the growth characteristics after a certain germination period in optimum conditions by evaluating a combination value of seedling uniformity and percentage of germination	-	- lodgpole pine and white spruce (Huang, 1988)	- variation due to aging	-	-

Table 2.3 Stress tests

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
A. Accelerated aging test (Baskin, 1981; AOSA, 1983)	- to evaluate seed germination after treating seeds under high temperature and high relative humidity	- variation in moisture content (Baskin, 1981) - variation in fungicide treated or untreated seeds (Baskin, 1981)	- soybean (Fiala, 1987)	-	-	- good correlations (r values not less than 0.70) at 2 stations, but quite low correlations (r less than 0.70) at other 5 stations
			- soybean (Kulik and Yaklich, 1982)	-	-	- good correlation (r not less than 0.70) at 2 planting sites and 3 planting dates in 1976, but low correlations (r less than 0.70) at those in 1975
			- cotton, pea, bean and soybean (reviewed by AOSA, 1983)	-	-	- gave good prediction in stand establishment
B. Controlled deterioration test (Matthews and Powell, 1981)	- to evaluate seed germination after treating seed under high temperature and high seed moisture content	- the test is potentially used for small seeded crops (Matthews and Powell, 1981)	- turnip, kale, sprout (Matthews and Powell, 1981)	-	-	- correlated highly and significantly
			- swede, onion (Matthews and Powell, 1981)	-	-	- correlated highly and significantly with 1977 trial, but low correlation with 1978 trial
			- sugar beet (Matthews and Powell, 1981)	-	-	- well correlated with 1977 and 1978 trial
			- lettuce (Matthews and and Powell, 1981)	-	-	- low correlation with 1977 and 1978 trial
			- turnip, onion, lettuce carrot (Matthews and Powell, 1981)	-	-	- good reproducibility within the same station

Table 2.3 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
C. Methanol or ethanol stress	- to evaluate seed germination after treating seed under methanol or ethanol vapour	-	- soybean (Mugnisjah and Nakanura, 1986)	-	-	-
D. Cold test	- to estimate seed emergence by early planting season by evaluating seed germination at 10°C for 7 days and at 25°C for 4 days (AOSA, 1973) or 13 days (Fiala, 1981)	-	- soybean, cotton, onion carrot, and sorghum (reviewed by AOSA, 1983)	-	-	- had ability to forecast seed performance
			- peas, corn (Fiala, 1987)	-	-	- poor correlation with field performance
			- peas, corn, and broad bean (Fiala, 1987)	-	-	- significant difference among stations
			- soybean (Kulik and Yaklich, 1982)	-	-	- inconsistent result between 2 dates of planting (1975 and 1976)
E. Cool germination test (AOSA, 1983)	- to estimate the capacity of seed to grow in cool soil by evaluating seed germination at 18°C	-	- cotton (AOSA, 1983)	- cool sensitivity	-	-
F. Hiltner test (Fuchs, 1981)	- to evaluate seed emergence in a brick grit media in a dark germination at temperature of 20°C	- lack of distinction between effects of pathogens and physical causes	cereals (reviewed by Fuchs, 1981)	- cereals injured by sprouting, hot water treatment, threshing damage and excessive chemical treatment	-	-
G. Osmotic stress test	- to evaluate the ability of seeds to emerge under drought condition by evaluating seed germination under osmotic solution	-	- ponderosa pine (Larson and Schubert, 1969)	- variation due to pre-sowing treatment	-	-

Table 2.3 (continued)

Test	Principles of the test	Conditions which can affect the validity of the test	Species	Quality differences which can be detected among seed lots	Correlation with other vigour indices	Correlation with field performance/ reproducibility
G. Osmotic stress test (continued)			- ponderosa pine (Moore and Kidd, 1982)	- variation due to seed collection zones	-	-
			- corn (Muchena and Grogan, 1977)	- variation due to seed size	-	-
H. the complex stressing vigour test	- to measure the ability of seeds to emerge under oxygen deficiency stress by evaluating seed germination after soaking the seeds at 20 or 25°C for 48 hours followed by further soaking at 2 or 5°C for 48 hours	-	- maize (Barla-Szabo and Dolinka, 1988)	-	-	- gave high correlation with early sowing but quite low correlation with mid and late sowing
I. Combined stress test	- to evaluate seed ability to emerge under water, oxygen and mechanical stress	-	- wheat and barley (Stormonth, 1978)	-	-	- well correlated with field emergence

III. MATERIALS AND METHODS

3.1. Deterioration in *P. radiata* seeds

The material used in this study was a Grade 2 *P. radiata* seed lot (20-25,000 seeds/kg) collected in 1988. This came from a mix of genotypes collected from Gwavas and Kaingaroa open pollinated seed orchards, and certified by the Seed Certification Service as *Pinus radiata* Code GF16. GF is the breed code for Growth and Form, and 16 is the improvement rating. The details of planting years and clone number of parent trees can be seen in Table 3.1 below.

Table 3.1 Planting years and clone number of parent trees of the seed lot used in the aging study of *P. radiata* seeds.

Location	Planting years	Clonal series[a]
Gwavas	1964, 1966	850
Kaingaroa	1972, 1974	850
	1976, 1978	268
	1977	875

[a] The clonal series number refers to a particular group of parent clones selected for breeding (Vincent, 1987). The first digit of the number refers either to the regional origin of the clone (2 = Rotorua, 6 = Canterbury, 7 = South Island) or if 8 signifies other selection programmes conducted by the New Zealand Forest Research Institute. The second two digits refer to the years of selection (Shelbourne, 1986).

Seed were treated by Accelerated Aging (AA) after the method of Baskin (1981) and AOSA (1983) at 100% RH for up to 14 days at 40 or 45°C. Germination tests

were conducted on samples of 50 or 25 seeds for 28 or 24 days at 20°C in continuous low light using the top of the paper method. Seeds and germination media were placed in plastic boxes which have semitransparent lids. Either two or four replications were used in each experiment.

Parameters of observation included: % radicle emergence, % normal seedling, T_{50} (median germination time) of radicle emergence, T_{50} normal seedling, and normal seedling dry weight at 28 days after sowing (das). Normal seedling dry weight was measured after drying at 60°C for 4 days; seed coats, if any, were removed from seedlings prior to measurement.

Time to reach 50% radicle emergence or normal seedling (T_{50}) were obtained by using the following formula (Coolbear *et al.*, 1980, cited in Rimbawanto, 1987):

$$T_{50} = t_i + \frac{\frac{N+1}{2} - n_i}{(n_j - n_i)} \times (t_j - t_i) \quad \text{, where}$$

N = total number of germinating seeds (radicle emergence) or normal seedlings

n_i, n_j = adjacent cumulative radicle emergence or normal seedling counts at times t_i, t_j

t_i, t_j = successive counts where $n_i < \frac{N+1}{2} < n_j$

A seed was classified as having an emerged radicle if it had successfully emerged at least 2 mm and was classified as a normal seedling if it had shown a well developed primary root, shoot axis and a varying number of cotyledons (ISTA, 1985). In later experiments the criterion of a normal seedling was the same as above with an additional requirement that the cotyledons should have developed to a length at least as long as the seed coat.

This series of experiments was conducted from November 1988 to April 1989 at the Seed Technology Centre Laboratory, Massey University, Palmerston North.

3.2. Vigour tests evaluation in *P. radiata* seeds using 5 seed lots which varied according to seed size and age

The material used in this study was two seed lots of *P. radiata* seed which had different weights: grade 2 (20-25,000 seeds/kg) and lighter seed from grade 3 (25-30,000 seeds/kg) collected in 1988. The seed source was the same as that explained in section 3.1.

The experimental procedure was divided into 4 stages that were: (a) seed lot preparation, (b) vigour testing, (c) a glasshouse test, and (d) a winter field test.

3.2.1. Seed lot preparation

1. The heavy seed lot was divided into 3 lots and each of them was treated by an aging treatment at 45°C, 100% RH for 0 (control), 8 and 10 days, respectively, producing lots A, B and C.

2. The light seed lot was divided into 2 lots and each of them was treated by an aging treatment at 45°C, 100% RH for 0 (control) and 8 days, respectively, producing lots D and E.

3. Seeds visibly infected with fungi, broken seeds, and inert matter were removed from each seed lot.

3.2.2. Vigour testing

Three groups of tests were used: (1) seedling growth tests, (2) tests not involving germination, and (3) stress tests.

3.2.2.1. Seedling growth tests

The procedure of the test was as follows:

1. Four replications of 25 seeds from each lot were sown in plastic boxes with semi-transparent lids, using moist blotter paper as germination medium. Then they were placed at a constant temperature of 20°C for 28 days (ISTA, 1985) with continuous low light. A second set of boxes (also with 4 x 25 seeds) were placed at 5°C for 7 days (pre-chilling treatment), then germinated at 20°C as above.

2. Observations were generally carried out every two days by counting the numbers of emerged radicles and normal seedlings. Criteria used were the same as explained in section 3.1. Both percentages and median germination times were calculated in each case.

3. At 28 days after sowing (das), normal seedling dry weight was determined as previously described (section 3.1).

3.2.2.2. Tests not involving germination

(a) Conductivity test

The procedure of the test was as follows (adapted from AOSA, 1983; Matthews and Powell, 1981a):

1. Four replicates of 50 or 100 seeds for each lot were weighed and placed (at 20°C) in 125 ml beakers with 100 ml deionised water. The beakers were covered to reduce evaporation and dust contamination.

2. Conductivity was measured 2, 4, 8, 10 and 24 hours after the start of imbibition (HAI) by means of a conductivity meter, conductivity of deionised water only (without seed) was also measured to determine background which was subtracted from all values.

3. The conductivity reading was expressed as $\mu S.cm^{-1}.g\ seed^{-1}$.

(b) Topographical tetrazolium test

The procedure of this test was as follows (adapted from ISTA, 1985):

1. Four replicates of 25 seeds were soaked in water for 18-24 hours.

2. Each individual seed was cut transversely at the radicle end and longitudinally (in the middle of seeds) along about 3/4 of the seed length.

3. Seeds were soaked in 1% w/v 2,3,4 triphenyl tetrazolium chloride (in buffer solution pH 6.5-7.5) for 18-24 hours. The buffer solution 0.0267 molal KH_2PO_4 and 0.04 molal Na_2HPO_4.

4. Seeds were washed with tap water, then their cut surfaces were observed to distinguish vigorous seeds from non-vigorous ones. Vigorous seeds were seeds which showed red staining in all parts including megagametophyte and embryo,

whereas non-vigorous ones were those which showed an unstained area in any part of embryo or megagametophyte.

(c) Quantitative tetrazolium test

The procedure followed in this test was basically adapted from that used by Gorecki and Harman (1987) for peas, which incubated seed powder in tetrazolium solution for 7 hours at 25°C. The basic test procedure was as follows:

1. About 0.5 g powdered seed tissue (obtained by hand grinding) was incubated in 10 ml 0.7% 2,3,5 triphenyl tetrazolium chloride (in buffer solution as in (b)) at 35°C, for various times.

2. The samples were centrifuged at 3000 x gravity for 10 minutes.

3. The pellets were extracted thrice with 15 ml acetone.

4. The optical density of the acetone solution was then measured by spectrophotometer at 510 nm.

(d) Radiographic test

This test procedure was basically adapted from Simak and Kamra (1963), and aims to evaluate vigour through $BaCl_2$ impregnation degree in seed tissue. The higher the degree of impregnation, the lower the seed vigour. The basic procedure of the test was as follows:

1. Four replicates of 25 seeds from each seed lot were soaked in water at 20°C for 24 hours.

2. The seeds were then surface dried by using paper towels, and then transferred to 20% $BaCl_2$ solution for 2 hours at 45°C, before washing in running tap water for 3-5 minutes, followed by drying at 30-35°C for 4 hours.

3. Seeds were photographed by soft x-ray with 20 kv, 2.8-3.1 mA for 3 minutes. Radiographs were evaluated by calculating the number of vigorous seeds. Vigorous seeds were seeds which were free from impregnation, their embryo length more than three quarters of the embryo cavity, and their endosperm almost filling the seed coat.

3.2.2.3. Stress tests

(a) The controlled deterioration (CD) test

The procedure of the test was as follows (Matthews and Powell, 1981b; AOSA, 1983):

1. The moisture content of seed lots was measured by using the method of ISTA (1985) with 2 replicates of 15 seeds.

2. Seeds were placed in aluminium foil bags, and a calculated amount of deionized water was added into the bag in order to raise seed moisture content to about 20%. The bags were then heat sealed. The formula to obtain the amount of water was as follows:

$$v = \frac{100 - mc_o}{100 - 20} \times w - w \quad \text{, where}$$

v = amount of water to be added

mco = initial moisture content (in %)

w = weight of seed in foil packet (g)

3. Bags were placed at a temperature of 5°C for 1 night to equilibrate, then they were held at 45°C for 2 or 4 days. Moisture contents were checked again at the end of the aging period. This measurement (Table 3.2) showed that there was uniform moisture content among seed lots after 4 days aging. However, there was variation after 2 days aging, where the moisture content of lot D (and possibly lot A) was lower than the others.

Table 3.2 Moisture contents after the controlled deterioration test. Figures with the same letters are not significantly different ($P \leq 0.05$).

Aging period of Controlled Deterioration test	moisture content (%) of lot					
	A	B	C	D	E	$lsd_{0.05}$
2 days	18.4^{bc}	19.8^{ab}	19.6^{ab}	17.6^{c}	20.4^{a}	1.8399
4 days	18.5^{a}	18.6^{a}	18.4a	19.1^{a}	17.6^{a}	2.4306

4. Seeds (4 x 25 seeds) were sown as described in section 3.2.2.1. and then number of normal seedlings was recorded at 28 days after sowing.

(b) The complex stressing vigour test (CSVT)

The procedure of this test was as follows (adapted from work with wheat by Barla-Szabo and Dolinka, 1988):

1. Seed samples were soaked in water containing 0.15% sodium hypoclorite at 20°C for 2 days, then they were soaked further in the same solution at 5°C for another 2 days. Next, they were washed in running tap water for about 1 minute.

2. Samples (4 x 25 seeds for each lot) were sown by the same method as in the seedling growth test. The number of normal seedlings was recorded at the end of the test period (28 days after sowing).

(c) Low temperature/osmotic stress test

The procedure of the test was as follows:

1. Seed samples (normally 3 x 25 seeds for each lot) were sown in plastic boxes with Kimpack and blotter paper as germination support (top of paper method) at 3 temperatures: 10°C, 15°C and 20°C, and two osmotic potentials: -5 bar, and 0 bar. A randomised complete block design was used in this experiment. An osmotic potential of -5 bar was obtained using polyethylene glycol (PEG) 6000 with the following concentrations: 219.98 g/kg H_2O for 10°C, 220.91 g/kg for 15°C, and 224.54 g/kg H_2O for 20°C (using the formula of Michel and Kaufman, 1973). An osmotic potential of 0 bar was obtained by using deionized water as the germination medium. The volume of the solution poured into each plastic box was 25 ml.

2. Observations of radicle emergence were done at frequent intervals for 24 weeks and the germination medium was changed at 14, 40, 77 and 125 days after sowing (das) for replication 1, at 15, 49, 72 and 123 das for replication 2 and at 15, 50, 73 and 123 das for replication 3. Seeds were classified as having emerged radicles if these were at least 2 mm long. At the end of the test period the number of fresh ungerminated seeds was also recorded.

3.2.3. Glasshouse test

The procedure of this test was as follows:

1. Seed samples (10 replicates of 25 seeds from each lot) were sown in the glasshouse using plastic trays and a commercial potting mix which contained sphagnum peat moss and pumice as germination media. Samples from the same replication were sown within the same tray, so the experiment had a randomised complete block design. Germination media were initially covered with moist paper in order to reduce evaporation, but owing to fungal development the use of the paper was discontinued after 8 days. Germination media were kept moist by frequent watering. Maximum and minimum temperatures were recorded daily where possible during the progress of the trial.

2. Normal seedling emergence was observed every 2 days for 30 days and at the end of the test period normal seedling (both roots and shoots) dry weight was measured after drying at 60°C for 3 days after washing the roots with tap water. An important point to be noticed was that not all of the root parts could be removed from soil.

3.2.4. Winter field test

The procedure of this test was as follows:

1. Seed samples (10 replicates of 25 seeds from each lot) were sown in the field during winter using plastic trays and potting mix (as in section 3.2.3.) as germination media using a randomised complete block design. Trays were placed within an iron cage to protect seedlings from bird attack. The test was conducted for 4 months (6 May 1989 to 5 September 1989).

2. Measurement included: % cumulative emerged seedlings, % cumulative normal seedlings, % surviving emerged seedlings, % surviving normal seedlings,

dry weight of shoots of normal seedlings, minimum-maximum temperature, and rainfall. Seedlings were classified as emerged if they had appeared at least 2 mm above the soil surface, and were classified as normal if their shoot and cotyledons had grown to more than four times their seed length and their cotyledons were at least as long as the seed. Shoot normal seedling dry weight measurement was done by cutting the top part of normal seedlings (excluding roots) then they were dried at 60°C for 4 days. Minimum-maximum temperatures was observed daily where possible. At periods when there was no observation, data were represented by temperature data which were observed on the next following day. Rainfall height was observed by using glass funnel and measuring cylinder which were placed next to the sowing area. Observations were again conducted daily where possible. At periods when there was no observations, the daily data were represented by an average daily value during the intervening period.

3.3 Vigour tests evaluation in *P. radiata* seeds using 16 seed lots which varied according to type of mother tree and collection date

The material used in this study were 16 lots of *P. radiata* seeds which varied according to type of mother tree and collection date. Differences in mother tree types were clonal series, propagation and/or pollination systems used, health status, and location. Details are given in Table 3.3. In each lot, seeds were collected from 5 cones for each of 10 clones.

The experimental procedure was divided into two activities: (1) seed weight and vigour tests which were conducted at the Seed Technology Centre, Massey University, Palmerston North in October-December 1989, and (2) nursery and

standard germination tests which were conducted in 1988 by the Forest Research Institute (FRI), Rotorua. All 10 clones were represented in the FRI work, but in the vigour tests carried out at the Seed Technology Centre, not all of the seed lots were represented by all 10 clones due to lack of seeds, lots 4, 9, 10, 11 and 15 being represented by 9, 9, 8, 8 and 9 clones respectively.

Table 3.3 Description of *P. radiata* seed lots

Group	Seed Lot No.	Year of Collection	Clonal Series	Mother Trees
1	1	1987	875	OP, seedling ortets
	2	1987	875	OP, cutting ramets
	3	1987	876	OP, grafted ramets
2	4	1987	268	OP, healthy cuttings, Kaingaroa seed orchard
	5	1987	268	OP, unhealthy cuttings Kaingaroa seed orchard
	6	1987	268	OP, healthy grafts, Kaingaroa seed orchard
	7	1987	268	OP, moderately unhealthy grafts, Kaingaroa seed orchard
	8	1987	268	OP, very unhealthy grafts, Kaingaroa seed orchard
3 A	9	1987	268	CP, collected May, Amberley seed orchard
	10	1987	268	CP, collected June, Amberley seed orchard
	11	1987	268	CP, collected July, Amberley seed orchard
B	12	1987	268	OP, collected May, Kaingaroa seed orchard
	13	1987	268	OP, collected June, Kaingaroa seed orchard
	14	1987	268	OP, collected July, Kaingaroa seed orchard
4	15	1987	268	OP, first cone crop, Kaingaroa seed orchard
	16	1987	268	OP, mature cone crops, Kaingaroa seed orchard

OP = open-pollinated CP = control-pollinated

3.3.1. Seed weight and vigour tests

3.3.1.1. Seed weight test

From each lot, 39 seeds per clone were taken and weighed. Moisture measurements were also done with 2 replicates of 15 seeds using the method of ISTA (1985). The seed weight of each lot was then converted into 100 seed weight at a moisture content of 7.69% (the average moisture content of all seed lots).

3.3.1.2. Vigour tests

Three types of vigour tests were used in this study: the seedling growth test with prechilling treatment (SG + pr test), the controlled deterioration test with 2 days aging treatment (CD2d test), and the complex stressing vigour test (CSV test). The basic procedure of these tests was the same as explained in section 3.2.

In each seed lot, seeds of all clones were bulked to form one seed lot. For germination purposes, the tests used 3 replicates of 25 seeds (for each lot). A randomised complete block design was used in SG + pr test, whereas a randomised complete design was used in CD2d and CSV tests. Kimpack, blotter paper, and 50 ml deionised water were used as germination media. Observations were generally made every two days by recording the numbers of emerged radicles and normal seedlings. At the end of the test period (28 days) normal seedlings dry weight was measured after drying at 65°C for 4 days, and the number of fresh ungerminated seeds was also recorded.

3.3.2 Nursery and standard germination tests carried out by FRI (the New Zealand Forest Research Institute)

Procedures used in this study are enclosed in Appendix 2.

IV. RESULTS

4.1. Deterioration in *P. radiata*

The results of this study indicated that, in general, conditions of 40°C, 100% RH for up to 14 days did not reduce % radicle emergence, % normal seedling, and normal seedling dryweight (Figs 4.1.A, B and E), but from 8 days onwards, reduced vigour (measured by increasing T_{50} radicle emergence and T_{50} normal seedlings, Fig. 4.1.C and D). An exception from this general pattern happened after 2 days aging (Figs 4.1B and E) when the conditions reduced % normal seedling and normal seedling dry weight. Another important exception happened at 10 days aging when the conditions tended to increase the apparent vigour level of the seed by reducing T_{50} normal seedling (Fig. 4.1.C). However, at this aging period, the T_{50} radicle emergence was significantly greater than the control (Fig. 4.1.C).

45°C, 100% RH had a greater effect in reducing vigour and viability indices of the seeds than 40°C. As shown in Fig. 4.1.D, the condition already had an effect on vigour fall at 6 days by increasing T_{50} radicle emergence. The effect on reducing viability indices (% radicle emergence and % normal seedling) happened at 8 days (Fig. 4.1.A and B). At 10 days the percentage of radicle emergence and normal seedling were 28 and 25% (experiment b) and 9 and 0% (at experiment c).

An important point to be noticed was that these three experiments (a, b and c) showed a quite good reproducibility as within the same aging conditions the deterioration pattern was quite similar especially concerning the initial day when the vigour or viability started to decrease.

Figure 4.1.A.
Changes in % radicle emergence, during accelerated ageing at 40°C and 45°C with 100% RH up to 14 days. Bars indicate lsd at p. 0.05.

○ : experiment a at 40°C
● : experiment a at 45°C
□ : experiment b at 40°C
■ : experiment b at 45°C
◆ : experiment c at 45°C
—— : average of all experiments at 40°C
----- : average of all experiments at 45°C
* : differs significantly from control (0 day)

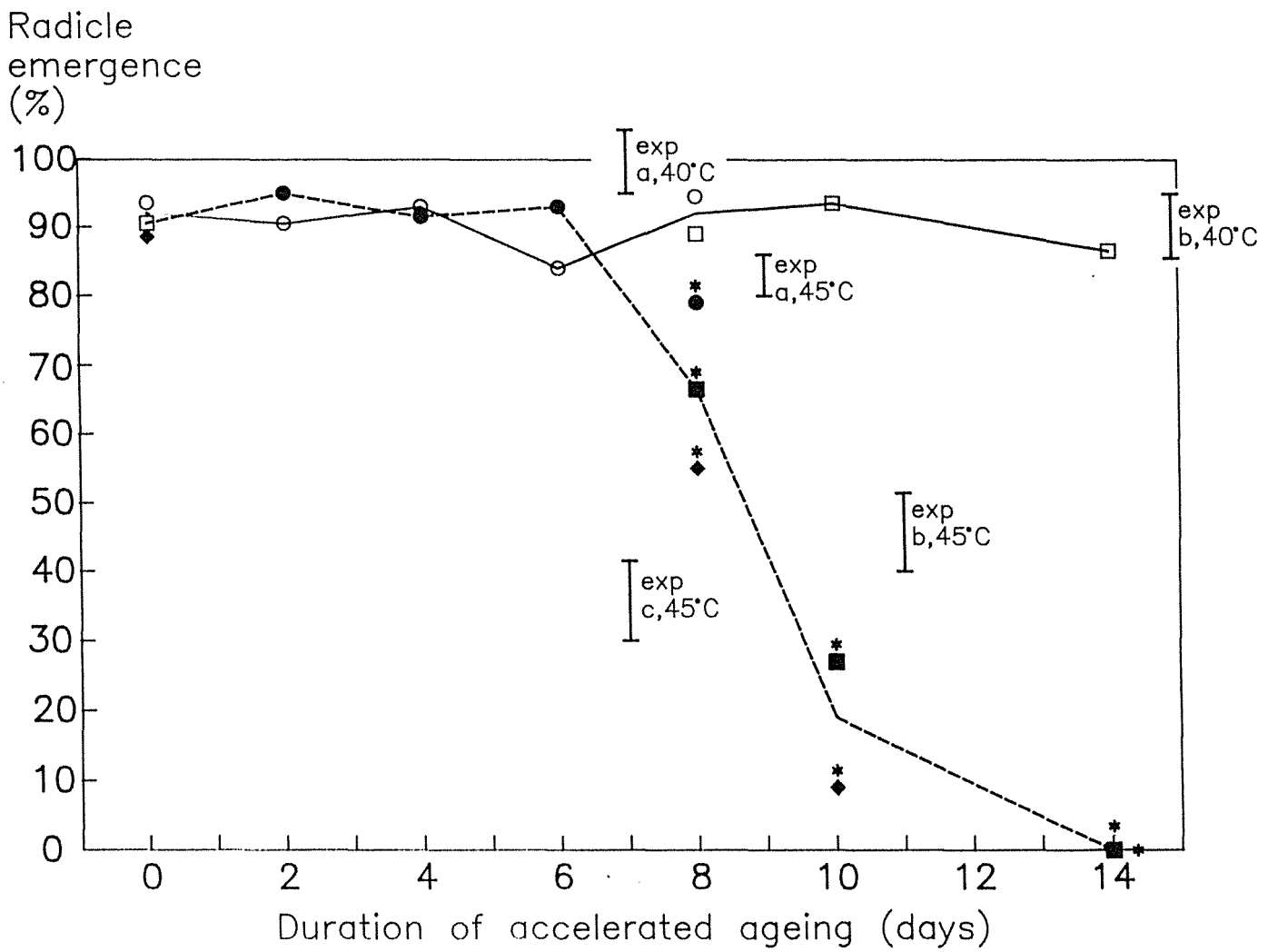


Figure 4.1.B.
Changes in % normal seedlings during accelerated ageing at 40°C and 45°C with 100% RH up to 14 days. Bars indicate lsd at p. 0.05.

○ : experiment a at 40°C
● : experiment a at 45°C
□ : experiment b at 40°C
■ : experiment b at 45°C
◆ : experiment c at 45°C
—— : average of all experiments at 40°C
----- : average of all experiments at 45°C
* : differs significantly from control (0 day)

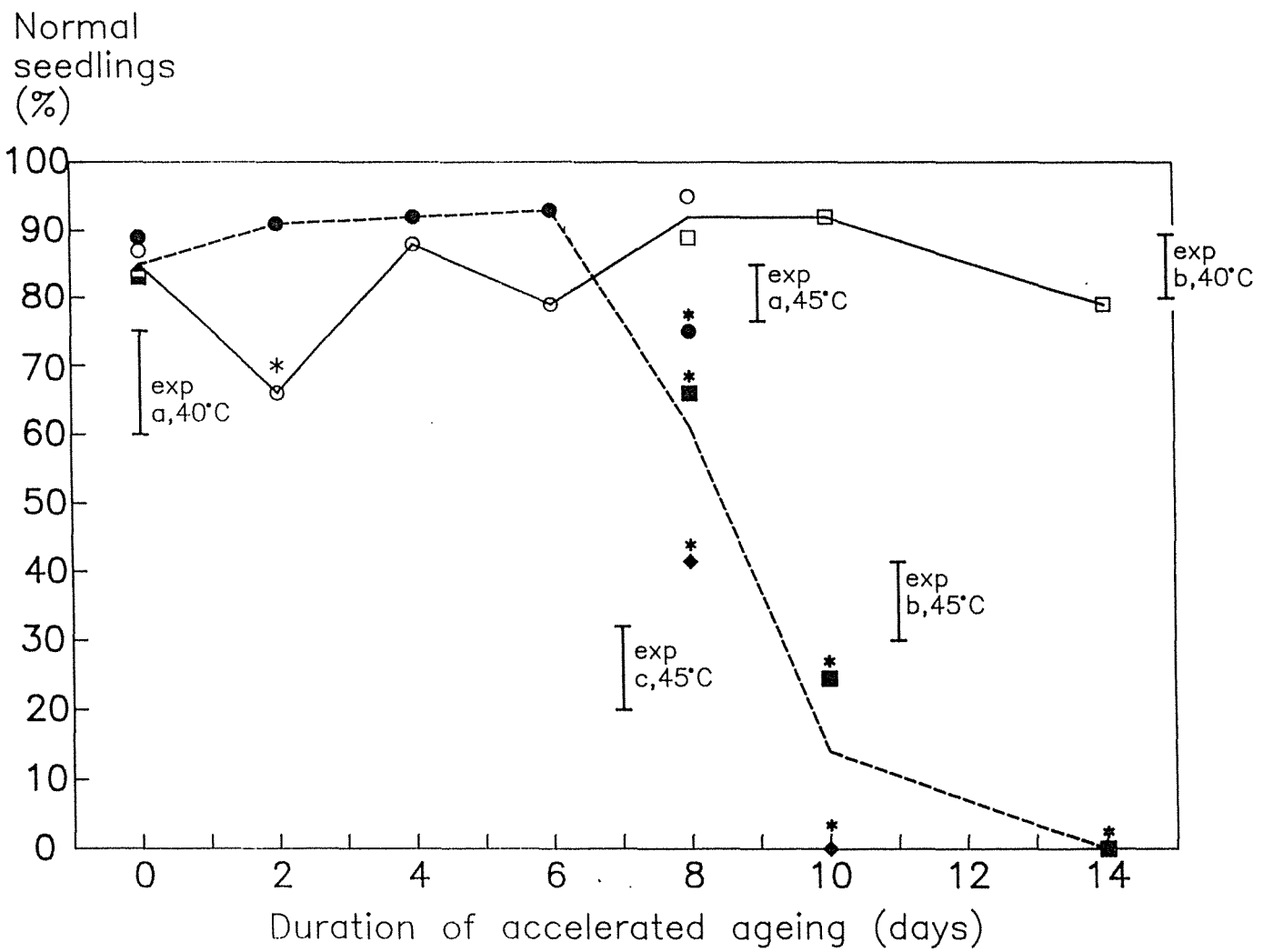


Figure 4.1.C.
Changes in t50 radicle emergence and t50 normal seedlings during accelerated ageing at 40°C with 100% RH up to 14 days.
Bars indicate lsd at p. 0.05.

o : experiment a at 40°C

□ : experiment b at 40°C

—— : average of all experiments at 40°C

* : differs significantly from control (0 days)

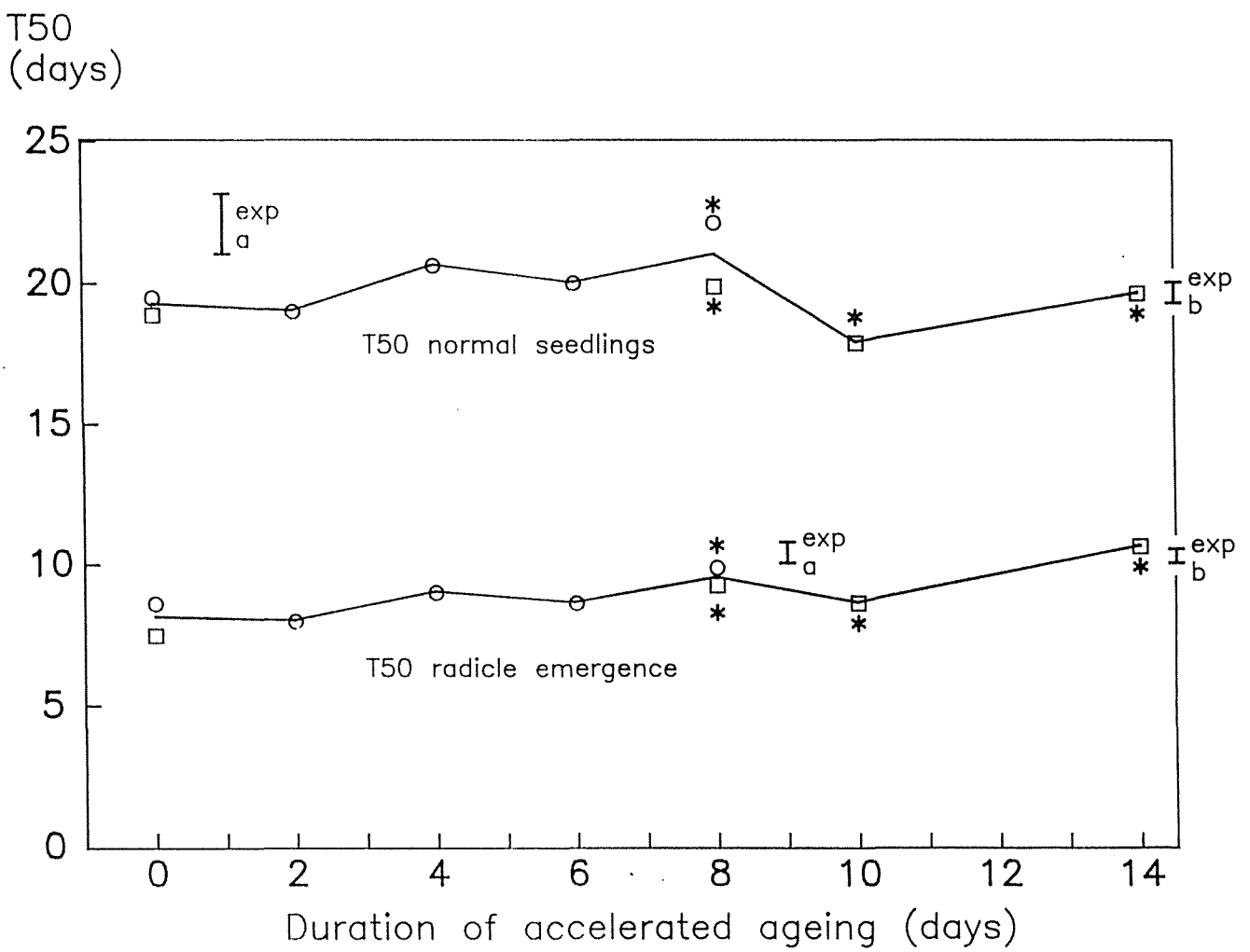


Figure 4.1.D.
Changes in t50 radicle emergence and t50 normal seedlings during accelerated ageing at 45°C with 100% RH up to 14 days.
Bars indicate lsd at p. 0.05.

- ● : experiment a at 45°C
- ■ : experiment b at 45°C
- ◆ : experiment c at 45°C
- ----- : average of all experiments at 45°C
- * : differs significantly from control (0 days)

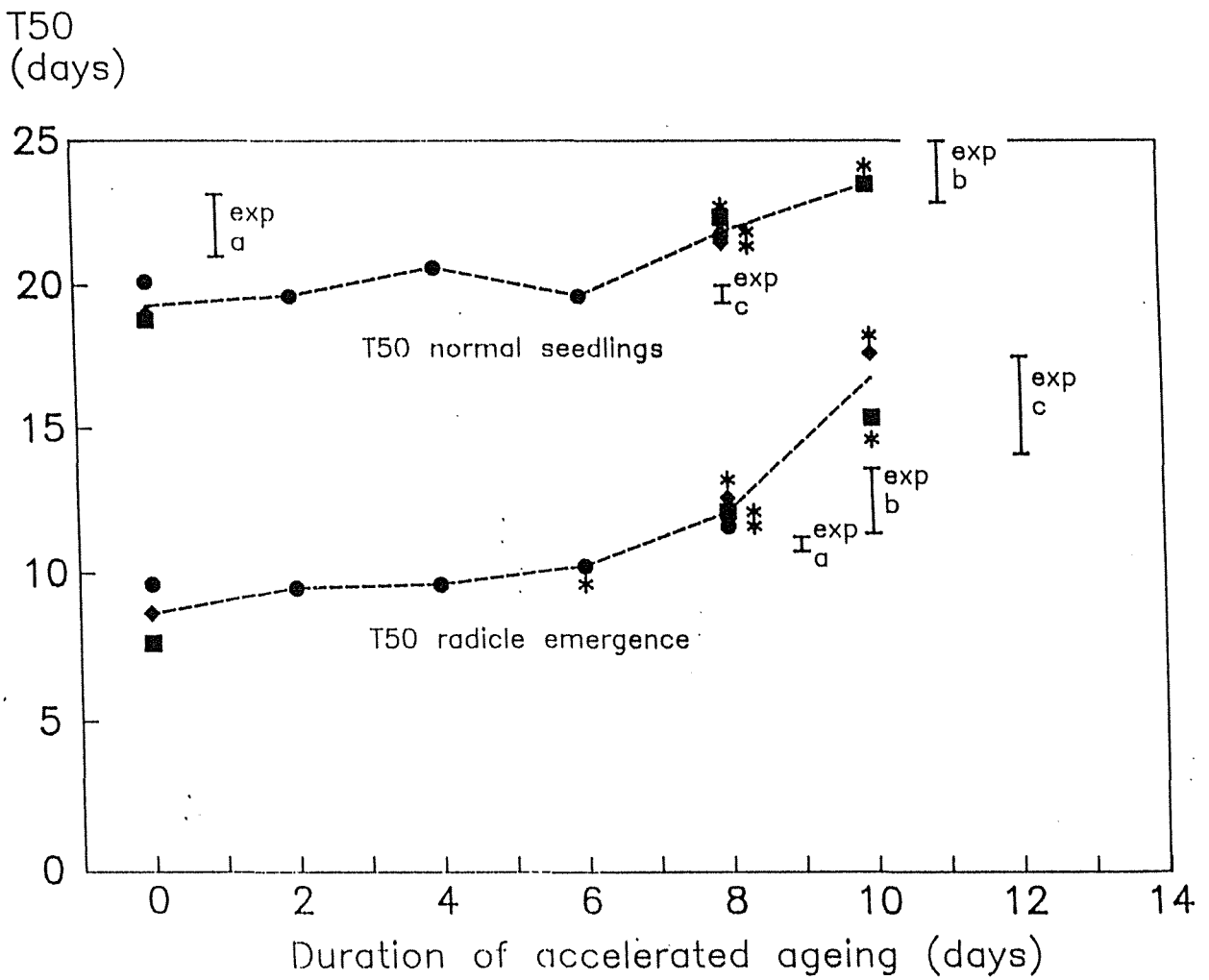
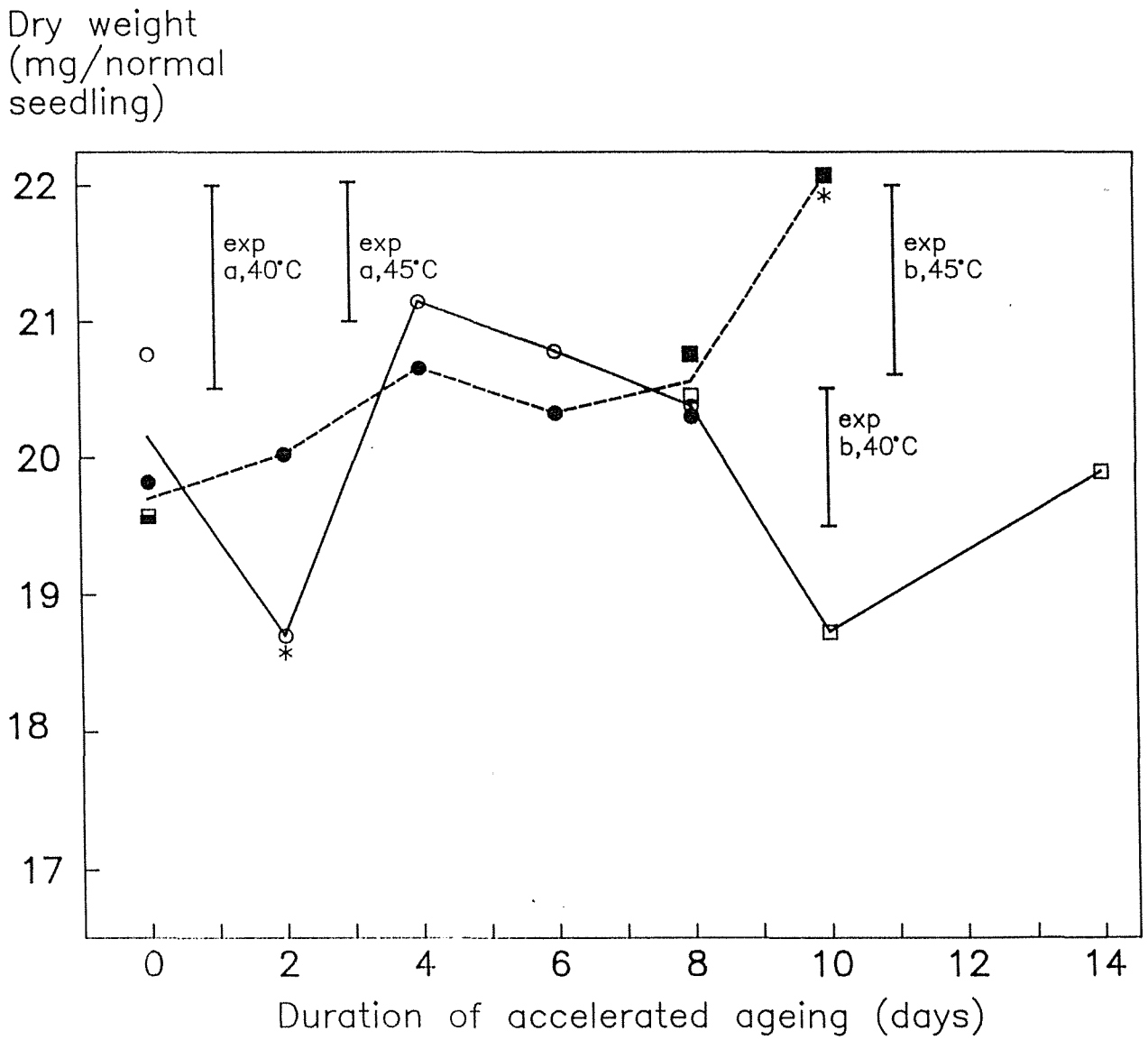


Figure 4.1.E.
Changes in normal seedlings dry weight during accelerated ageing at 40°C and 45°C with 100% RH up to 14 days.
Bars indicate lsd at p. 0.05.

- o : experiment a at 40°C
- ● : experiment a at 45°C
- □ : experiment b at 40°C
- ■ : experiment b at 45°C
- —— : average of all experiments at 40°C
- ------ : average of all experiments at 45°C
- * : differs significantly from control (0 day)



4.2 Vigour test evaluation in *P. radiata* seeds using 5 seed lots which varied according to seed size and age

Appendices 5 and 6 show the data for seed lot performance in the glasshouse test (good conditions), the winter field test (stress conditions), and vigour tests.

4.2.1 Seed lot performance in the glasshouse test

The microclimate of the glasshouse seemed to be quite optimum, since it had enough water and quite warm temperature. The minimum temperature was about 10-15°C, whereas the maximum temperature was around 25-35°C (Appendix 3).

Lot C had the lowest and slowest emergence. As shown in Figs 4.2A and B. It had the lowest percentage of normal seedlings and the highest T_{50} value. For seedling dry weight, however, the lowest performance was shown by lots D and E, where dry weight 17 mg/normal seedling, whereas lots A, B and C ranged from about 20-21 mg/normal seedling (Fig. 4.2.C). Another important point was that the dryweight of lot C was significantly lower than lot A.

4.2.2 Conditions and seed lot performance in the winter field test

The microclimate of the field test environment seemed to be sub-optimal for temperature and, for part of the period, water supply also. As shown in Appendix 4A, except for a very short period (91-96 days after sowing), the maximum temperature was about 15-17°C, and the minimum temperature range from -3 to 10°C. From the period of 46 days after sowing (das) onwards the overall minimum temperature was lower than the previously, with ground frost occurring several times later in the trial (at 46, 47, 48, 51, 68, 75-81, 85-87, 119 and 120 das).

Figure 4.2.A
Percentage of normal seedlings in glasshouse test.
Bar charts with same letter are not significantly different.

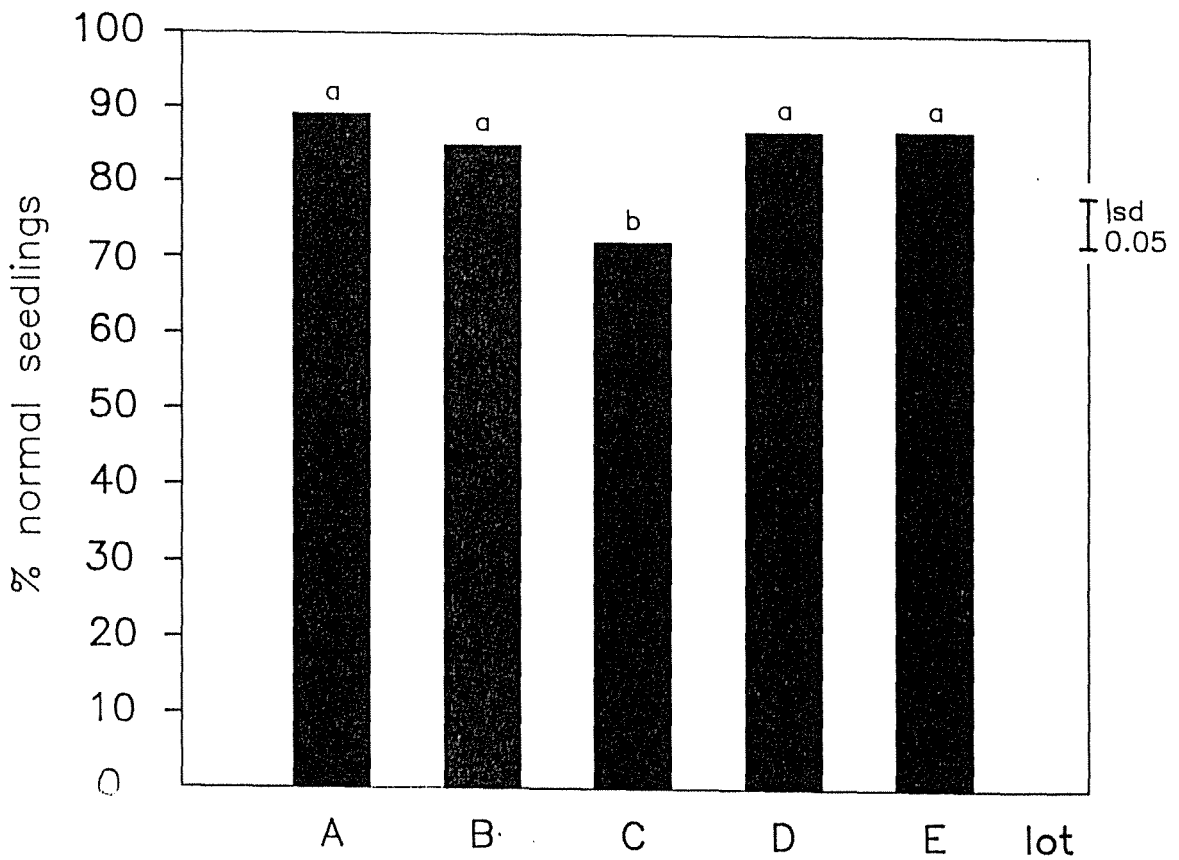


Figure 4.2.B
T50 normal seedlings in glasshouse test
Bar charts with same letter are not significantly different.

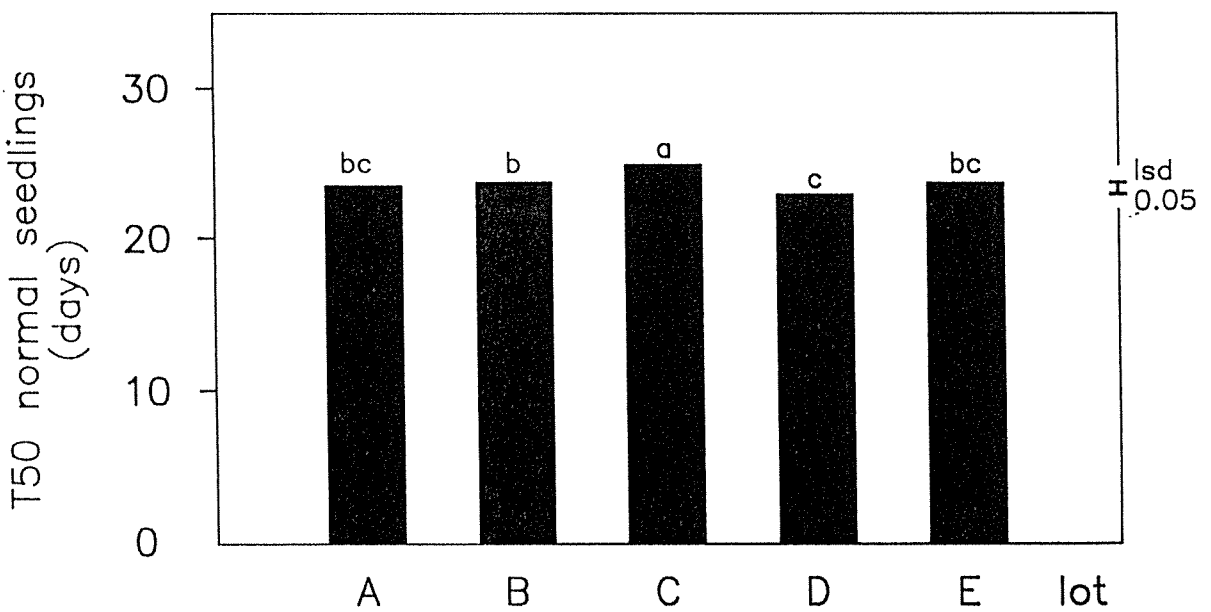


Figure 4.2.C
Normal seedlings dry weight in glasshouse test
Bar charts with same letter are not significantly different.

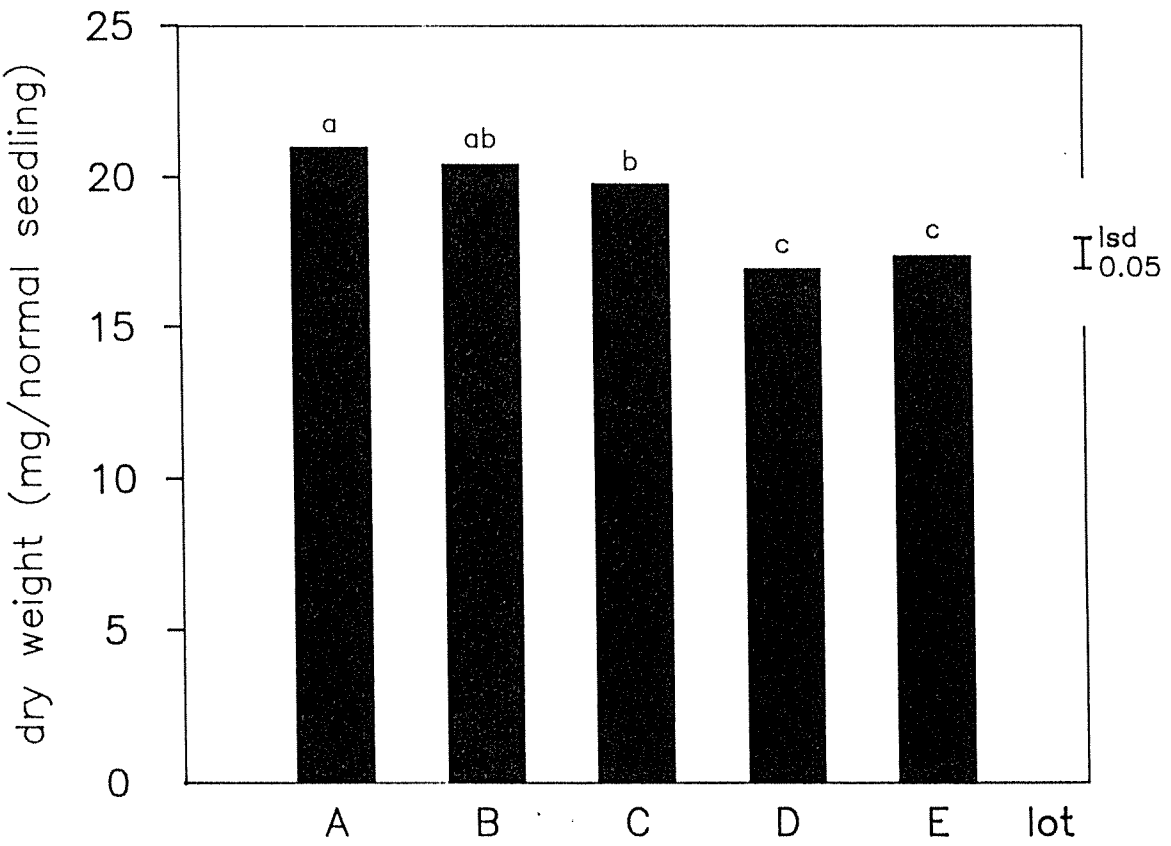


Figure 4.3.A
Cumulative emerged and normal seedlings in the winter field test. Bar charts with the same letter are not significantly different

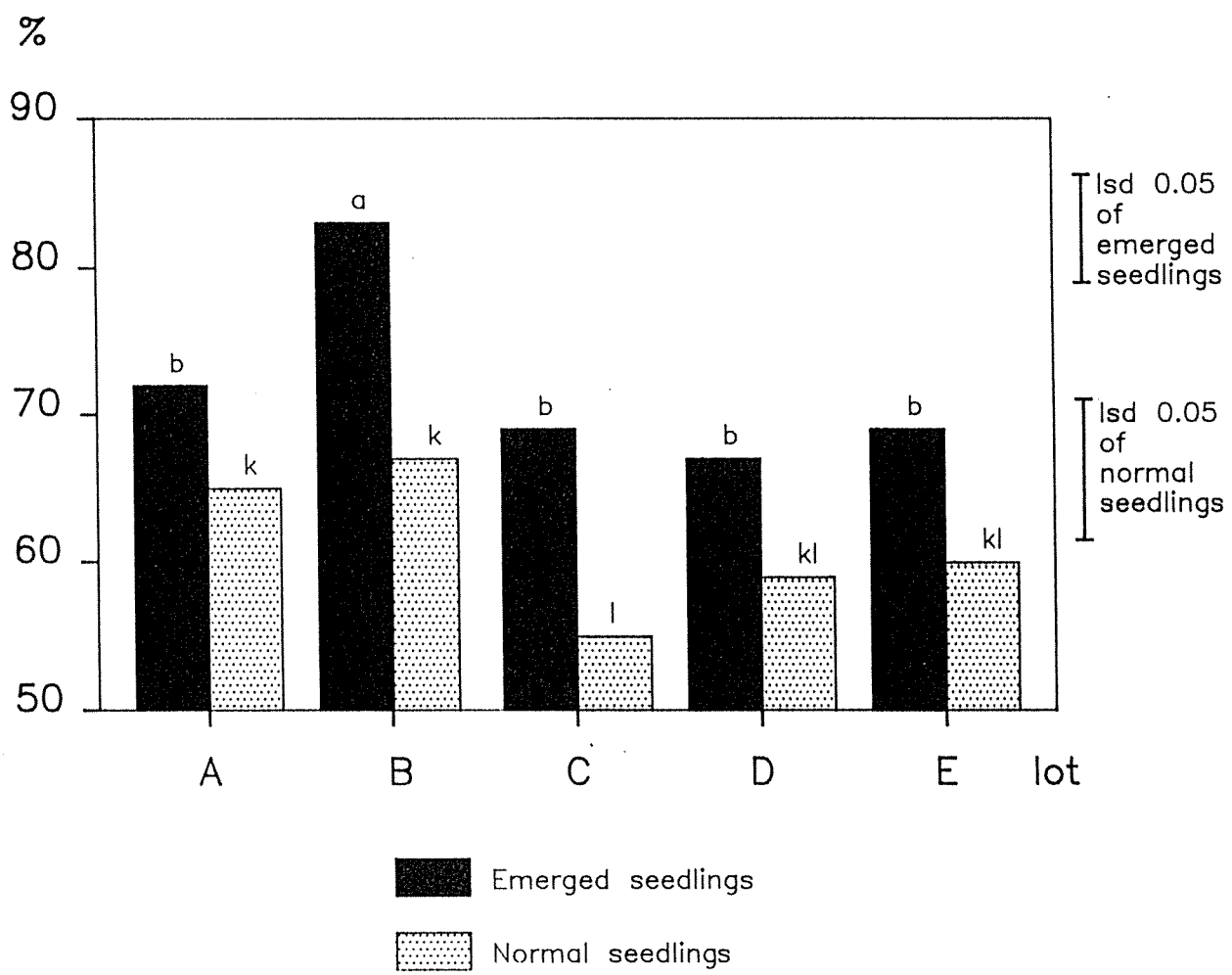


Figure 4.3.B
Surviving emerged and normal seedlings in the winter field test. Bar charts with the same letter are not significantly different

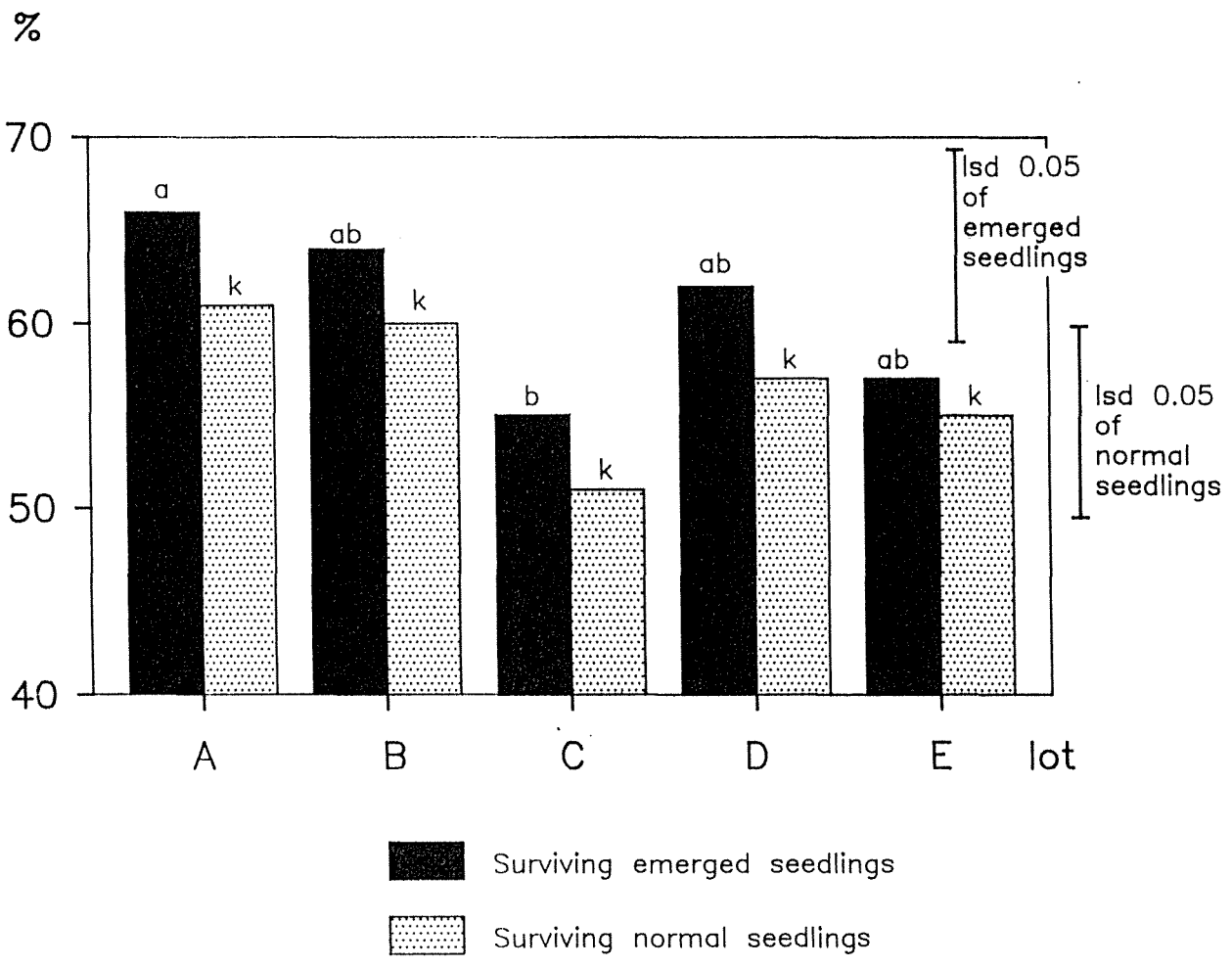


Figure 4.3.C
Time to 50% emergence and 50% establishment of normal seedlings in the winter field test.
Bar charts with the same letter are not significantly different.

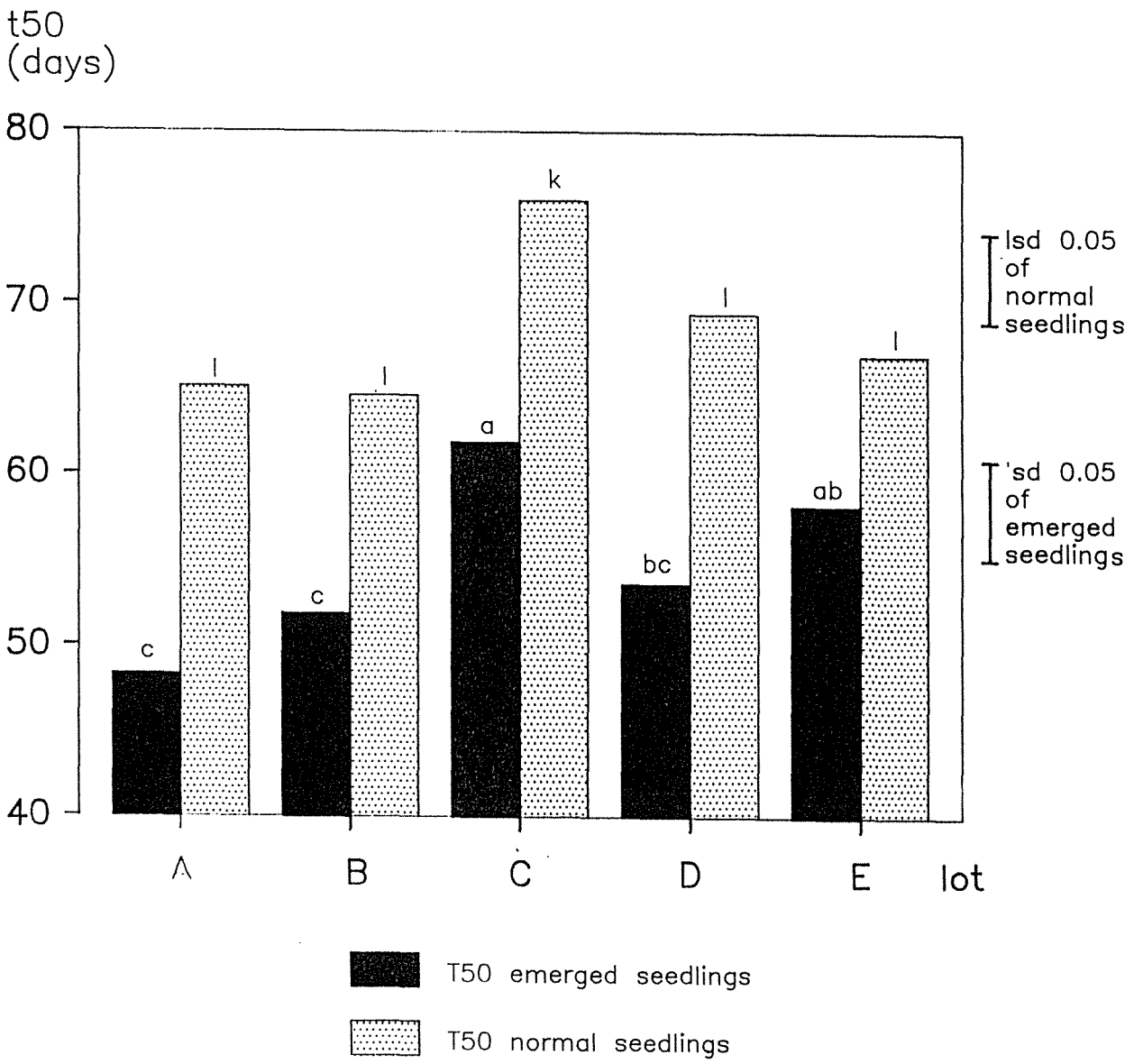
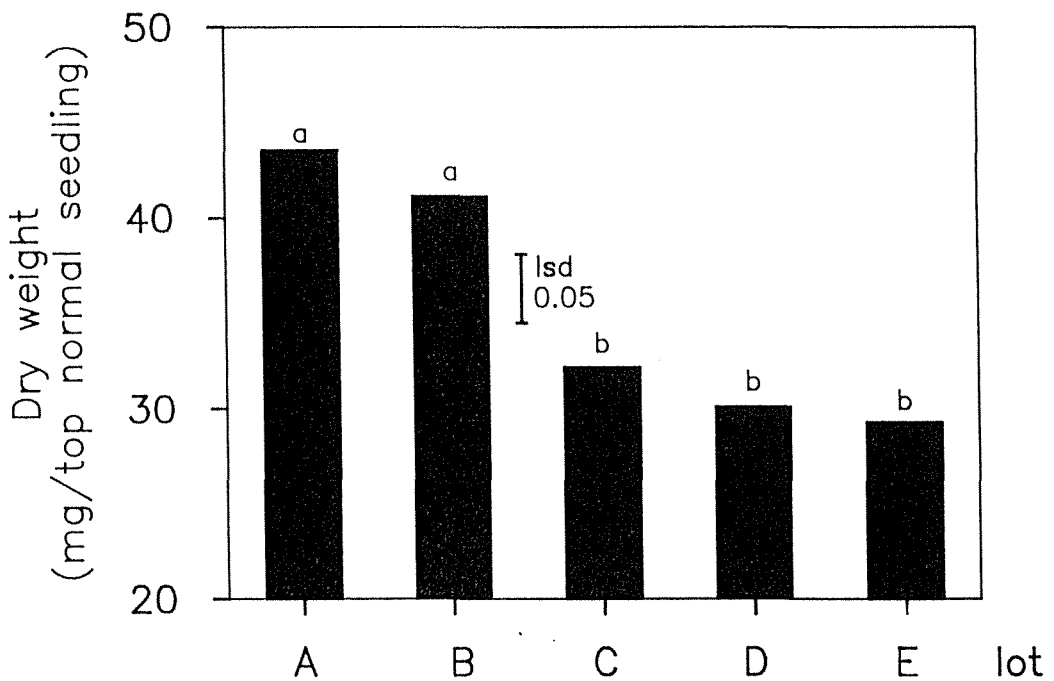


Figure 4.3.D
Dry weight of shoots of normal seedlings in the winter field test. Bar charts with the same letter are not significantly different



Water supply was adequate except for 76-96 das when there was very low rainfall (Appendix 4B). Some wilted seedlings were visually observed during germination observation. Another important factor affecting seed survival was some fungal attack on seedlings which caused damping off.

Figures 4.3.A, B, C and D show the performance of the five seed lots in the winter field test. Variation of data is high for most parameters measured. Quality differences between the three heavy seed lots are shown in that seeds of lot C produce significantly fewer normal seedlings and have reduced survival rates of emerged seedlings compared to lot A (Figs 4.3.A and B). Emergence time and shoot normal seedling dryweight are significantly reduced compared to lots A and B (Figs 4.3.C and D). In general, lot B appears to have slightly reduced performance compared to lot A, but differences are not significant. One interesting result was the increased total emergence from seeds of lot B (83%) compared to 72% for lot A (Fig. 4.3.A). Apart from resulting in 30% lighter seedling, seeds of lot D and E did not perform markedly more poorly than those of lot A, except that lot E showed significantly slower seedling emergence (Figs 4.3.C and D).

4.2.3 Seed lots performance in seedling growth test

Figure 4.4 shows the performance of the different seed lots in the seedling growth test. Within the heavy seed lots, a difference is shown in that lot C produce fewer radicles and slower radicle emergence than lot A (Figs 4.4.A and C), the light seed lot generally did not perform worse than the heavy ones, except they produced lighter seedlings. As shown in Fig. 4.4.D, seedling dry weight of lot D and E was about 15-17 mg/seedling, whereas lot A, B and C were in the range 19-21 mg/seedling.

Figure 4.4.A
Percentage of radicle emergence in seedling growth test.
Bar charts with same letter are not significantly different.

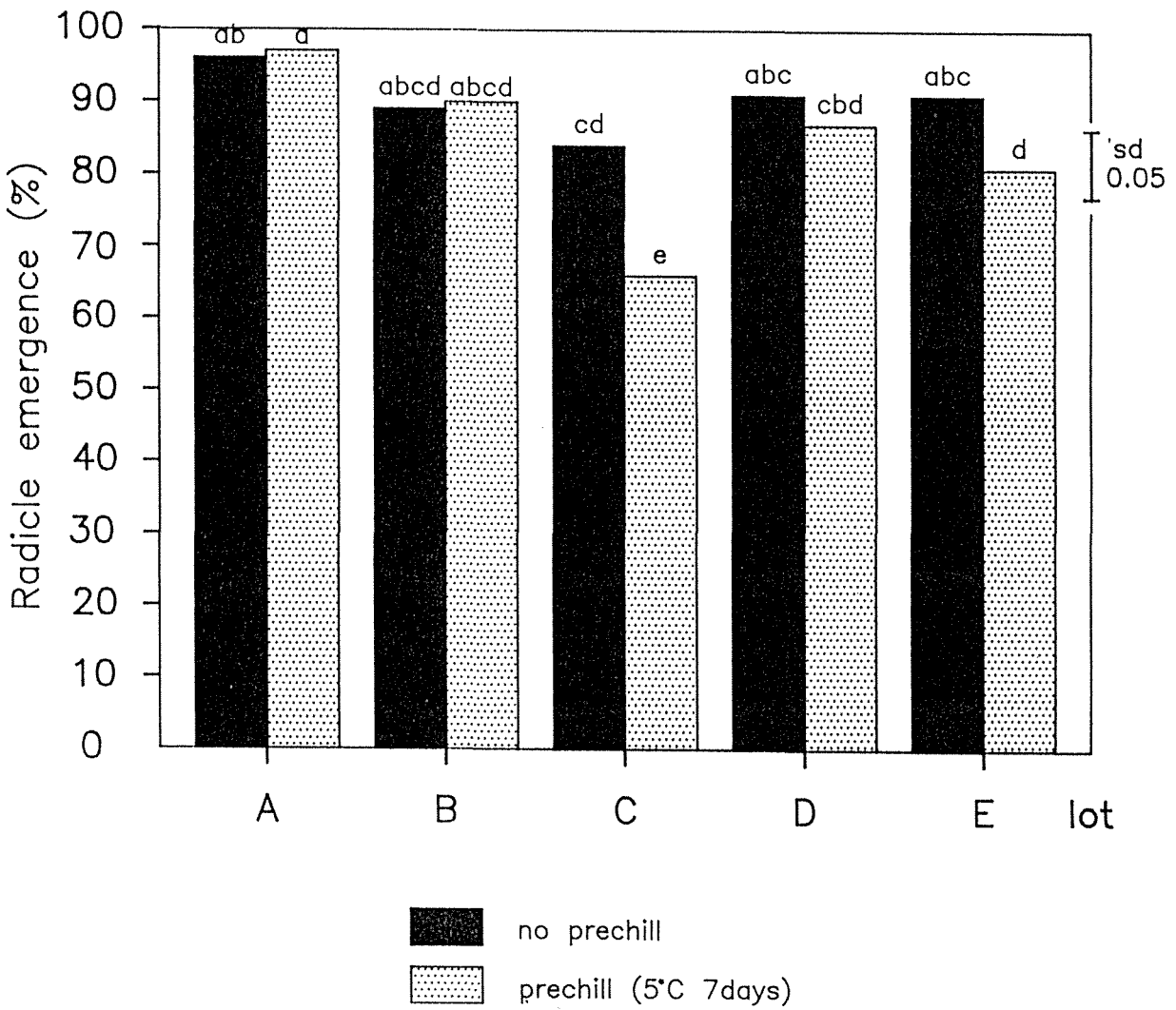


Figure 4.4.B
Percentage of normal seedlings in seedling growth test.
Bar charts with same letter are not significantly different.

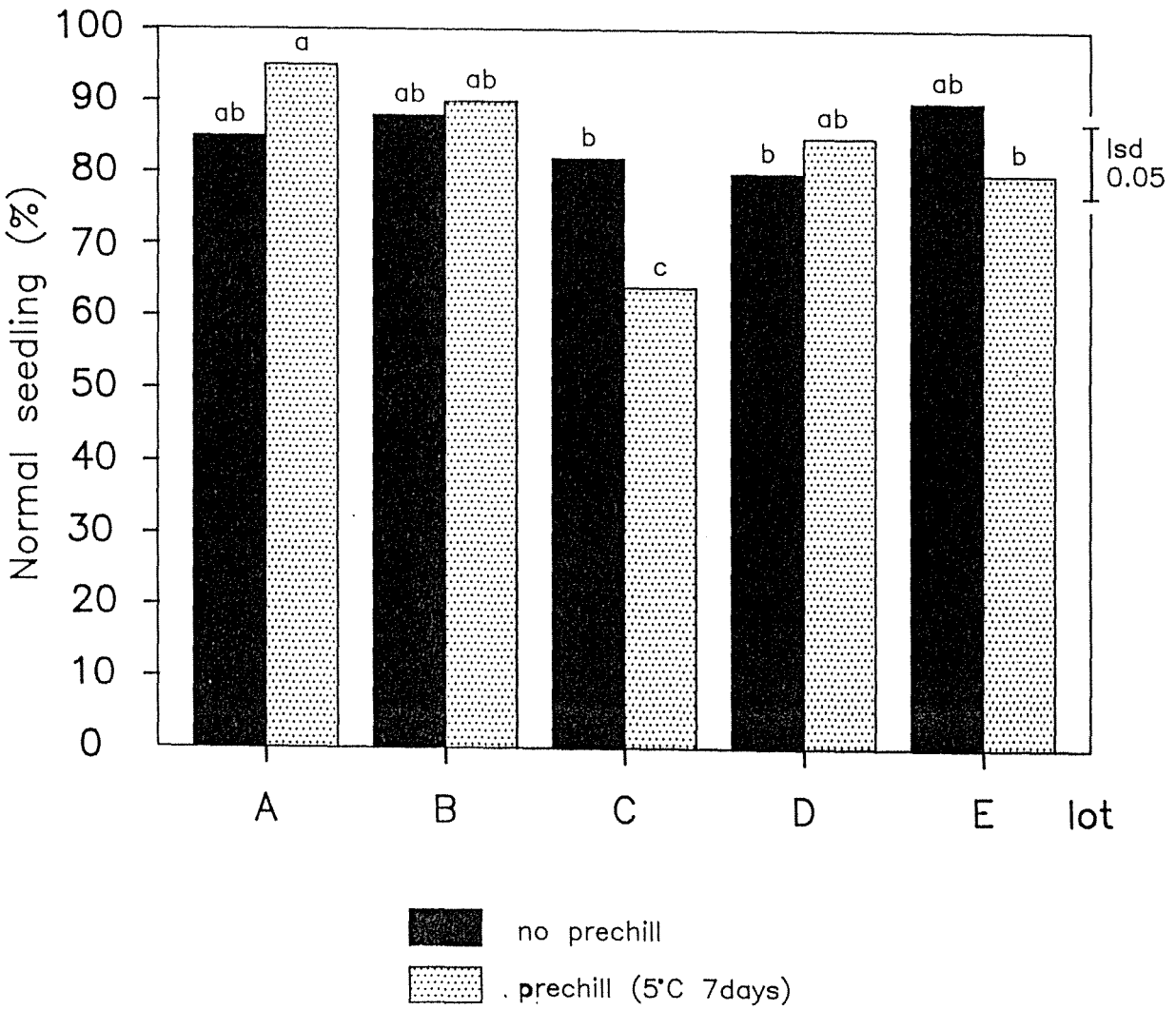


Figure 4.4.C
T50 radicle emergence in seedling growth test.
Bar charts with same letter are not significantly different.

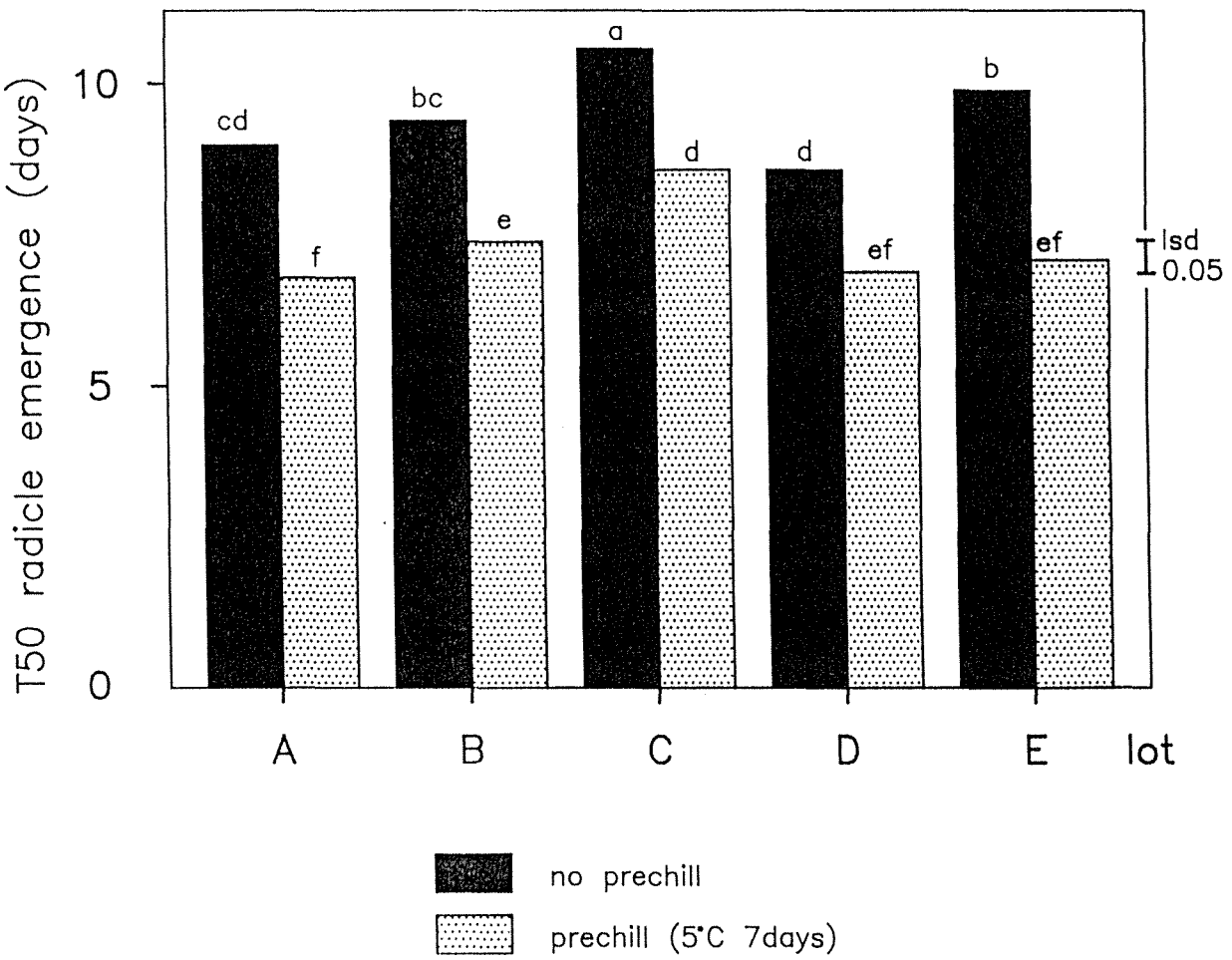


Figure 4.4.D
T50 normal seedlings in seedling growth test.
Bar charts with same letter are not significantly different.

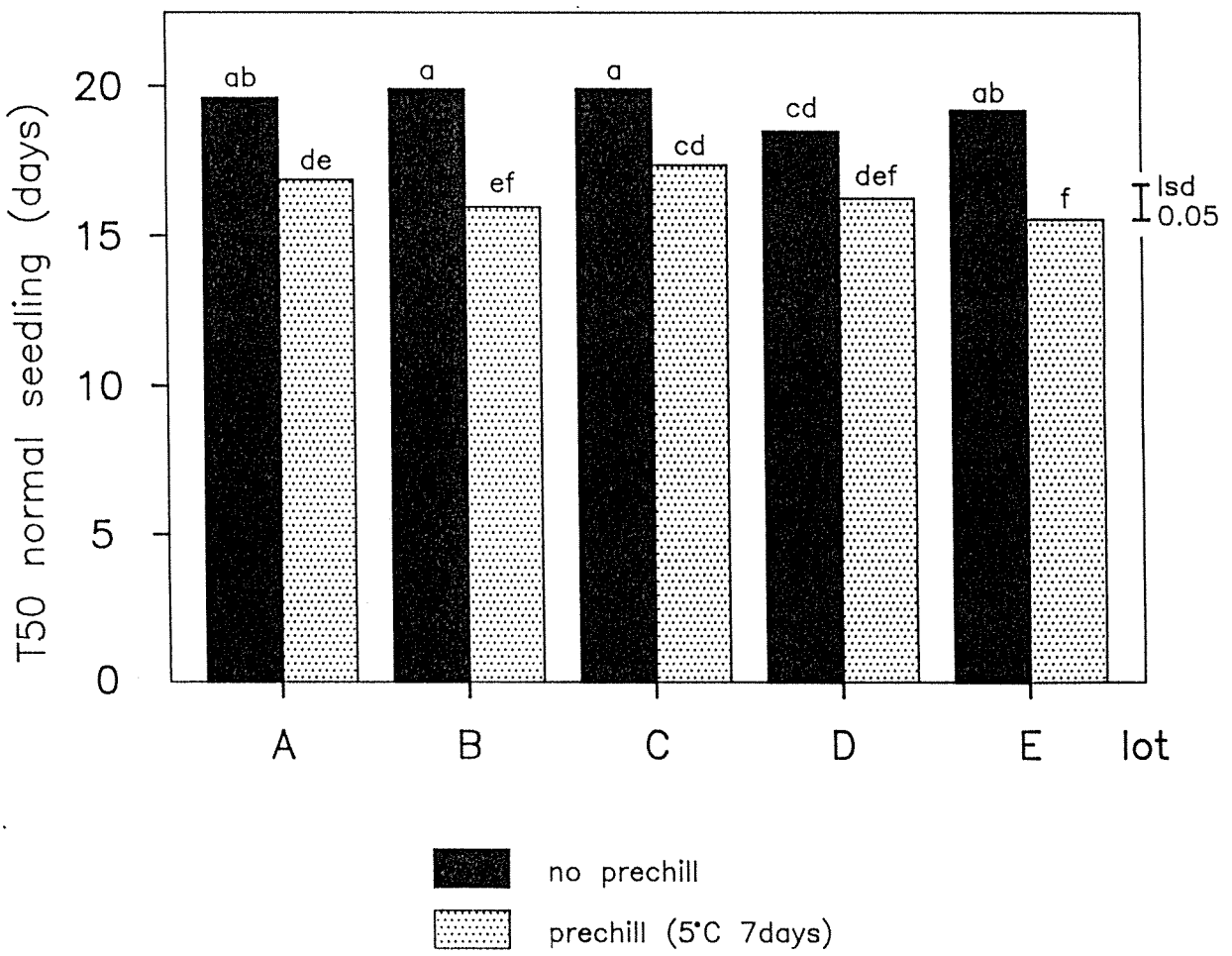
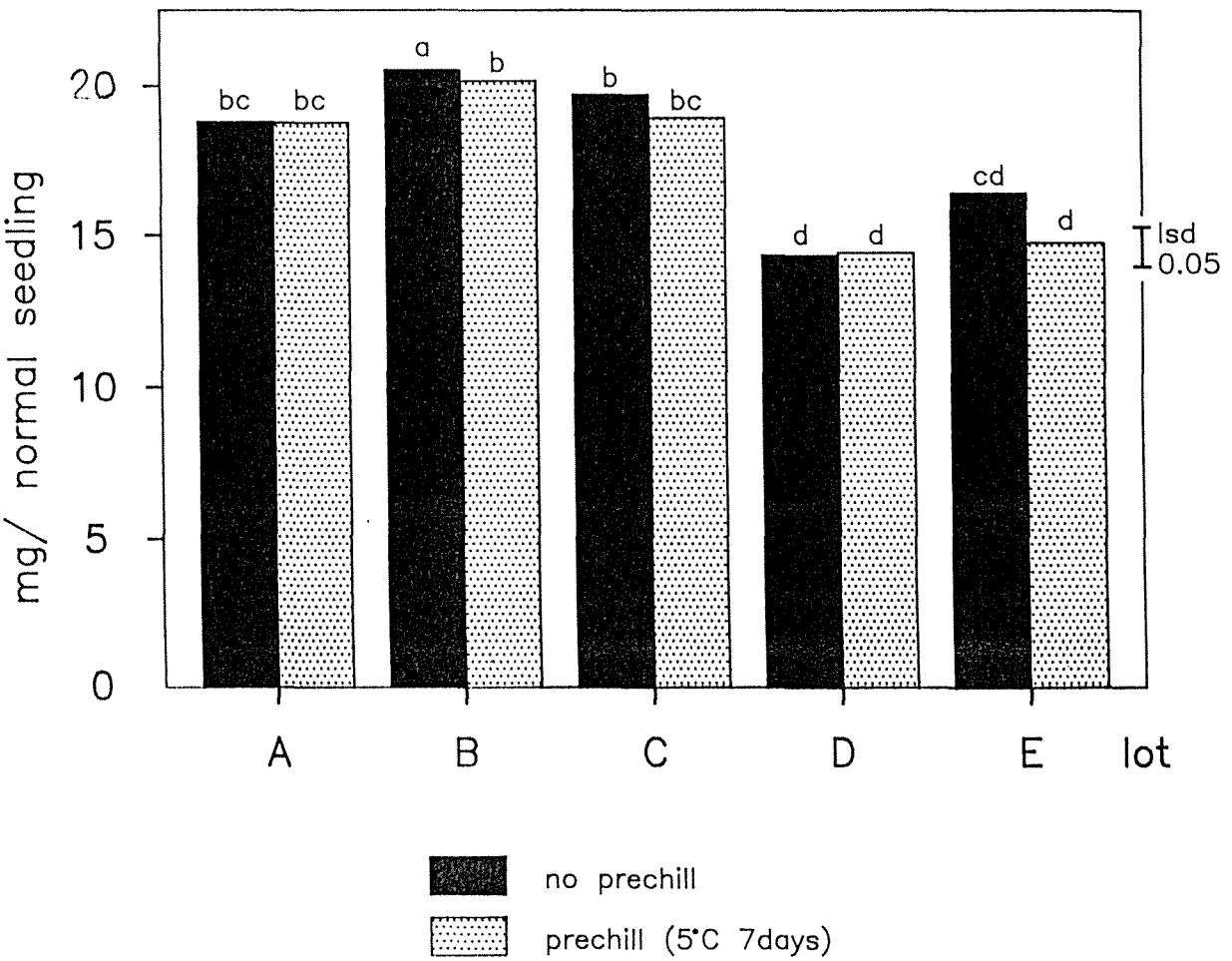


Figure 4.4.E
Normal seedlings dry weight in seedling growth test.
Bar charts with same letter are not significantly different.



Apart from speeding up germination (Figs 4.4.C and D), prechilling had an interesting effect on altering the seed lots' performance pattern. Prechilling reduced radicle emergence and numbers of normal seedlings of lot C, so the difference with lot A and B became more pronounced (Figs 4.4.A and B). Prechilling also caused a significant difference between lots A and B in T_{50} which was not detected in unchilled material (Fig. 4.4.C). For the light seed lots, prechilling removed the differences between lot D and E in emergence speed (Fig. 4.4.C) and seedling dryweight (Fig. 4.4.E), which were detected in the earlier test without prechilling.

4.2.4 Seed lot performance in radiographic test

No significant differences were apparent among lots A, B, C, D and E (Appendix 6.A). All seeds showed very low levels of $BaCl_2$. This condition might be caused by the difficulty of $BaCl_2$ solution in penetrating the seed coat as prolonging seed soaking in $BaCl_2$ solution for up to 48 hours still produced only very few $BaCl_2$ impregnated seeds.

4.2.5 Seed lot performance in topographical tetrazolium test

The test result (Appendix 6.A) indicated that lot C shows lower performance than lot A, even though percentages of vigorous seeds in these two lots were quite high. As shown in the Appendix vigorous seeds of lot A was 100%, whereas lot C was 95%. There were no differences detected among lots A, B, D and E. An important point to be noticed was that all seed lots produced quite high vigorous seeds, those were in the range of 95-100%.

Qualitative tetrazolium test result (Appendix 1) produced quite high variation among replicates which was probably caused by fungal development during

tetrazolium incubation. Surface sterilisation treatment reduced fungal development, but it semded to inhibit formazan development in incubated seeds.

4.2.6 Seed lot performance in the conductivity test

The result of the test (Appendix 6.A) pointed out that aged seed tended to have a higher conductivity reading than unaged ones. As shown in the appendix, lot C (in test using 50 seeds, at all period of imbibition) and lot E (in test using 100 seeds, especially at 24 hours after imbibition) had the highest conductivity reading. However, it seems that this test method faces the problem of reproducibility, since there were great differences in seed lot rankings between test using 50 and 100 seeds.

4.2.7 Seed lot performance in the controlled deterioration (CD) test

Figure 4.5 shows seed lot performance in the CD test using 2 and 4 days aging treatments. In the test using 2 days aging treatment, lot C shows the lowest performance, but there was no difference between lots A and B, and between lots D and E. In the test using 4 days aging treatment, however, lot B and C both show lower performance than lot A, and also lot E lower than lot D. In both aging methods, there was no appreciable effect of seed weight.

4.2.8 Seed lot performance in complex stressing vigour test

Data in Figure 4.6 show that this test can distinguish between deteriorated seed lots, but does not differentiate seed quality parameters which are a function of seed weight. Exposure of seeds of lots A and D reduced the percentage of normal seedlings to 83 and 73% respectively from 95 and 90% (data of prechilled seedling growth test). It also reduced performance of lot C and E to 56% from 64 and 80%

Figure 4.5
Seed lot performance in controlled deterioration (CD) test. Bar charts with same letter are not significantly different.

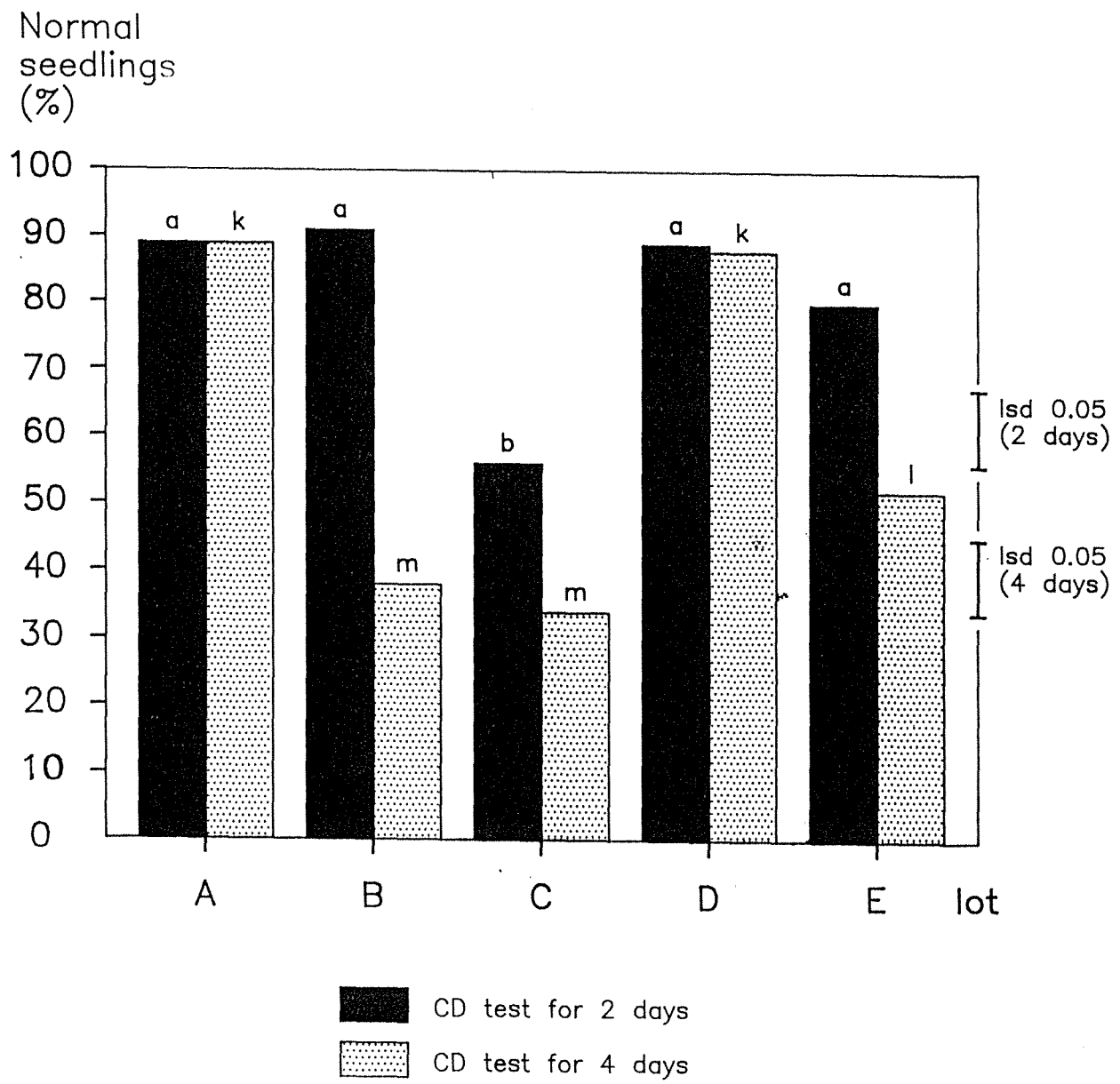
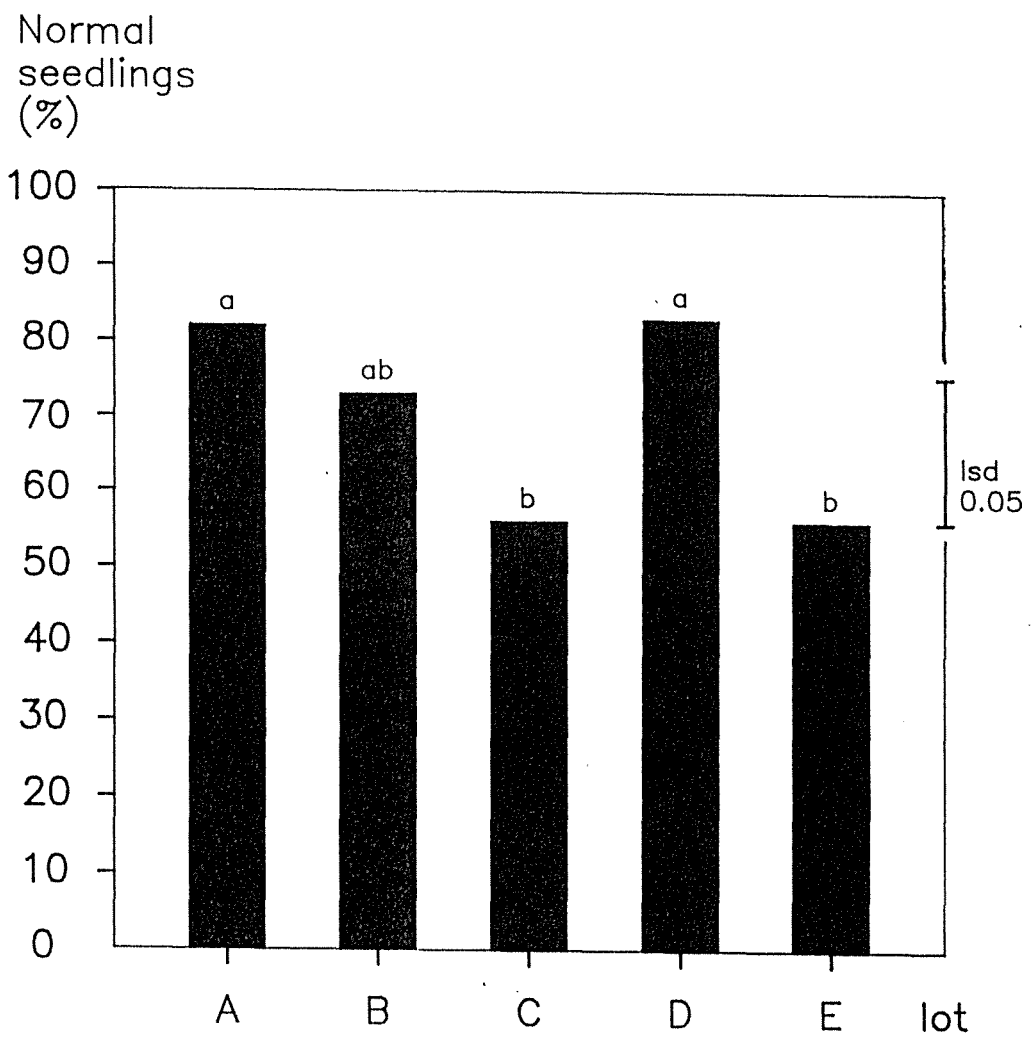


Figure 4.6
Seed lot performance in complex stressing vigour test.
Bar charts with same letter are not significantly different.



(in prechilled seedling growth test) respectively. Lot B was of intermediate performance between A and C.

4.2.9 Seed lot performance in low temperature/osmotic stress test

Seed lot performance in all of the test combinations can be seed in Appendix 6B. Conditions which can differentiate quality differences seem to be at a temperature of 10°C (Figs 4.7A and B), or at 15°C with respect to T_{50} radicle emergence (Fig. 4.7C). Data in the figures show that generally aged seeds had faster emergence than unaged ones (Figs 4.7B and C), and also had higher radicle emergence at -5 bar (Fig. 4.7A). An interesting result is that there is a shift in the percentage radicle emergence shown by lots A and C at different osmotic potentials at 10°C. As shown in Fig. 4.7A, at -5 bar, lot C produces 79%, while lot A only 61%. At 0 bar, however, lot C only 69% whereas lot A 92%. Another important point is that there was a difference in germination speed which is a function of seed weight at 10°C at both osmotic potentials (Fig. 4.7A) and at 15°C only at -5 bar (Fig. (4.7C). Under these conditions generally lot D had slower emergence than lot A.

4.2.10 Correlation between vigour tests and glasshouse/winter field test

Appendix 7 shows correlation between vigour tests and glasshouse/winter field tests. The best correlation seems to be shown by the prechilled seedling growth test and the controlled deterioration with 2 days aging treatment.

As shown in the appendix, four parameters of prechilled seedling growth test gave significant correlations with the glasshouse test result and two of these correlated significantly with some of the results of the winter field test. Correlation of % radicle emergence in prechilled seedling growth test with surviving normal seedling in the winter field test can be seen in Figure 4.8. The radicle emergence

Figure 4.7.A
Percentage of radicle emergence at 10°C.
Bar charts with same letter are not significantly different.

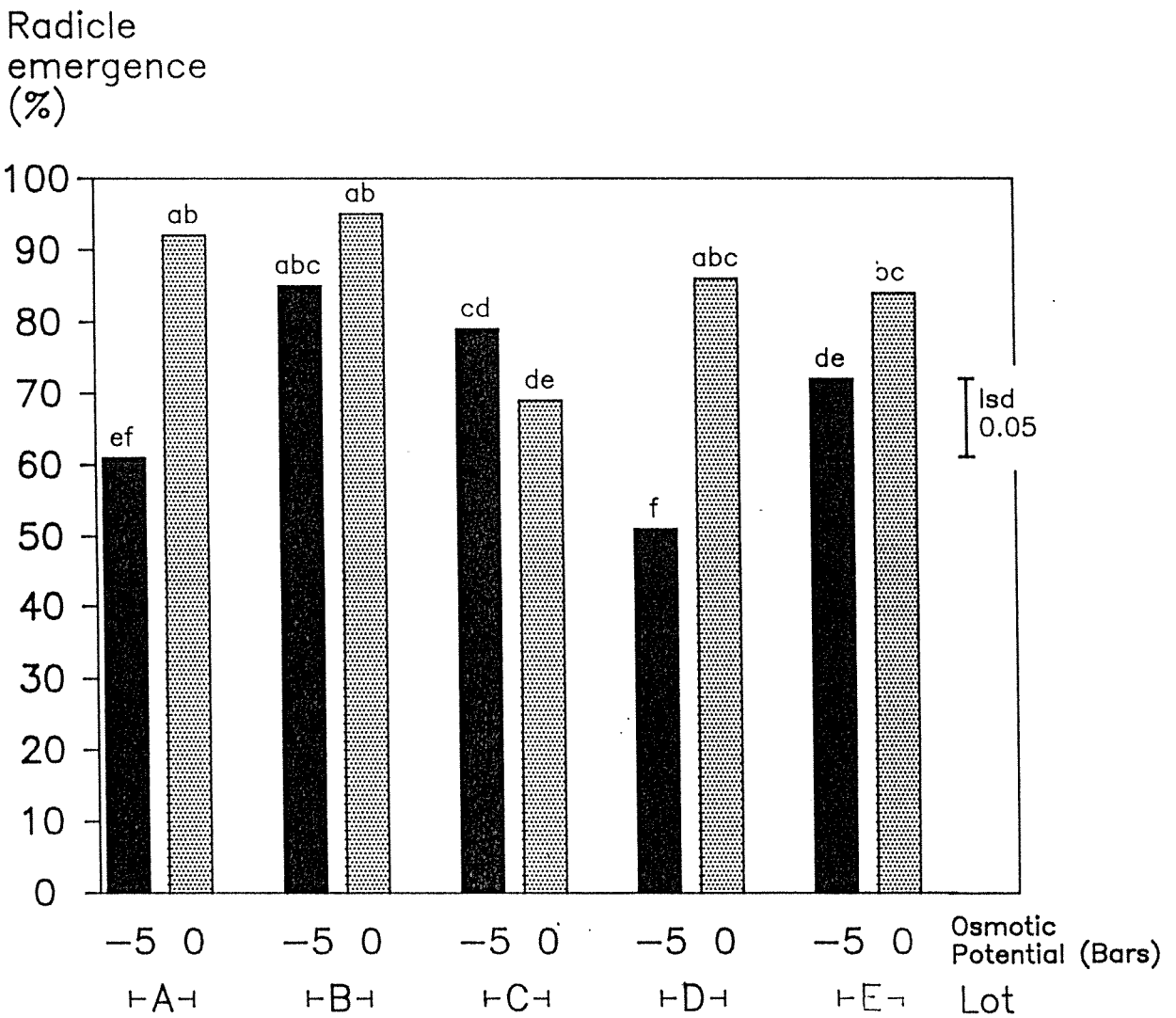


Figure 4.7.B
T50 radicle emergence at 10°C.
Bar charts with same letter are not significantly different.

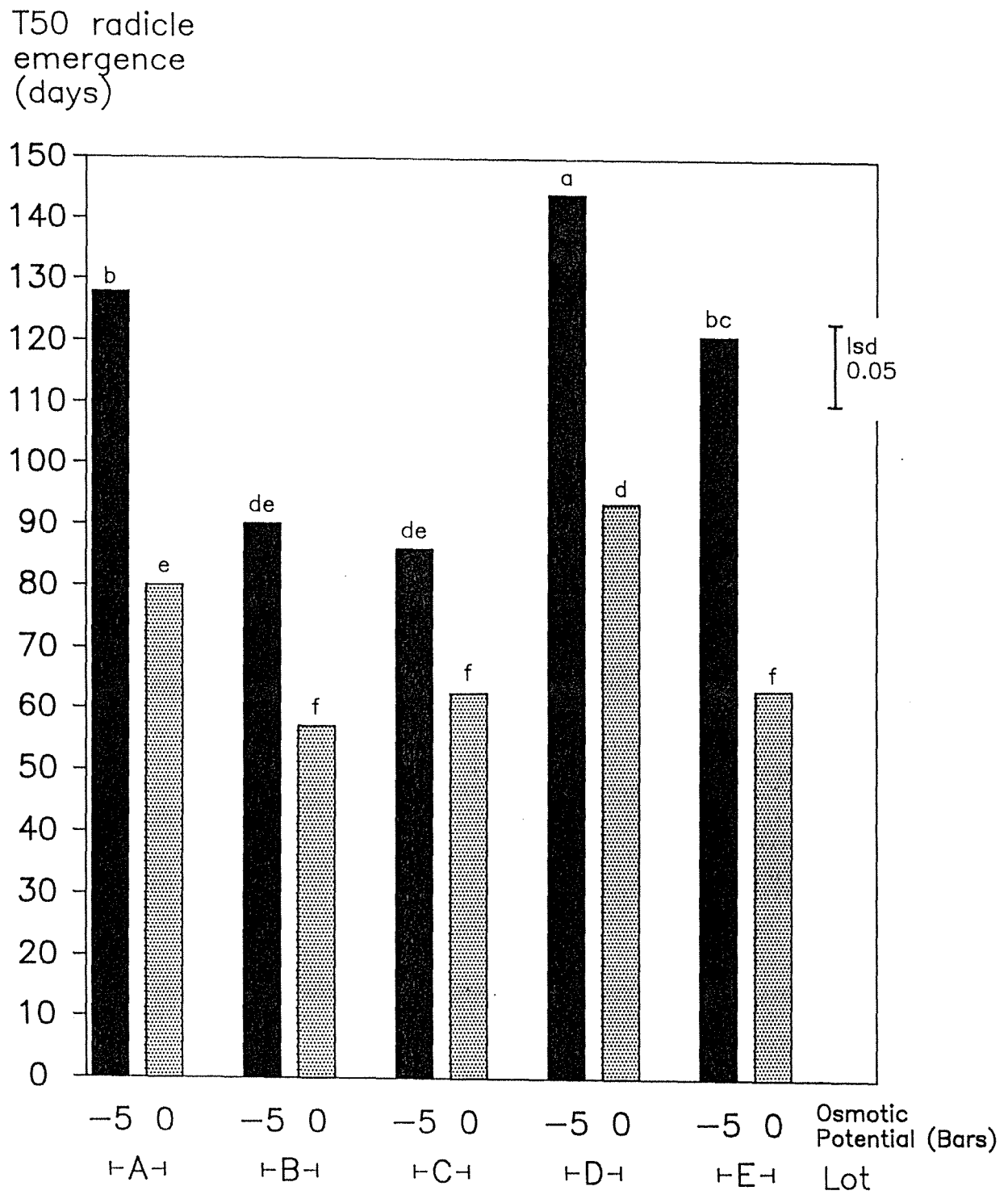
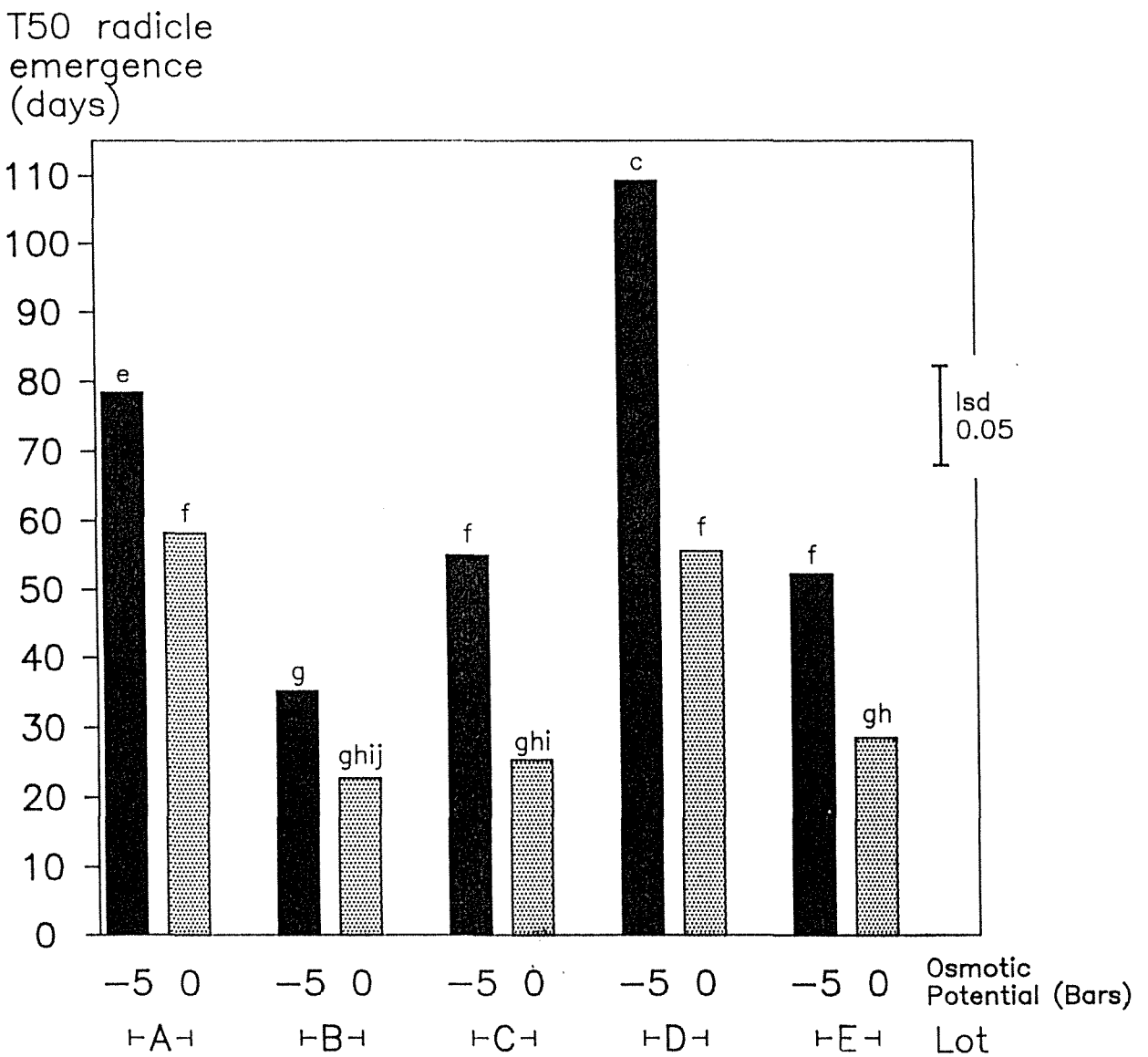


Figure 4.7.C
T50 radicle emergence at 15°C.
Bar charts with same letter are not significantly different.



measurement in prechilled seedling growth test predicts seedling survival in the field by a regression equation of Y = 0.34X + 28.44 with a coefficient of determination (R^2) of 94%. This prediction is much better than radicle emergence measurement in unchilled seedling growth test which do not correlate significantly (correlation coefficient = r = 0.76^{ns}) with seedling survival in the field (Fig. 4.9).

Of most interest is the measurement of percentage normal seedlings resulting from the controlled deterioration with 2 days aging treatment test, since this single measurement correlated significantly with % normal seedling and T_{50} normal seedling in glasshouse test (Figs 4.10 and 4.11), and also with surviving normal seedling and T_{50} normal seedling in field (Figs 4.12 and 4.13). As shown in the figures, the coefficients of determination of the controlled deterioration with 2 days aging treatment test are quite high either with glasshouse (87 and 79%) or with the winter field test (76 and 83%).

Even though the complex stressing vigour test can differentiate deteriorated seed lots (Fig. 4.6), it does not give any significant correlation with glasshouse nor with the winter field tests (Appendix 7.C). In relation to seedling survival in the field, it gave a correlation coefficient of 0.75^{ns} (Fig. 4.14).

Another test which has some predictive value is the low temperature test at 10°C, 0 bar. This test gave high and significant correlations with 4 parameters of the winter field test, namely cumulative normal seedling (r = 0.93*), surviving emerged seedling (r = 0.90*), surviving normal seedling (r = 0.96**), and T_{50} normal seedling (r = -0.96*). In relation to surviving normal seedling in the field, this method gave a regression equation of Y = 0.38X + 24.16 with coefficient of determination 92% (Fig. 4.15). This method was much better than test using 15°C,

Figure 4.8
Radicle emergence from seedling growth test + prechilling treatment vs surviving normal seedlings in the field

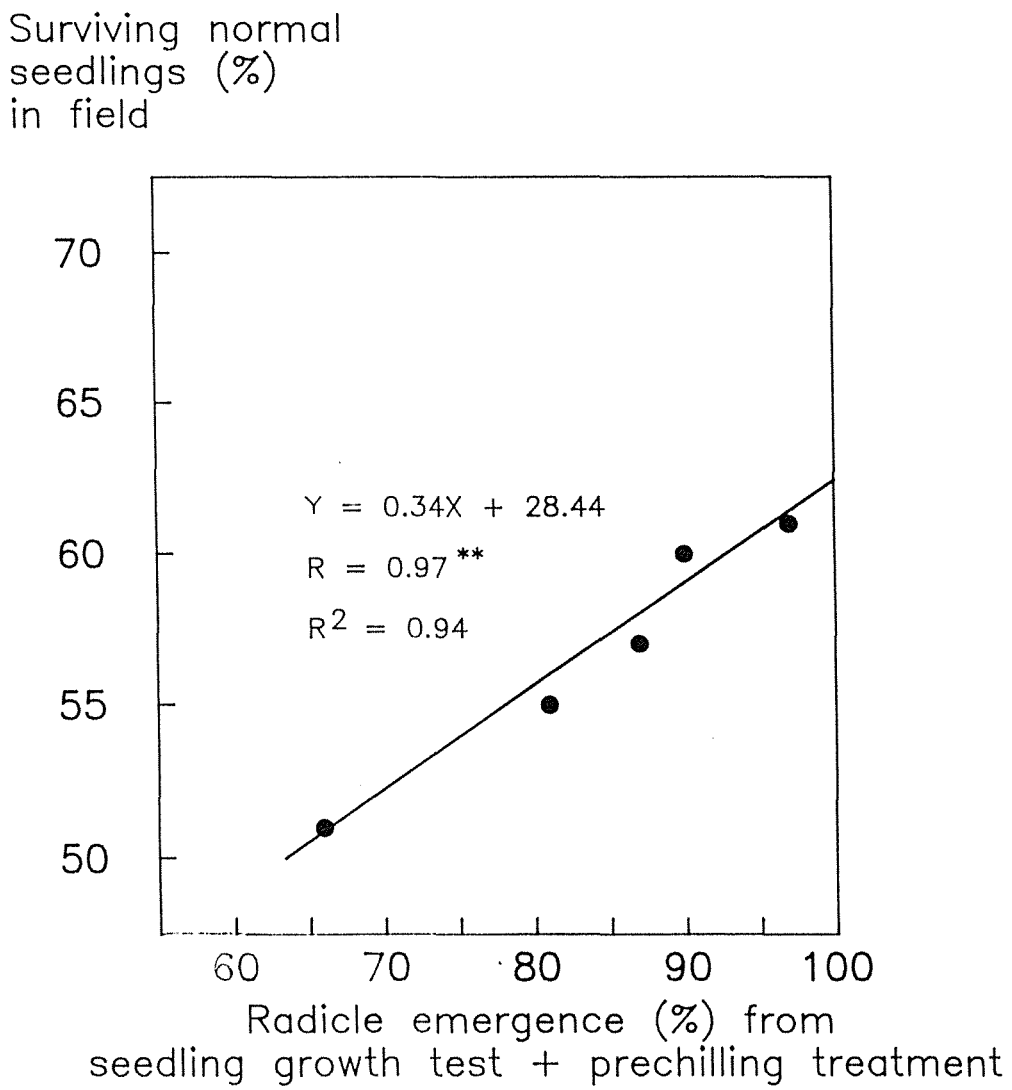


Figure 4.9
Radicle emergence from seedling growth test without prechilling vs surviving normal seedlings in the field

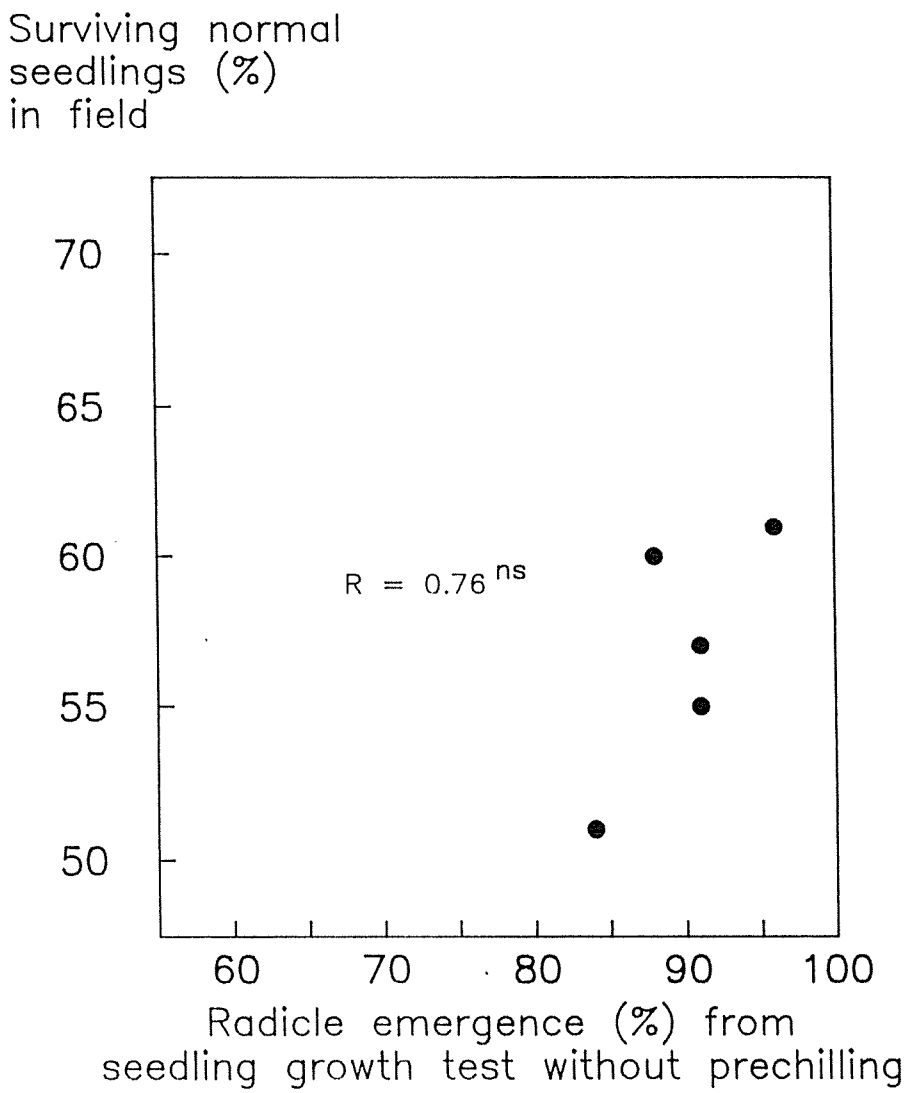


Figure 4.10
Normal seedlings from the controlled deterioration test with 2 days ageing treatment vs normal seedlings in the glasshouse

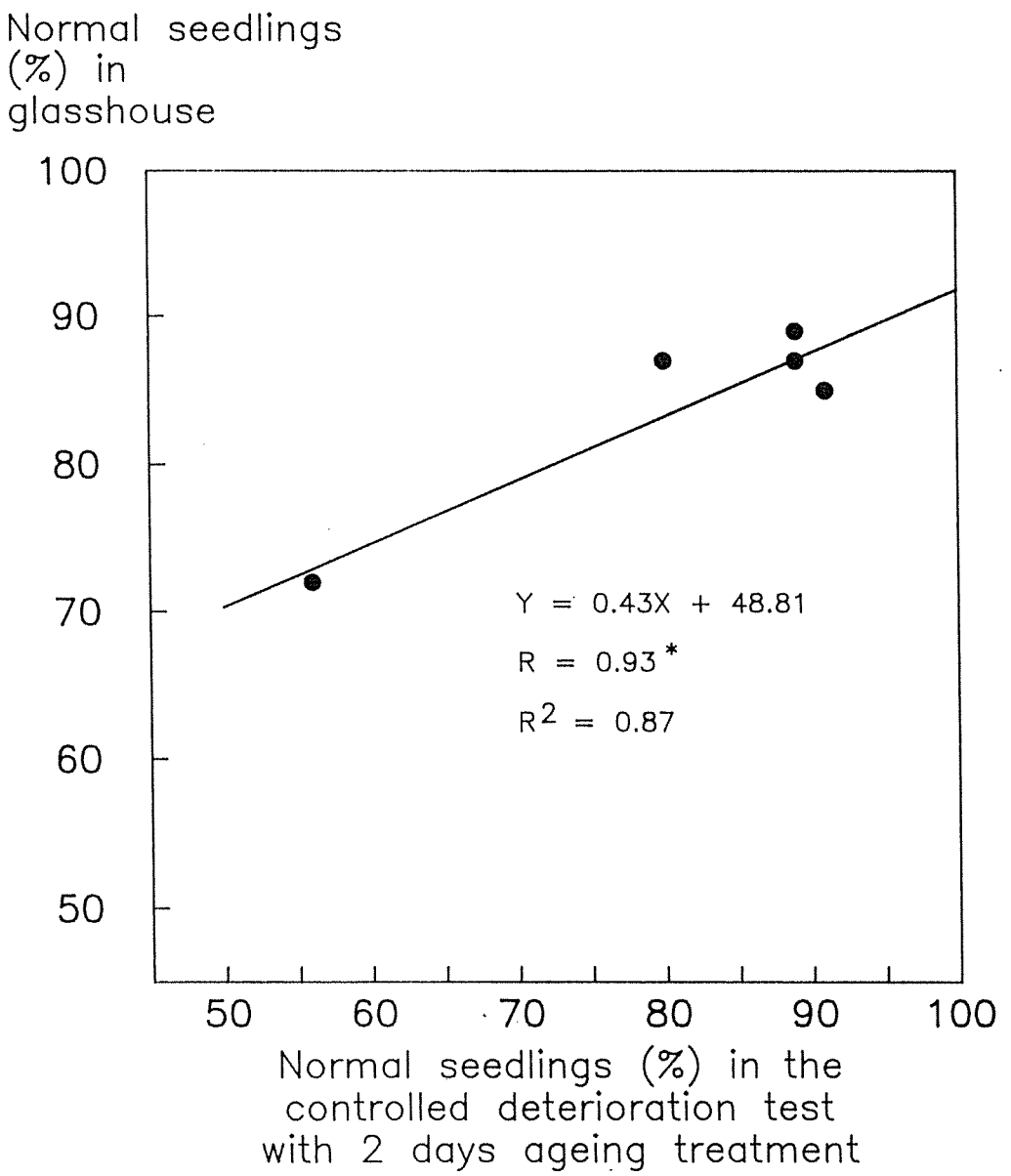


Figure 4.11
Normal seedlings from the controlled deterioration test with 2 days ageing treatment vs T50 normal seedlings in the glasshouse.

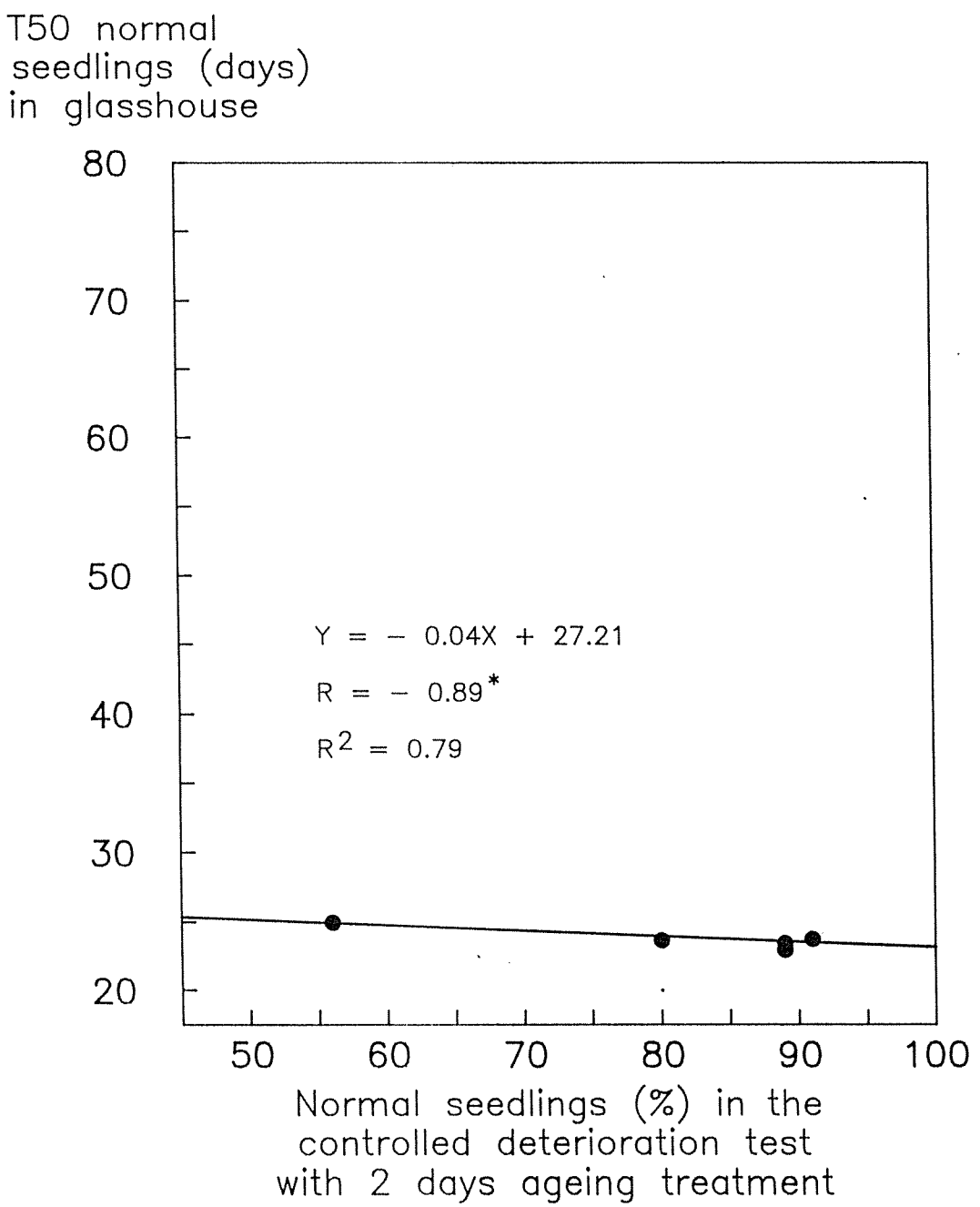


Figure 4.12
Normal seedlings from the controlled deterioration test with 2 days ageing treatment vs surviving normal seedlings in the field.

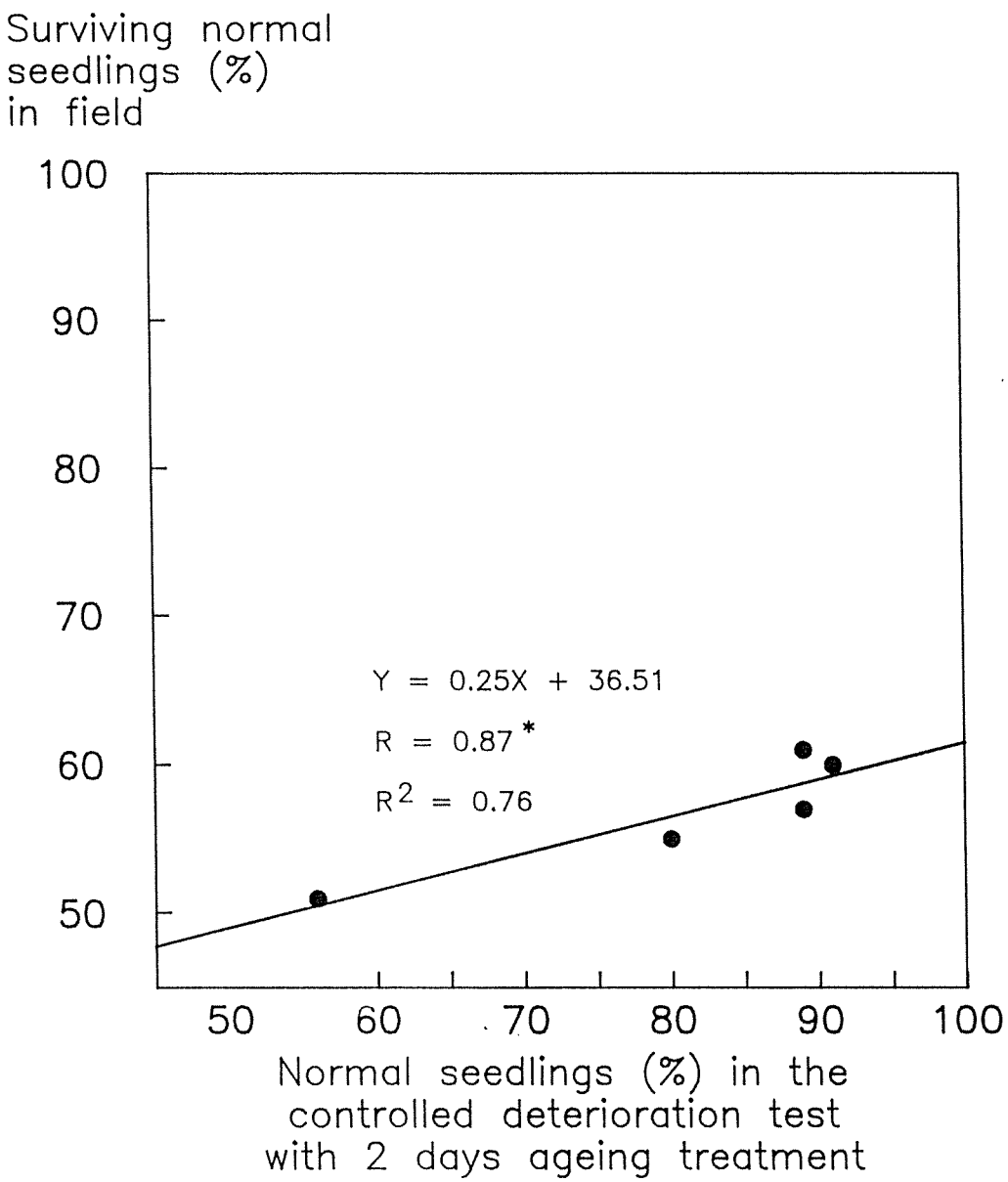


Figure 4.13
Normal seedlings from the controlled deterioration test with 2 days ageing treatment vs T50 normal seedlings in the field.

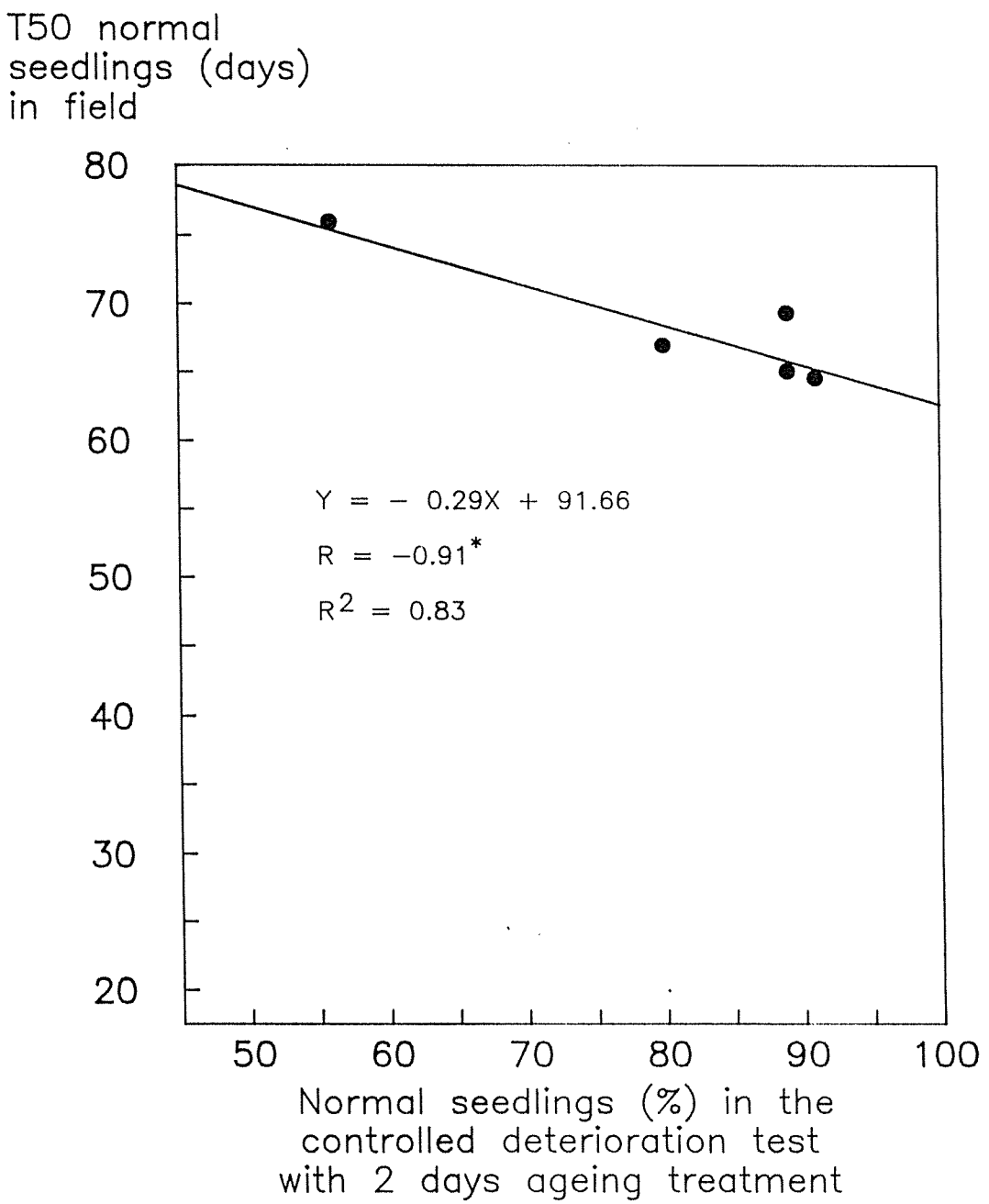


Figure 4.14
Normal seedlings from the complex stressing vigour test vs surviving normal seedlings in the field.

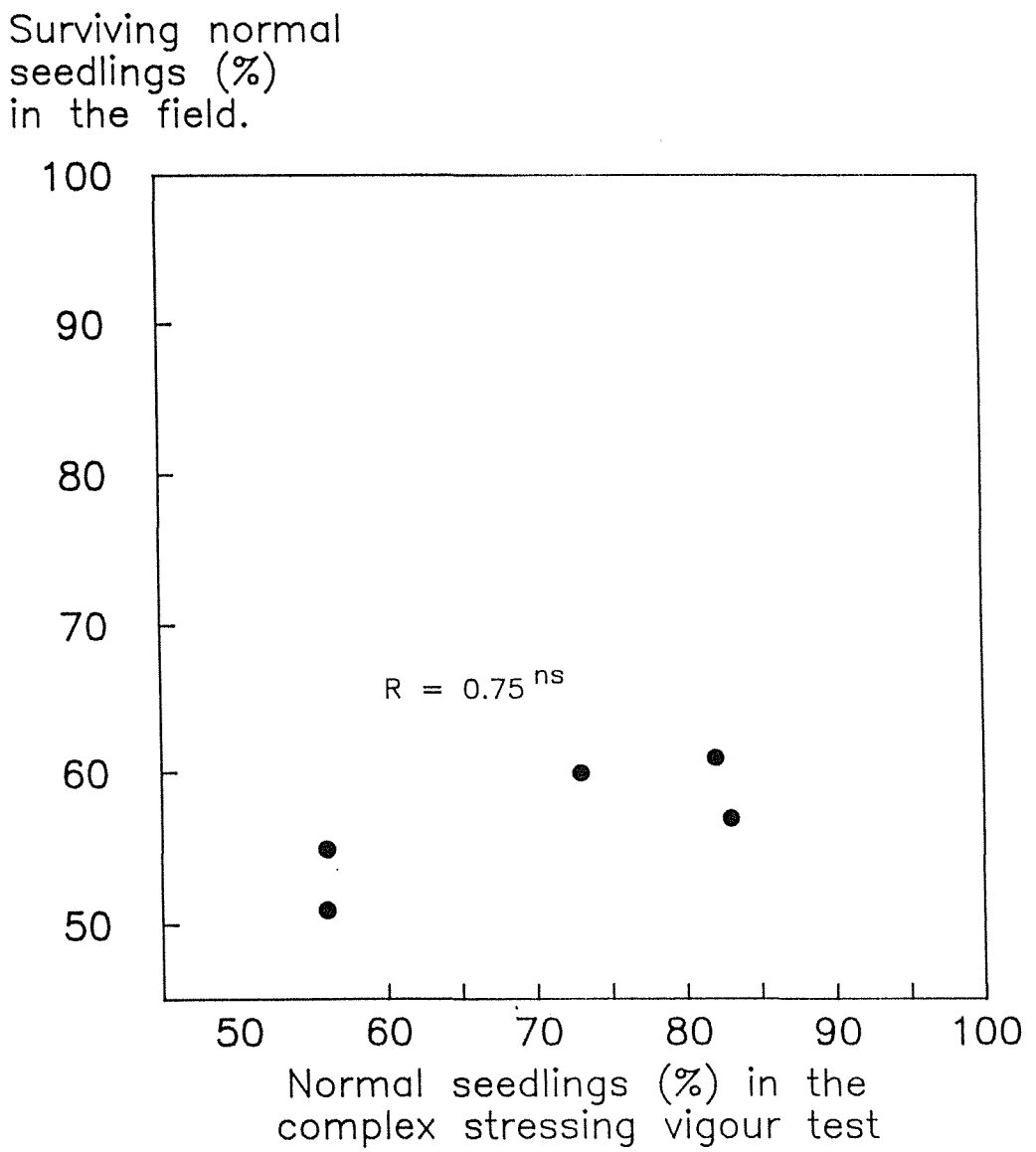


Figure 4.15
Radicle emergence in 10°C, 0 bar vs surviving normal seedlings in the field.

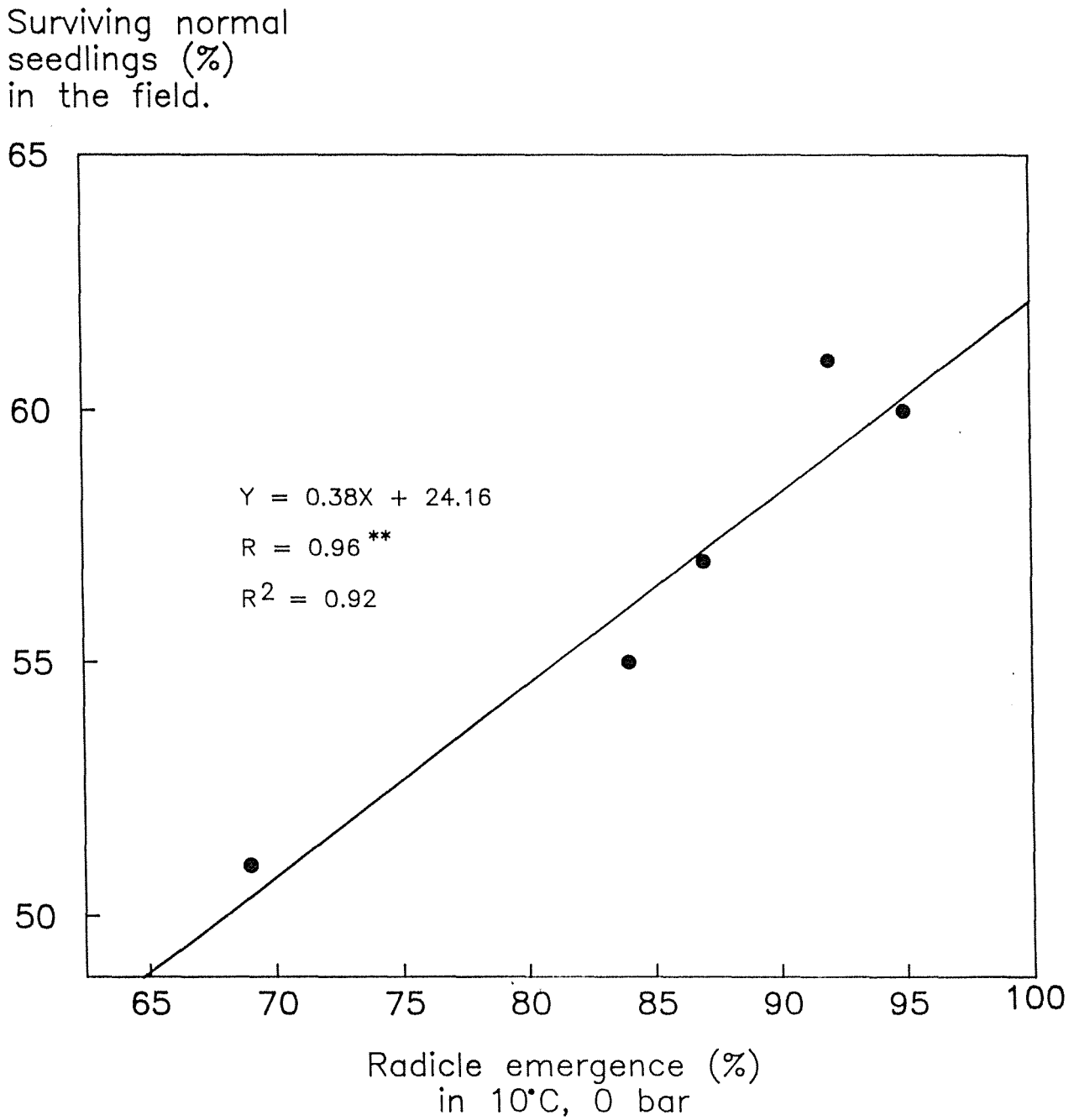


Figure 4.16
Radicle emergence in 15°C, 0 bar vs surviving normal seedlings in the field.

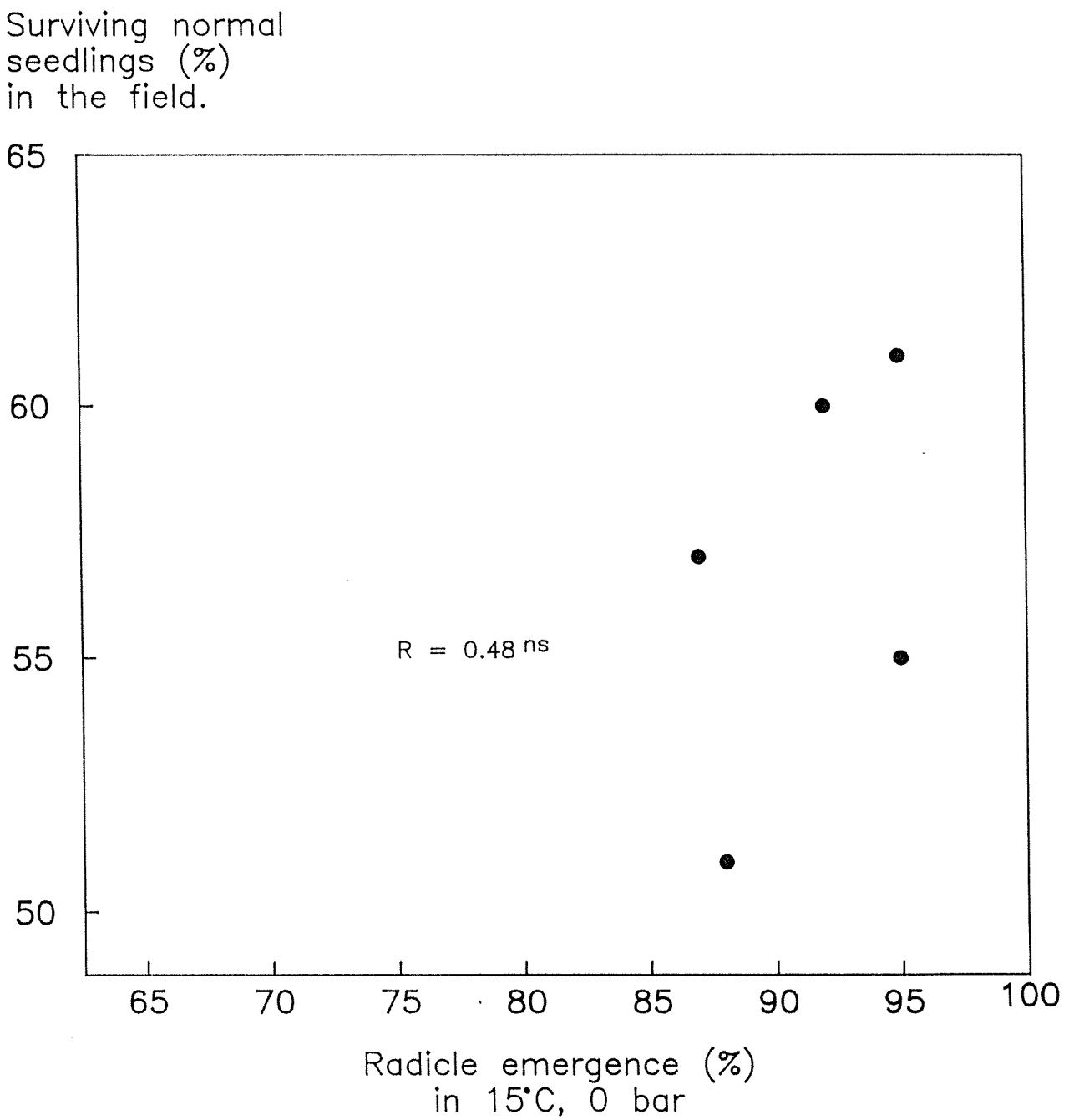
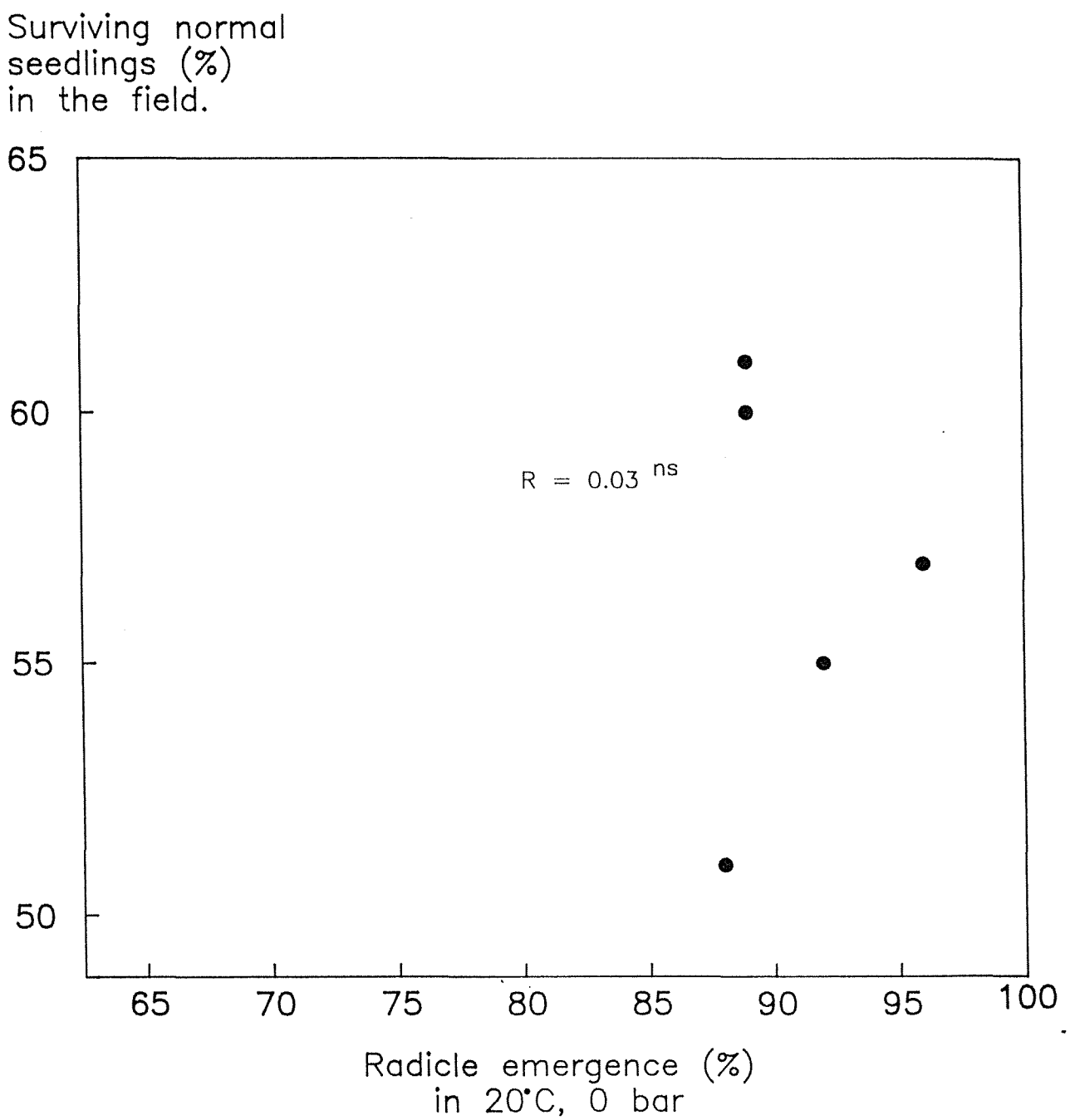


Figure 4.17
Radicle emergence in 20°C, 0 bar vs surviving normal seedlings in the field.



0 bar or 20°C, 0 bar which only gave correlation coefficients respectively 0.48^{ns} and 0.03^{ns} with surviving normal seedling in the field (Figs 4.16 and 4.17). Not surprisingly, the 10°C, 0 bar test did not give a significant correlation with the glasshouse test result.

4.3 Vigour evaluation test in *P. radiata* seeds using 16 seed lots which varied according to type of mother tree and collection date

4.3.1 Seed lot performance in the standard germination and nursery tests carried out by FRI

Appendices 8 and 9 show seed lot performance and seed lot ranking according to relative vigour score. Within groups, the best performing seed lots are quite similar in these 2 tests except for lots 7 and 14 (Appendix 10). Lot 7 (within group 2) produced the highest performance in the standard germination tests, but it dropped into the lowest performance in the nursery test. In contrast, lot 14 (within group 3B) had low performance in the standard germination test (with 45% germination), but it rose markedly in the nursery test and reached the highest performance level with field germination 82.8% (Appendix 9).

Overall, the best performance in standard germination test was produced by lots 6 and 7 (Appendix 10), but in the nursery test was produced by lot 16. The lowest performance in these two tests was produced by the same seed lots, i.e. lot 12.

An important point to be noticed is that percentage germination in the laboratory were generally lower than germination in the field, except for lots 5, 6 and 7. As

shown in Appendix 8, laboratory germination of lots 9-14, for example, are about 34-55%, whereas those in the nursery are about 67.4-82.8%.

4.3.2 Seed weight

Table 4.1 shows the mean of 100 seed weight of each seed lot at moisture content 7.69%. Data in the table revealed that within group, except for group 4 where lot 15 showed lower weight than lot 16, generally there was no significant difference among seed lots. This might be caused by great variation among clones within the same lots. Great variation can be seen, for example, in lot 9 which has minimum and maximum weight 2.109 and 5.102 gram respectively. Other examples in seed lots 13-16 can be seen in Figure 4.18.

Overall, the heaviest seed were produced by lot 6 (3.521 gram) whereas the lightest were produced by lot 15 (2.410 gram).

4.3.3 Seed lot performance in the vigour tests done at Seed Technology Centre

Appendix 12 shows seed lot performance in three vigour tests, namely seedling growth test with prechilling treatment (SG+pr test), controlled deterioration test with 2 days aging treatment (CD2d test), and complex stressing vigour test (CSV test), whereas Appendix 13 shows seed lot ranking according to relative vigour score (rvs).

Within groups, the best performing seed lots are not always the same for different vigour tests, except for group 4 where lot 16 is the best performing seed lot indicated by all three vigour test types (Appendix 14). For groups 1 and 2, for example, CD2d and CSV tests produced the same seed lots as the best

Table 4.1 100 seed weight data for the different seed lots (adjusted to seed moisture content 7.69%). Figures with the same letters are not significantly different according to $lsd_{0.05}$.

Group[1]	Seed lots	100 seed weight at m.c. 7.69% (gram)	Standard error	range of seed weight	
				minimum	maximum
1	1	2.957^{abc}	0.122	2.445	3.505
	2	3.088^{ab}	0.117	2.558	3.832
2	3	3.139^{ab}	0.146	2.467	4.145
	4	3.127^{ab}	0.202	2.447	4.214
	5	3.094^{ab}	0.167	2.436	3.902
	6	3.521^{a}	0.211	2.455	4.920
	7	3.134^{ab}	0.217	1.808	3.854
	8	2.951^{abc}	0.198	1.915	3.702
3 A	9	3.133^{ab}	0.283	2.109	5.102
	10	2.939^{abc}	0.264	1.999	4.488
	11	2.890^{bc}	0.301	1.804	4.629
B	12	2.936^{abc}	0.225	2.004	3.853
	13	2.955^{abc}	0.283	1.623	4.289
	14	3.089^{ab}	0.261	1.863	4.236
4	15	2.410^{c}	0.123	1.931	3.018
	16	3.017^{ab}	0.147	2.241	3.716

$lsd_{0.05}$ = 0.5868
(due to unequal cell sizes, harmonic mean of cell sizes = 0.50495 is used for computing lsd values)

[1] group description can be seen in Table 3.1.

Figure 4.18.A
100 seed weight of Pinus radiata clones of lot 13 at moisture content 7.69%

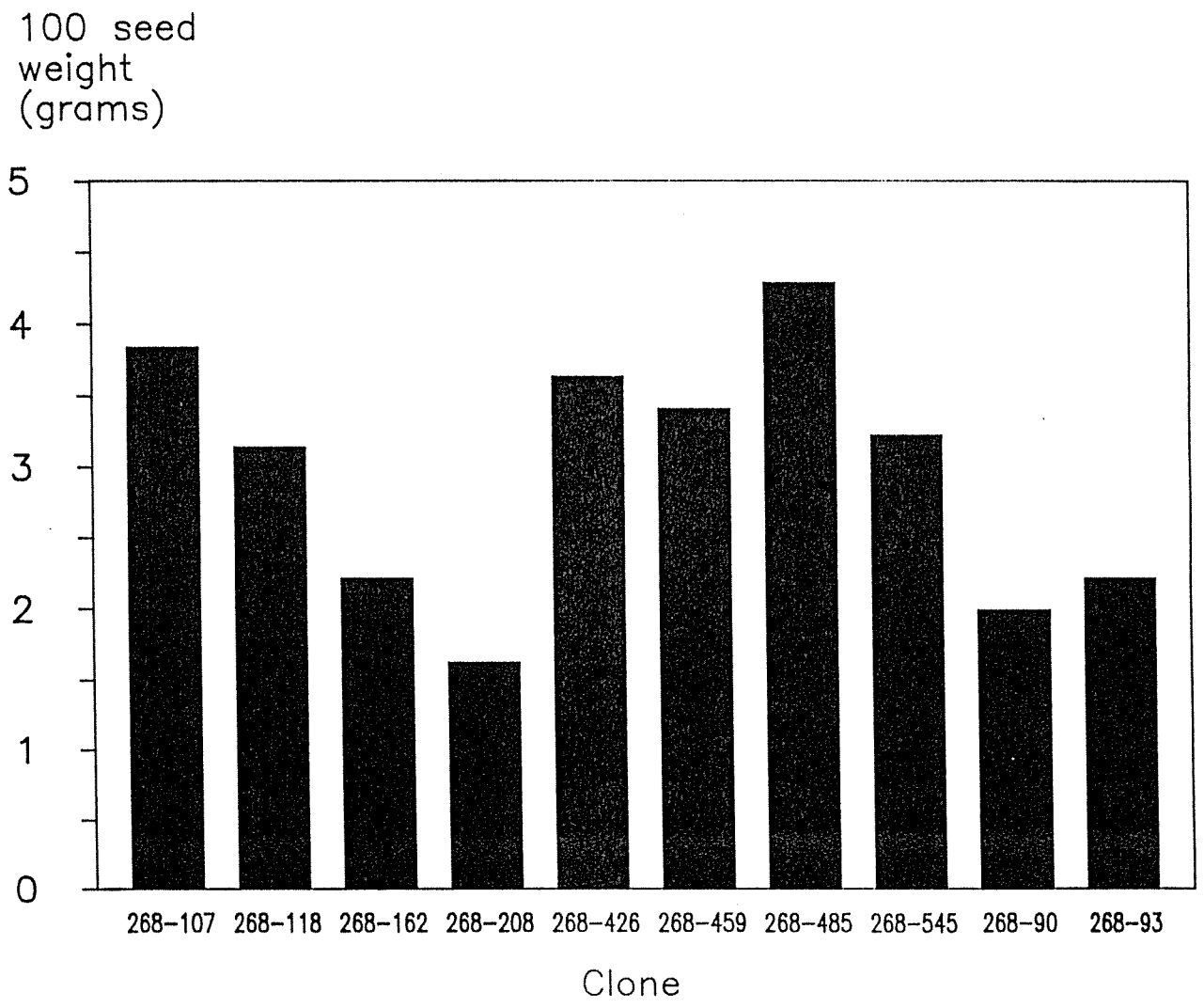


Figure 4.18.B
100 seed weight of Pinus radiata clones of lot 14 at moisture content 7.69%

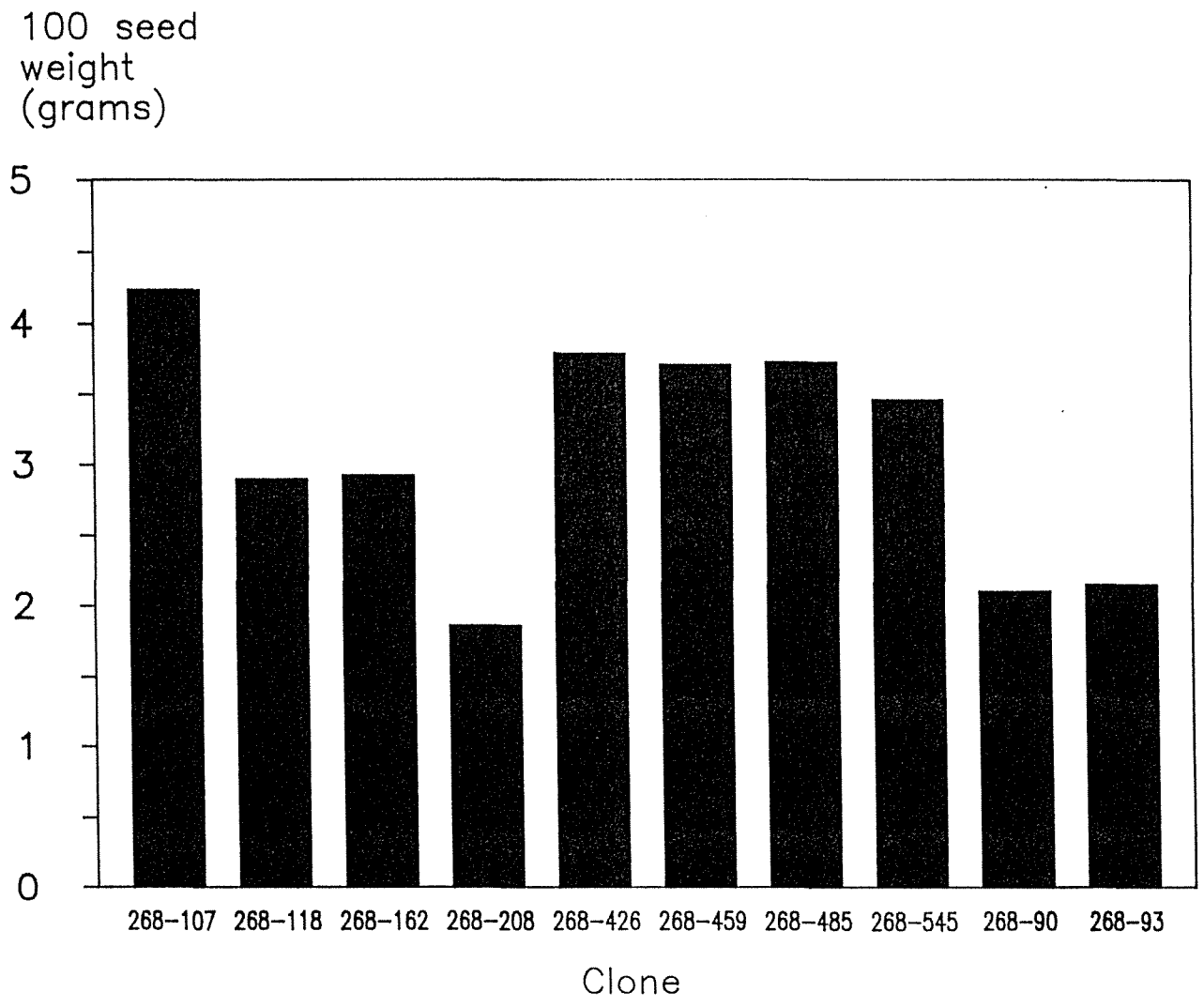


Figure 4.18.C
100 seed weight of Pinus radiata clones of lot 15 at moisture content 7.69%.

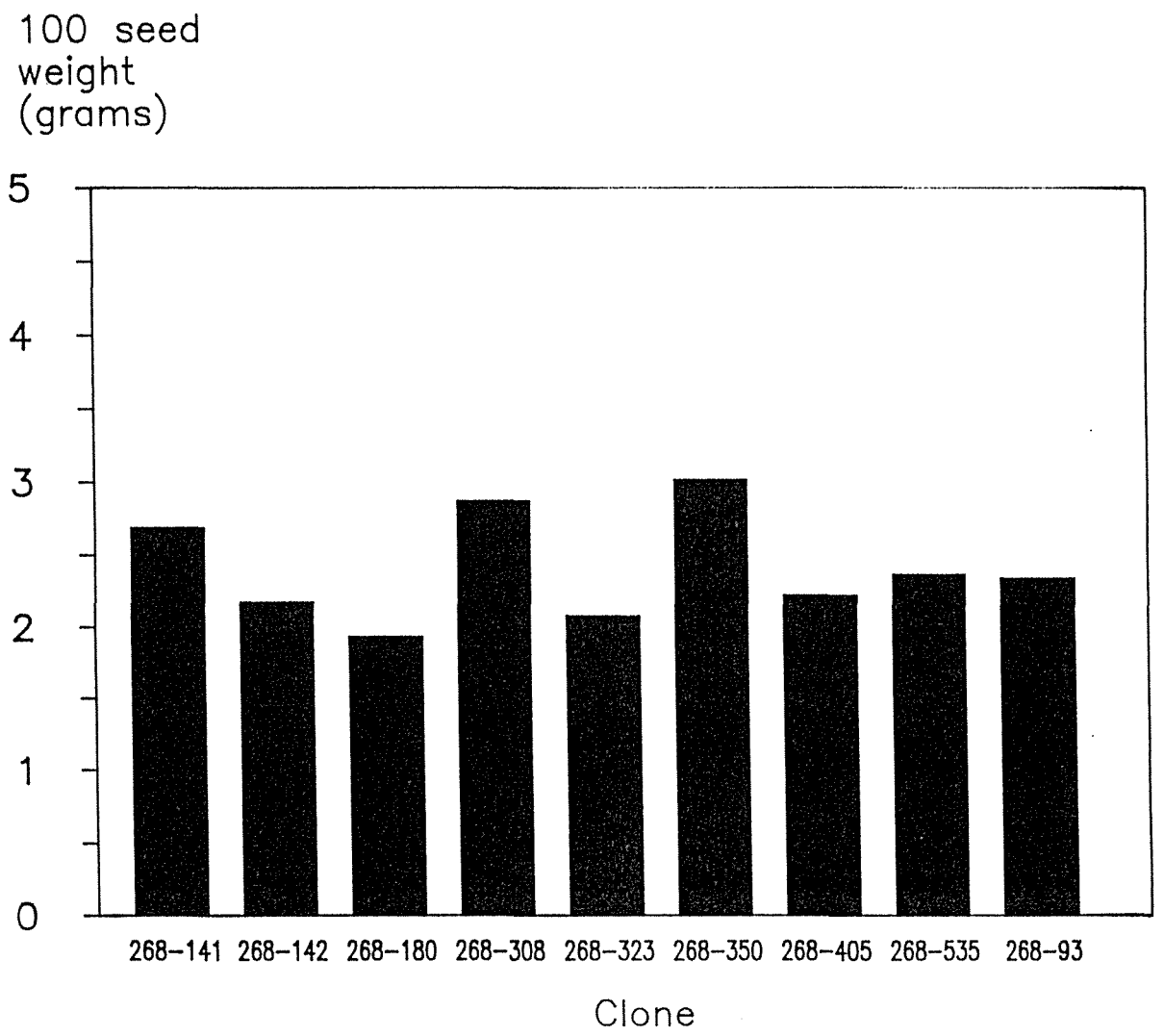
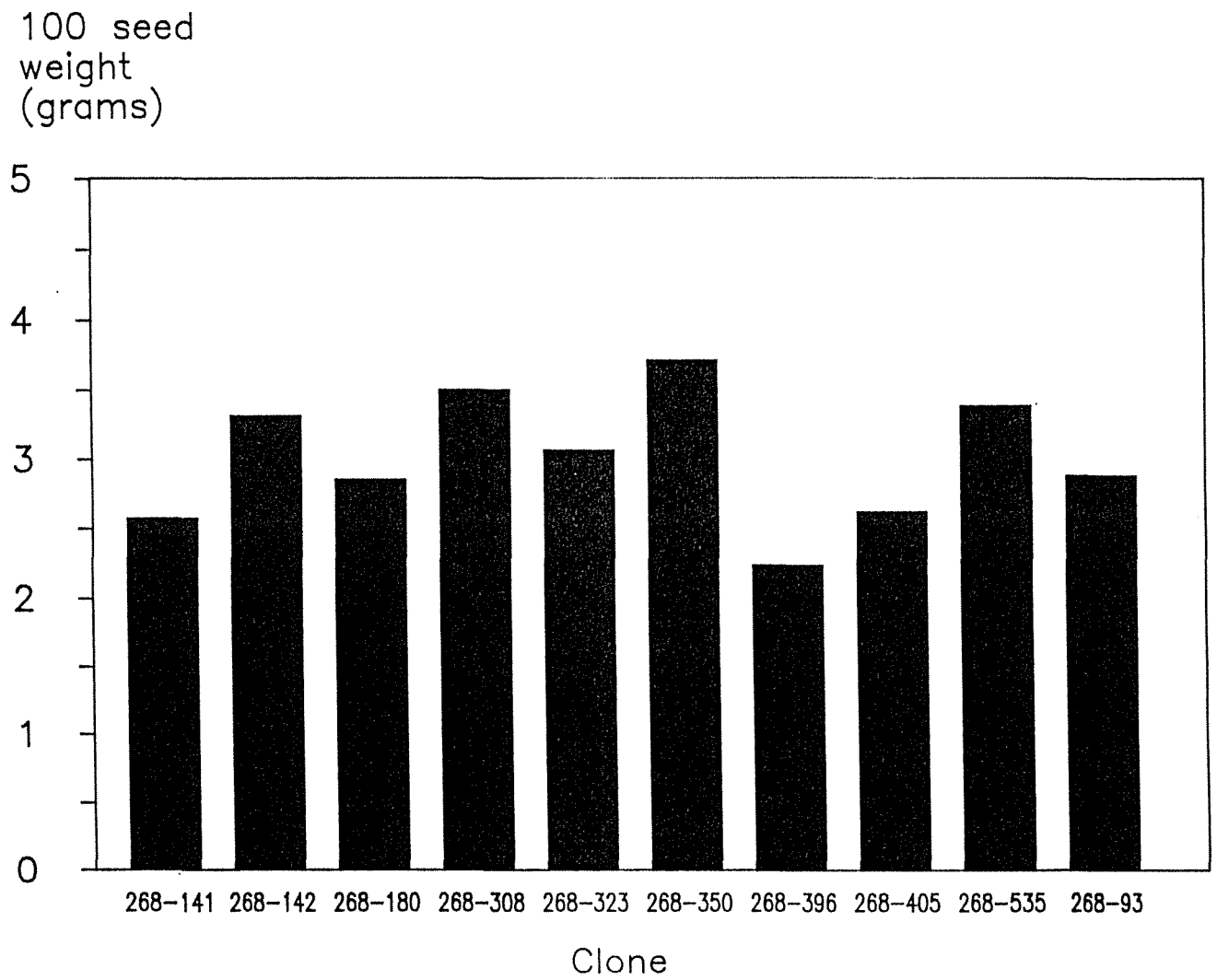


Figure 4.18.D
100 seed weight of Pinus radiata clones of lot 16 at moisture content 7.69%.



performance. While for group 3A, similar results are shown by SG+pr and CD2d tests.

In the overall seed lots (Appendix 15), lot 12 produced the lowest performance in all of the vigour tests. And lots 3 and 4 produced the best performance in SG+pr test. In CD2d and CSV tests, however, the best performance was shown by lots 6 and 16.

4.3.4 Correlation of seed weight, standard germination, and vigour tests with FRI nursery test

Appendix 17 shows correlations between seed weight, standard germination, and the different vigour tests with the nursery test results.

4.3.4.1 Correlation between seed weight and nursery test

Data in Appendix 17 show that seed weight gave significant correlation with seedling height and seedling diameter, but the r value with seedling height (0.82****) was higher than that with seedling diameter (0.56*). In relation to seedling height, seed weight had a regression equation of Y = 10.12X with a coefficient determination (R^2) of 67%, whereas that to seedling diameter had a regression equation of Y = 0.57X + 4.55 with a coefficient of determination only 31% (Figs 4.19 and 4.20).

Another important point to be noticed was that seed weight did not correlate with either shoot or root dry weight (Appendix 17).

Figure 4.19
Seed weight vs seedling height in the nursery

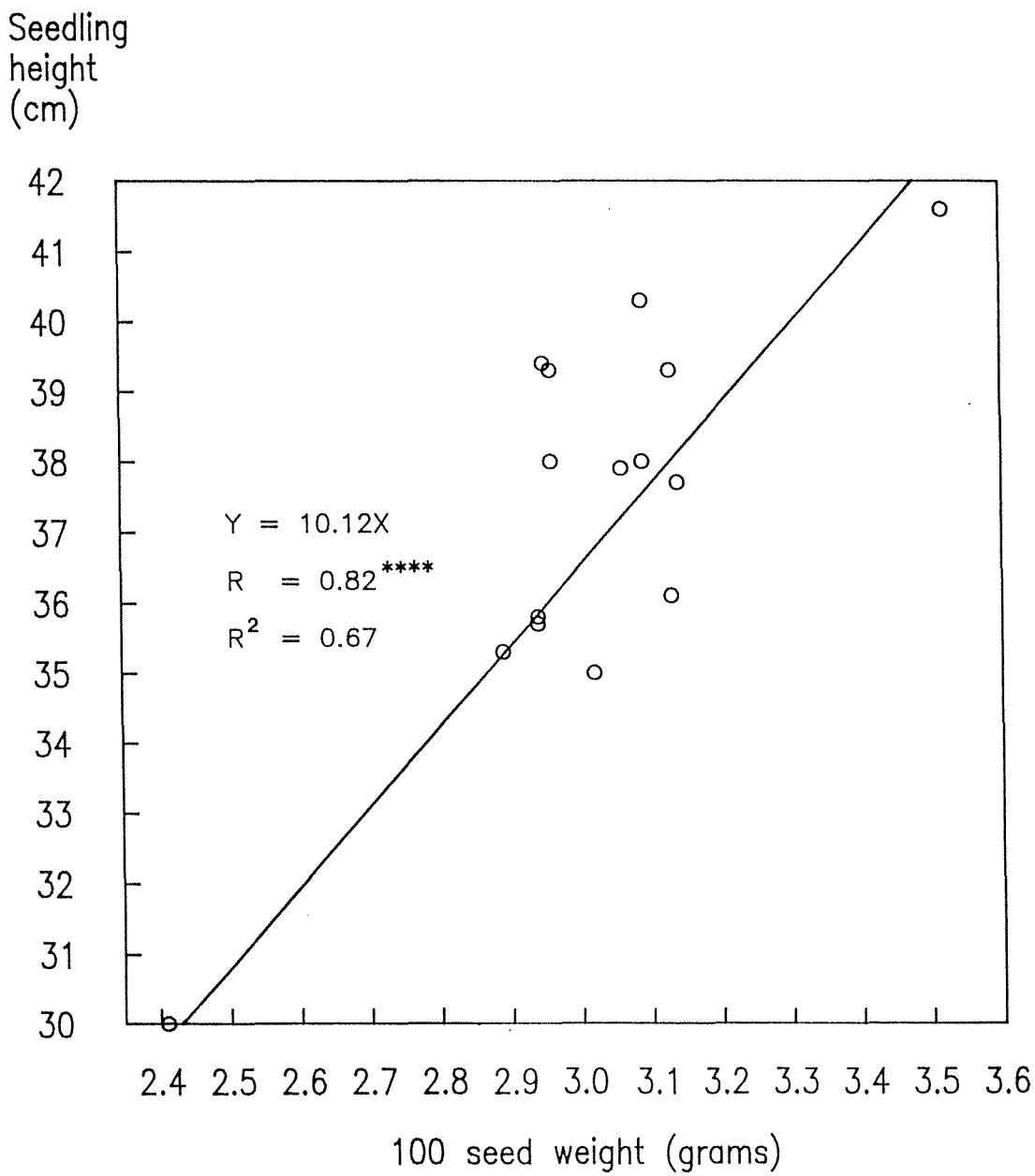
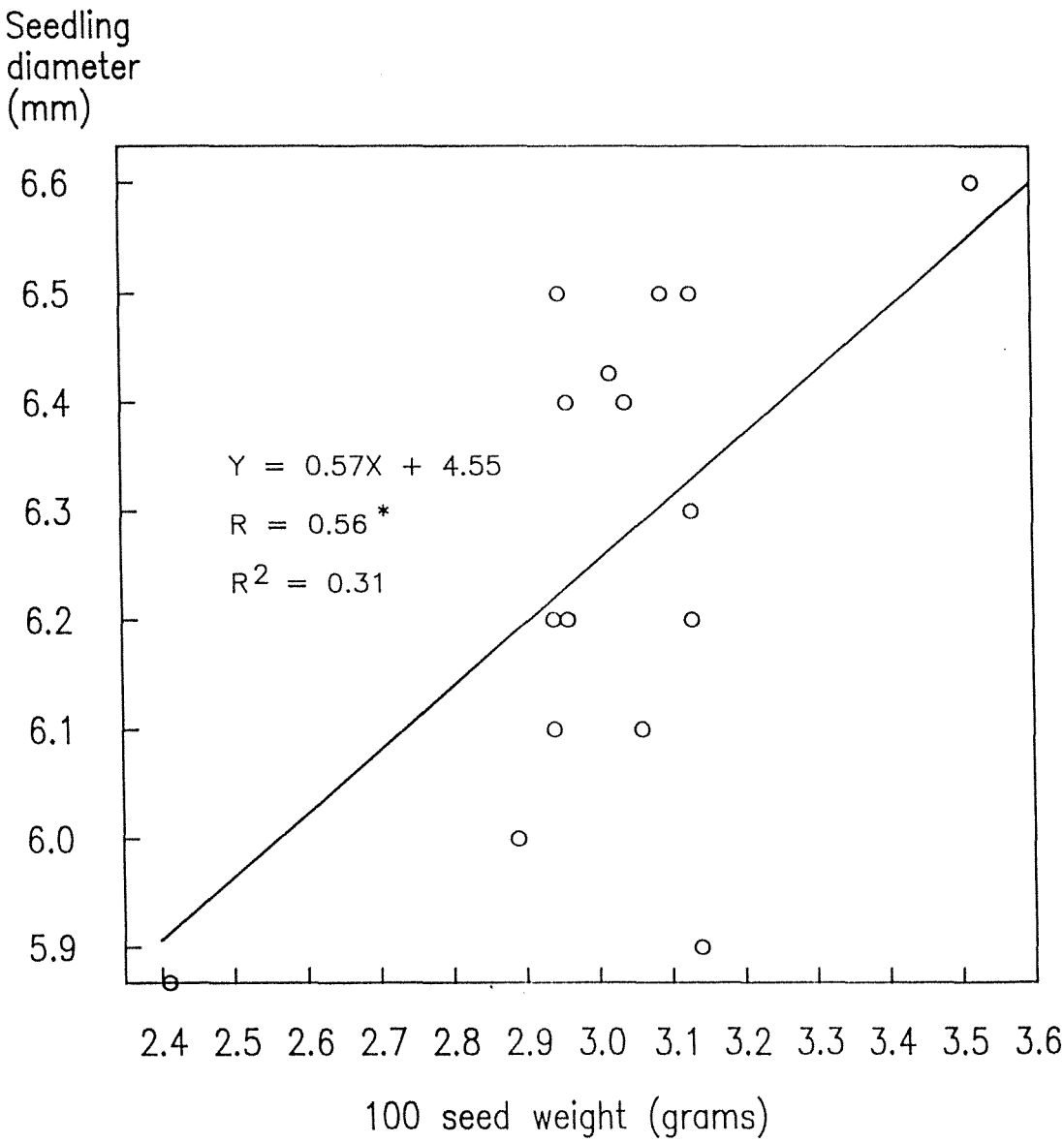


Figure 4.20
Seed weight vs seedling diameter in the nursery



4.3.4.2 Correlation between standard germination test (by FRI) and the nursery test

The standard germination test which accidentally faced watering problems (Appendix 2) and generally produced lower germination percentages than that in the nursery (section 4.3.1) quite surprisingly gave high significant correlation (r = 0.84****) with % plantable seedlings (Appendix 17). This r value was higher than those in other vigour tests. As shown in Appendix 17, % normal seedling measurement in SG+pr, CD2d, and CSV test gave r values of 0.45^{ns}, 0.78*** and 0.76***, respectively.

Another important point was that this test gave a highly significant correlation with another four parameters of nursery test, namely % field germination (0.80***), T_{50} field germination (-0.76***), % healthy seedling at 38 das (0.93***) and % healthy seedling at 66 das (0.87****).

4.3.4.3 Correlation between vigour tests and nursery test

Data in Appendix 19 indicate that generally all of the vigour tests gave good correlation with some parameters of nursery test, even though there was variation in coefficient of correlation (r) value. However, the CD2d test seems to have the best correlation with the nursery test since its parameters give the highest number of significant r values. As shown in the Appendix, this test gave 25 significant r values, compared to 16 in the SG+pr test and 24 in the CSV test.

In relation to percentage of plantable seedlings in the nursery, radicle emergence and normal seedling percentage in CD2d test gave regression equation of Y = 0.73X and Y = 0.70X respectively (Figs 4.21 and 4.22). Data in the figures show that coefficient of determination (R^2) of number of normal seedlings (61%) is higher than that of radicle emergence (55%).

Figure 4.21
Radicle emergence from the controlled deterioration test with 2 days ageing treatment vs plantable seedlings in the nursery.

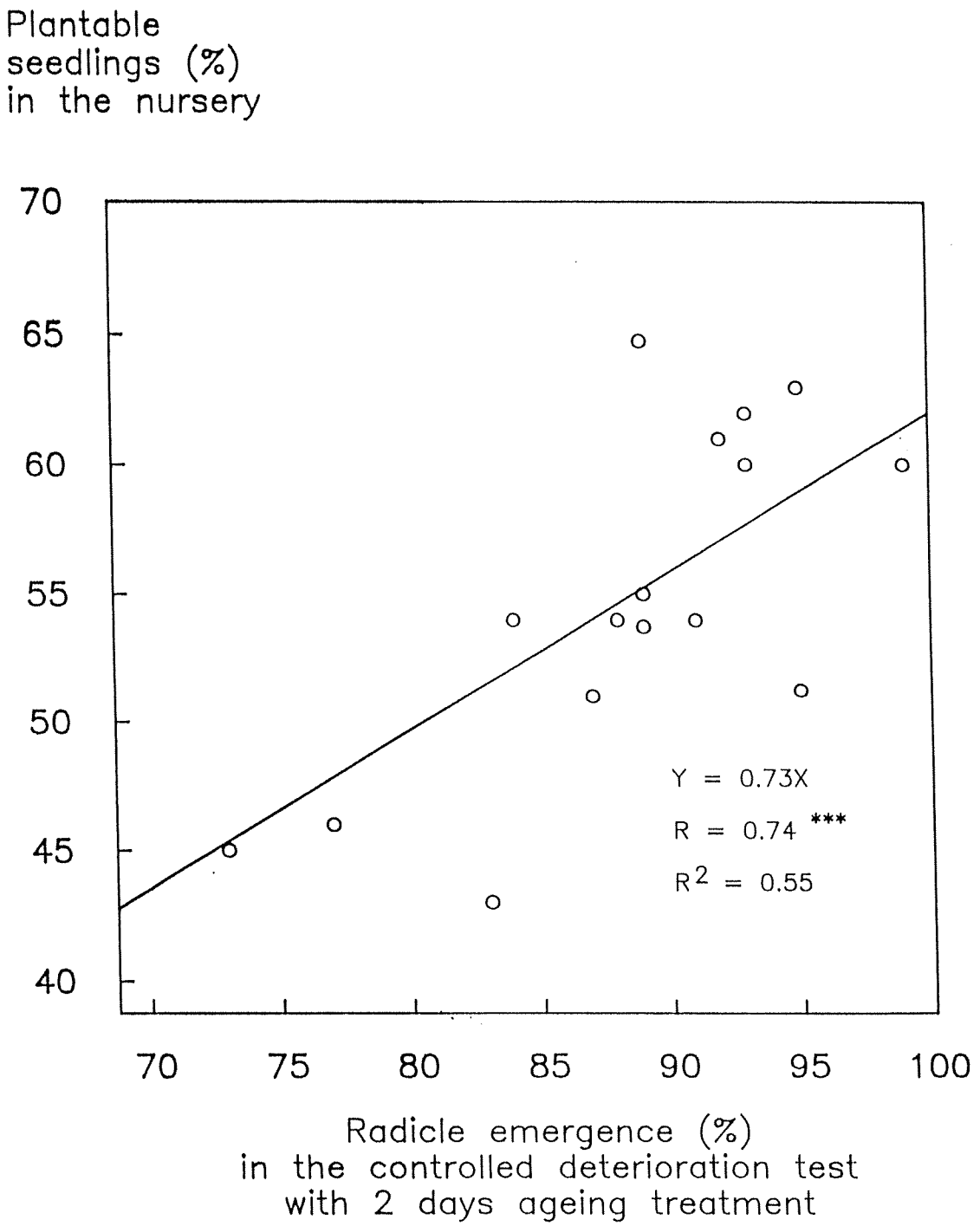
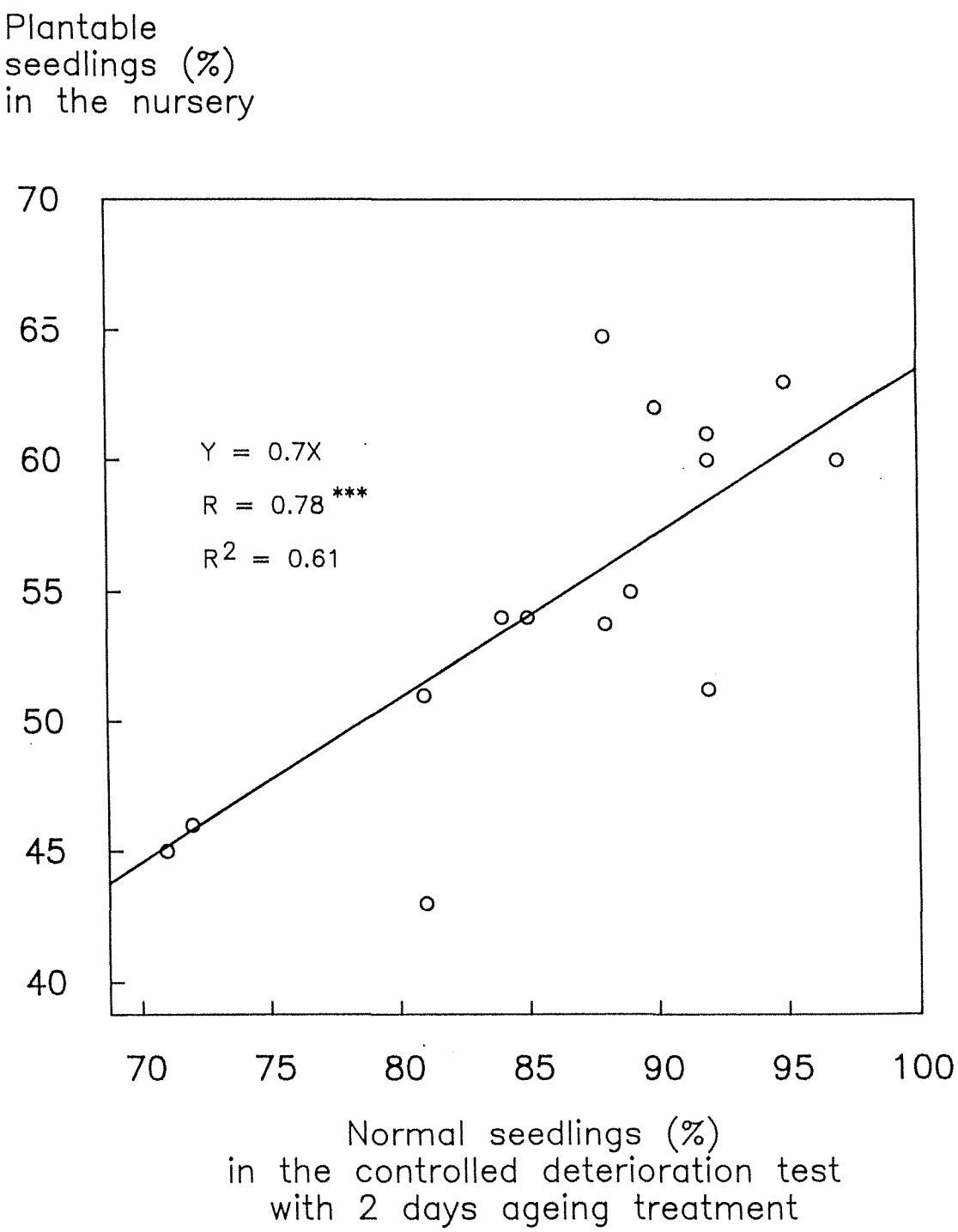


Figure 4.22
Normal seedlings from the controlled deterioration test with 2 days ageing treatment vs plantable seedlings in the nursery.



Measurement of median germination time after CD2d test also gave quite good correlation with T_{50} germination in nursery. As shown in Figures 4.23 and 4.24, T_{50} radicle emergence and T_{50} normal seedling in this test produced regression equations of Y = 1.31X + 7.07 and Y = 1.82X - 16.06 with coefficient of determinations 67 and 64% respectively.

An important point to be noticed was that median germination time measurement in SG+pr test generally produced the highest r value compared to those in CD2d and CSV tests. As shown in Appendix 17, absolute r values of median germination time in SG+pr test were in the range of 0.77-0.85, compared to 0.51-0.82 in CD2d test, and 0.65-0.79 in CSV test.

Another important thing was that normal seedling dry weight measurements in all of the vigour tests were poorly correlated with plantable seedling and seedling dryweight in the nursery. As shown in Appendix 17, absolute r values of these correlations were in the range of $0.25\text{-}0.43^{ns}$ in SG+pr test, $0.05\text{-}0.36^{ns}$ in CD2d test, and $0.01\text{-}0.30^{ns}$ in CSV test. However, the normal seedling dry weight parameter in SG+pr test gave significant correlation (0.78***) with seedling height in the nursery. In the CD2d test it also correlated significantly with seedling height (0.51*) and seedling diameter (0.67**) in the nursery.

Figure 4.23
T50 radicle emergence in the controlled deterioration test with 2 days ageing treatment vs t50 germination in the nursery

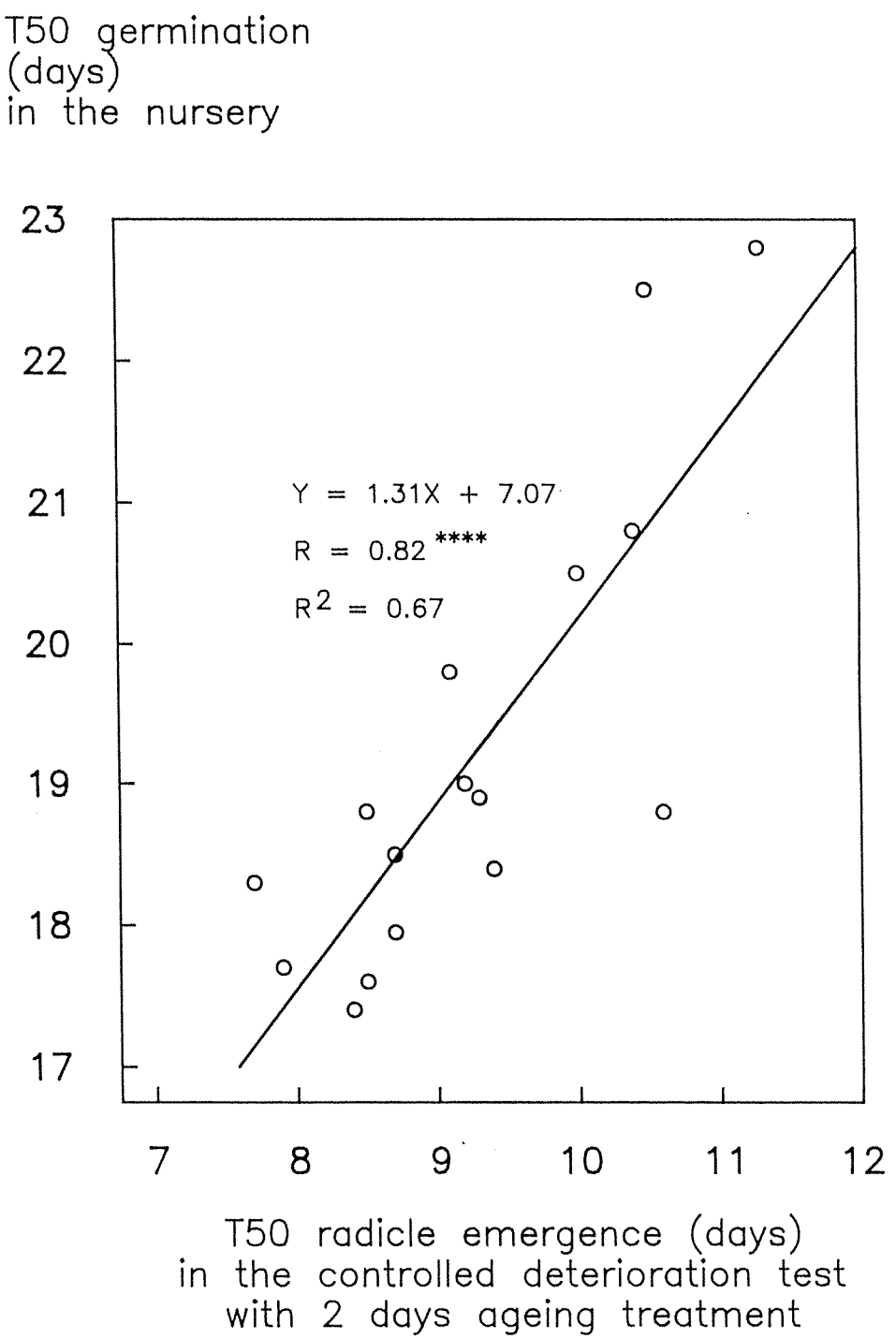
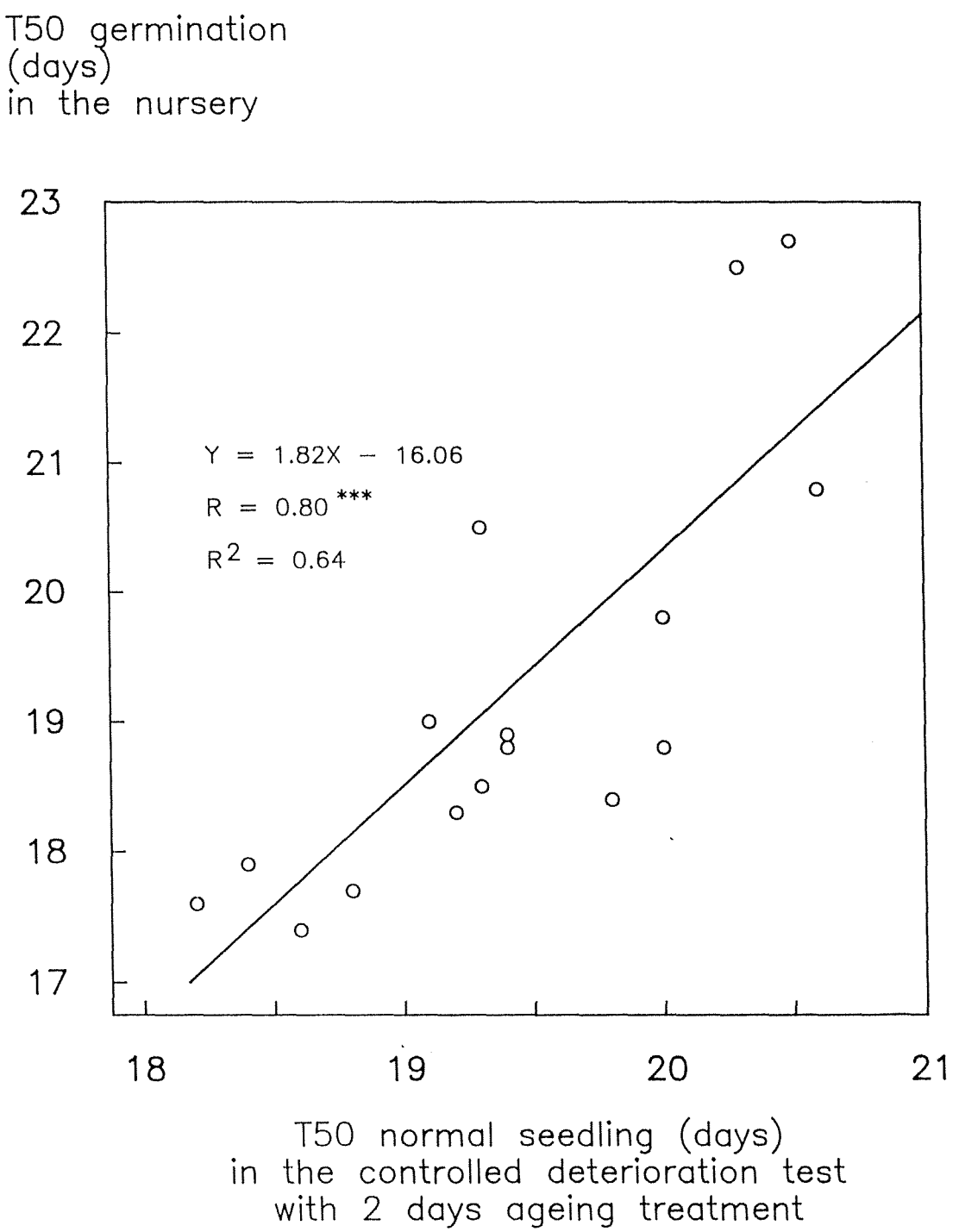


Figure 4.24
T50 normal seedling in the controlled deterioration test with 2 days ageing treatment vs t50 germination in the nursery



V. DISCUSSION

5.1. Deterioration in *P. radiata* seeds

5.1.1. The pattern of deterioration

The results of these experiments indicate that the conditions of 40°C, 100% RH did not generally reduce viability after treatment for up to 14 days, but that vigour was reduced from 8 days onward. Conditions of 45°C, 100% RH, however, had a greater effect: vigour being reduced after 6 days. The detrimental effect of high temperature and relative humidity on viability and vigour of *P. radiata* seeds follow the general pattern for orthodox seeds. For this kind of seed, the higher the temperature and relative humidity of the environment (and thus seed moisture content), the faster the rate of deterioration. Harrington (1959, cited in Harrington, 1972) devised two "rules of thumb" for orthodox seeds: (a) each 5°C increase in seed temperature (applied between at least 0 and 50°C) halves the storage life of the seeds, and (b) for each 1% increase in seed moisture (between 5 and 14%), the storage life of the seeds is also halved.

The fall in vigour (measured here by increasing T_{50} radicle emergence or T_{50} normal seedlings) which preceded the fall in viability (compare Figures 4.1.C and D to Figures 4.1.A and B) has a similarity to the general pattern of deterioration suggested by Delouche and Caldwell (1960). They showed that vigour will tend to reduce steeply before there is any great reduction of viability. In the initial stages of deterioration, differences between vigour and viability scores for a range of seed lots may not be very pronounced, but at later stages the difference becomes large.

One important point to be noticed in the vigour changes is that T_{50} radicle emergence seems to be more sensitive to aging treatment than T_{50} normal seedling especially at 45°C. As shown in Figure 4.1.D, the change of T_{50} radicle emergence was initially detected after 6 days aging whereas that of T_{50} normal seedling was after 8 days. At 40°C, however, changes in both parameters were detected at the same time (i.e. after 8 days).

Ching and Ching (1972) reported that in *Pinus ponderosa* gametophytes there was a rapid increase of ATP, and total adenosine phosphate (TAP) at the radicle emergence stage (after 5 days imbibition at 15-25°C) and this continued up to 9 days imbibition when the cotyledons emerged. ATP levels then rapidly decreased and were close to zero at 18 days. Based on this study, it is suggested that the more sensitive changes in the early growth in *P. radiata* (i.e. T_{50} radicle emergence) may be related to disruption of metabolic activity in the period when there was a rapid increase in ATP production. In the later stages, when ATP production decreases, the sensitivity of the growth rate parameters also decreases.

Another important point is that there was greater variation of viability results (among experiments a, b and c) in the later stages of aging than in the earlier ones despite the fact that they had good reproducibility for the initial time when the vigour or viability started to decrease (see also section 4.1, and, for example, Figures 4.1.A and B).

5.1.2. <u>The role of high temperature and relative humidity</u>

The role of high temperature and relative humidity in speeding seed deterioration, is suggested to have two kinds of effects in this case: firstly, affecting seed tissue directly and, secondly, it might have indirect effects by stimulating microbial

growth, especially storage fungi. This suggestion is based on the fact that there was no surface sterilisation applied before aging treatment, although the conditions of 40°C or 45°C and 100% RH are most likely to be conducive for fungal growth.

An important point to be noticed was that different fungal species grow at different temperatures. At 40°C (100% RH) the species which might grow include *Aspergillus restrictus*, *A. glaucus*, *A. candidus*, and *A. flavus*, and *Penicillium*, whereas at 45°C (100% RH) *Penicillium* is unlikely to develop as its maximum growth temperature is 35-40°C (Christensen and Kaufmann, 1974). The possible mechanisms of seed deterioration and the role of fungal invasion are reviewed in section 2.2.2 and discussed in the following section (5.1.3).

5.1.3. Changes which may be associated with the loss of vigour and viability

Ultrastructural changes which might cause vigour and viability loss in this seed probably involve membrane damage. Evidence of this was gained from another series of experiments (Appendix 6) which indicated that aged *P. radiata* seed tended to have higher conductivity readings than unaged ones after imbibition at 20°C for 24 hours. High conductivity readings in those aged seed indicated the inability of membrane components to become reorganised in a relatively short time. This condition causes solutes to leak out from cells into the imbibition medium. Membrane damage (which is indicated by increased leakage) as a result of aging has also been found in other seeds like soybean (Stewart and Bewley, 1980; Tilden and West, 1985) and pea (Harman and Granett, 1972).

Membrane damage has a great role in vigour and viability as it can cause loss of cellular compartmentalisation. It also can cause failure of mitochondrial activity

which leads to failure of dehydrogenases, transferases, and t RNA synthetases which affected protein synthesis. All these events can finally cause the seed to become non-viable (Osborne, 1980).

5.1.4. Changes in normal seedling dry weight

Except for 14 days aging at 45°C where none of the seeds were germinable, normal seedling dry weight was a parameter which was not greatly reduced by both aging treatments (40 and 45°C under 100% RH), it even rose at 8 and 10 days aging to 45°C. However, it is not suggested that seedling growth was not affected by aging treatment. Data from Figure 4.5D indicated that under field conditions, normal seedling dry weight of *P. radiata* seeds was affected by aging. Normal seedling dry weight is a very important component of seedling vigour, especially in forest establishment, since transplanted seedlings require enough food and water supply to maintain rapid root growth and survive the period when photosynthesis may be reduced or stop temporarily during the transplanting period (seed also section 2.1.1). It appears from these experiments, however, that impairment of seedling growth cannot be detected under optimal germination test conditions.

5.2. Vigour tests evaluation

The objectives of the discussion in this section are:

1. To investigate promising suitable vigour tests for *P. radiata*, their applicability to a wide range of field conditions, and to elucidate factors which may affect their sensitivity and predictivity.

2. To investigate specifically suitable vigour tests for assessing seedling establishment at the FRI nursery, Rotorua.

3. To elucidate the effects of seed coat conditions on germination behaviour and the conductivity test result.

4. To elucidate the relationship between seed weight and seedling performance.

5.2.1. Promising suitable vigour tests for P. radiata

AOSA (1983) describes some criteria for a practical vigour test. These include:

1. sensitivity, which means that the test should consistently rank seed lots in terms of field performance potential,

2. reproducibility of test results,

3. easily interpreted and correlated with emergence under certain field conditions,

4. rapidity,

5. objectivity,

6. simplicity, and

7. it should be economically practical.

Based on the sensitivity of the tests in differentiating quality differences among seed lots (see section 4.2.3-4.2.10) and the predictivity of the test to assess field emergence (especially surviving normal seedlings in the winter field test) which was indicated by the value of the correlation coefficients (see Appendices 6 and 7), it is suggested that some promising suitable vigour test for *P. radiata* include:

1. the controlled deterioration test with 2 days aging treatment,

2. the prechilled seedling growth test,

3. the complex stressing vigour test, and

4. the low temperature germination test at 10°C, -0 bar.

These will be discussed in turn.

5.2.1.1. <u>The controlled deterioration test with 2 days aging (CD2d test)</u>

The ability of aging treatment in the controlled deterioration test to predict seed performance in a field may be related to its ability to differentiate seed lots according to their deterioration level as seed lots from different vigour levels may respond differently under storage at elevated moisture contents and temperature (Matthews, 1980; see also section 2.3.2.3 and Table 2.3).

Section 4.2.7 and Figure 4.5 shows that the CD2d test has quite good sensitivity as it can differentiate seed lot (lot C) from the others, although it could not detect quality differences which were a function of seed weight. In addition, section 4.2.10 and Appendix 7 show that this method has a good predictivity for a wide

range of field conditions as measurements of percentage of normal seedling in this test were correlated highly and significantly with two parameters of the glasshouse test (optimum conditions) and three parameters of the winter field test (sub-optimum conditions). The coefficients of determination (R^2) of regression equations between the percentage of normal seedling in this test and percentage normal seedling or T_{50} normal seedlings in the glasshouse test (Figure 4.10 and 4.11), and between that in this test and surviving normal seedlings or T_{50} normal seedlings in the winter field test (Figures 4.12 and 4.13) were not less than 76%.

The wide applicability of this method was also supported by the results of a further study (see section 4.3.4.3 and Appendix 17) which indicated that this method gave significant correlations with some parameters of the Rotorua nursery test results. For example, measurement of percentage normal seedlings in this method gave an r value of 0.78** (R^2 = 61%) with the percentage of planatable seedlings from the nursery (Figure 4.22).

An important point to be noticed is that this method does not require complicated equipment or procedures and can be done in a relative short time (see section 3.2.2.3 a). Therefore, it indicates that the CD2d test for *P. radiata* meets all AOSA's criteria (see section 5.2.1), although reproducibility still needs further investigation.

The direction for further study would seem to be evaluate the reproducibility of the CD2d test results using the same seed lots within the same laboratory and to evaluate the reproducibility of their correlations with field emergence under a limited range of different conditions. In later stages, the evaluation of reproducibility of the test results between laboratories would also be important.

There appear to be no reports concerning the value of the controlled deterioration (CD) test in *P. radiata* seed or other tree species. However, Matthews (1980) reported that CD tests were usually reproducible in the laboratory and gave highly significant correlations with field emergence for a range of crops. However, this method faced a reproducibility problem in some species, e.g. swede where a 1978 sowing test results only gave an r value of 0.18^{ns}, despite having an r = 0.89*** in the previous year's sowing.

The suitability of the aging conditions seems to be crucial for the sensitivity, predictivity, and generalisability of the controlled deterioration test. The results of this study indicated that a combination of about 20% moisture content at 45°C was suitable for predicting seed emergence in the glasshouse and winter field tests, if it was applied for 2 days. Four days application, however, seemed to be too severe as it produced very low germination for deteriorated seed lots (Figure 4.5).

5.2.1.2. The prechilled seedling growth test (SG+pr test)

Section 4.2.3 indicated that the SG+pr test is a sensitive one as it could differentiate quality differences due to aging (Figures 4.4.A, B and C) and also seed weight factors (Figure 4.4.E). In addition, section 4.2.10 and Appendix 7A show that this method also has a good predictivity and is applicable to a wide range of field conditions. Parameters of this test gave high and significant correlation with some of the results of the glasshouse test (optimum conditions) and the winter field test (sub-optimum conditions). For example, radicle emergence measurement in this test could predict seedling survival in the winter field test (Figure 4.8) with a coefficient of determination of, R^2 = 94%. Furthermore, parameters of the test also gave a significant correlation with some results of the Rotorua nursery test (section 4.3.4.3 and Appendix 17). The possibilities for wider

application of this method in standard seed testing laboratories is quite promising, meeting most of the criteria discussed previously.

An important point to be noticed is that the selection of suitable germination parameters to be measured seems to have a significant role in the predictivity of SG+pr test. Results of the previous study for the glasshouse or the winter field tests (Appendix 7) indicated that % radicle emergence and % normal seedling were the most reliable parameters as they gave the best r value with the glasshouse and the winter field test. In the later FRI Nursery study where the seeds had been given a pre-sowing treatment (seed soaking in water for 48 hours at 10°C), however, the most reliable parameters were shown to be T_{50} values for radicle emergence and normal seedlings (Appendix 17).

An hypothesis to explain the role of this pre-sowing treatment in affecting the suitability of the SG+pr test parameters may be that 48 h soaking enhances the germination speed of highly vigorous seeds but causes a stress effect on low vigour seeds. This would lead to the production of a gap in germination speed between high and low vigour seeds. As a result, the T_{50} measurements become the most predictive parameters.

The superiority of the seedling growth test with prechilling (SG+pr) compared to that without prechilling (SG-pr) in predicting seedling performance in the field (see Appendix 7 and compare Figures 2.8 and 4.9) might be related to the similarity of low temperature exposure in the SG+pr test and field conditions. In the SG+pr test, low temperature was imposed during prechilling treatment for 7 days at 5°C prior to germination at 20°C, whereas in the winter field test the mean daily temperature was 10.3°C, and the mean daily minimum and maximum

temperatures were 4.7 and 15.9°C respectively (calculated from data in Appendix 4A). The similarity in low temperature exposure might also have an important role in producing the significant correlations between the SG+pr test and the data from the Rotorua nursery test (Appendix 17). Weather observations (Appendix 18) indicated that in June, July and August the mean minimum temperatures were quite low, i.e. 5.3, 4.8 and 4.8°C respectively. In addition, there were some air and ground frosts between April and August.

There are not many reports concerning correlations (with field emergence) or reproducibility of this type of test in *P. radiata* or other tree species. However, Bonner (1974) reported that measurement of total seedling fresh weight in a seedling growth test (without a prechilling treatment) in cherrybark oak acorns (*Quercus falcata* var. pagodaefolia) gave quite a highly significant correlation with other vigour indices, i.e. peak value (r = 833*) and germination value (r = 859*). Rimbawanto *et al* (1988; 1989) reported that T_{50} radicle emergence measurement in seedling growth tests with or without prechilling treatment could differentiate quality differences due to collection date and artificial ripening of *P. radiata*. Bergsten (1988) also reported that T_{50} radicle emergence measurements (without prechilling) could differentiate quality due to invigoration treatment in *P. sylverstris* seeds. However, there was no information concerning its correlation with other vigour indices nor with field emergence. A summary of seedling growth tests and their varying results in other species can be seen in Table 2.2.

Apart from its effect in enhancing germination speed, low temperature exposure prior to the germination period (prechilling treatment) in the SG+pr test also has a detrimental effect on certain aged seed lots (see section 4.2.3, Figures 4.4.A, B

and C). In *Tsuga heterophylla* and *Pseudotsuga menziesii*, Allen (1958a; 1958b, cited in Edwards, 1980) reported that prechilling treatment reduces germination of immature seeds.

Prechilling treatment is usually used in tree species for overcoming dormancy or enhancing the germination speed of tree species. This treatment was suggested for the standard germination test of *P. radiata* in the ISTA Rules 1966 (ISTA, 1966), but this suggestion was removed from the Rules of 1976 and 1985 (ISTA, 1976; 1985).

5.2.1.3. The complex stressing vigour test (CSVT)

Section 4.2.8 and Figure 4.6 show that the CSVT has quite good sensitivity as it can distinguish between deteriorated seed lots, although it does not differentiate seed quality parameters which are a function of seed weight. The predictivity of this method is also quite good as Appendix 17 shows that CSVT gives a significant correlation with some results of the Rotorua nursery test. However, the general applicability of this method to a wide range of field conditions is not as good as the CD2d and SG+pr test as it did not give any significant correlation with the glasshouse and the winter field tests (Appendix 7.C). With surviving normal seedlings in the winter field test (Figure 4.4), it gave quite a high correlation coefficient, but the value is not significant ($r = 0.75^{ns}$).

The good predictivity of CSVT to seedling performance in the Rotorua nursery test might be related to the water soaking treatment in both tests. In the CSVT seeds were soaked at 20 and 5°C for 2 days respectively, whereas in the Rotorua nursery test seeds were soaked at 10°C for 48 hours. Both treatments might have

imposed a stress effect especially on poor vigour seeds as the treatments could cause oxygen deficiency to the seeds.

There are no reports concerning the use of this test in differentiating quality parameters in *P. radiata* or other tree species. In maize seed, however, Barla-Szabo and Dolinka (1988) reported that this test gave quite a high and significant correlation with early sowing emergence (r = 0.717**). But the correlations with mid and late sowings were quite low (r = 0.598** and 0.376 respectively).

5.2.1.4. The low temperature germination test at 10°C, 0 bar

Appendix 7.C shows that the percentage of radicle emergence measurements in this test gave a high and significant correlation with 4 parameters of the winter field test, i.e. percentage normal seedlings (r = 0.93*), surviving emerged seedlings (r = 0.90*), surviving normal seedlings (r = 0.96**, Figure 4.15), and T_{50} normal seedlings (r = -0.96*). In contrast, radicle measurements at 15°C, 0 bar and 20°C, 0 bar (Figure 4.16 and 4.17) did not correlate with surviving normal seedlings in the field (r = 0.48^{ns} and 0.03^{ns} respectively) nor with the glasshouse test. Other combinations of temperature and osmotic potential also did not correlate with the glasshouse and the winter field test (Appendix 7.C).

The ability of this test to predict field emergence might relate to the similarity of temperatures in the test and field. Calculation of data from Appendix 4.A indicated that mean daily temperature of the winter field test was 10.3°C.

The inability of other temperature and osmotic potential to predict seedling performance might be related to inability of those combination to simulate similar conditions in the field. It was true that there was water stress condition during 76-

96 days after sowing when the rainfall was very low (Appendix 4.B), but this event occurred in the later stages of seedling growth which was not equivalent to the period of osmotic stress where -5 bar was imposed in the initial stages of germination.

This test does not require complicated equipment or procedures, but it requires a relatively long time (up to 24 weeks) in germinating the seeds. This will cause difficulty in wide application of this test in a standard seed testing laboratory. However, this method may be useful for specific purposes like in breeding programmes for selecting low temperature or frost tolerant seed.

It is reported that the cold test has the ability to forecast seed performance in some agricultural and horticultural seeds like corn, soybean, cotton, onion, carrot and sorghum (reviewed by AOSA, 1983). An important point to be noticed is that in the cold test the seeds are placed in low temperature (10°C) for only 7 days before transfer to 25°C (Fiala, 1981; AOSA, 1983). The reports of the ISTA collaborative test on maize seed indicated this test gave significant correlations in 3 stations, i.e. Hungary (r - 0.828***), USA (r = 0.856***), and the Netherlands (r = 0.616*), but there were no significant correlations in 7 other stations (Fiala, 1987). In soybean, Kulik and Yaklick (1982) reported that the results of the cold test gave significant correlations in the range 0.60-0.75 with field emergence in 1975 and 1976 (for 2 soil types and 3 planting dates).

5.2.2 Suitable vigour tests for assessing seedling establishment at Rotorua (FRI) nursery

Based on the correlation analysis results (Appendix 17), it is concluded that three of the vigour tests used in this study, i.e. the prechilled seedling growth (SG+pr),

the controlled deterioration with 2 days aging treatment (CD2d), and the complex stressing vigour (CSV) tests are quite suitable for assessing seedling establishment at the Rotorua nursery as all of them gave a good correlation with some of the nursery test parameters.

The standard germination test method done by FRI, although it gave high and significant correlation with 5 parameters of the nursery test (Appendix 17), is not suggested for assessment purposes as it involved an unplanned stress condition not prescribed by the test (see Appendix 2). Such conditions will be difficult to reproduce.

For application purposes, it is suggested that those parameters which gave the highest r value with percentage of plantable seedlings in the nursery shall be used as a reliable test parameter. Therefore, percentage of normal seedling shall be used either in the CD2d or the CSV tests ($r = 0.78^{***}$ and 0.76^{***}, respectively), whereas T_{50} radicle emergence or T_{50} normal seedling shall be used in the SG+pr test ($r = -0.77^{***}$).

As mentioned earlier (sections 5.2.1.2. and 5.2.1.3.), pre-sowing treatment (by soaking the seeds in water for 48 hours at 10°C) in the nursery seems to have an important effect especially in relation to the predictivity of germination parameter by the SG+pr and CSV tests. Therefore, removing or altering this pre-sowing treatment may alter the effectiveness of these tests.

The direction for further studies would seem to be to evaluate reproducibility of r values and regression equations given by CD2d, SG+pr and CSV tests in the same nursery site over several sowings. A consistent regression equation with high r and

R^2 values produced by any of these tests could then be used for assessing planting value or plantable seedlings.

For application in other nurseries, those three tests may still be valid especially if pre-sowing treatment and nursery conditions are about the same as in the Rotorua nursery. If there are some differences, however, the CD2d and SG+pr are more likely to be useful as they have better generalisability than CSV test (see section 5.2.1.).

In relation to reproducibility of regression equations produced by vigour tests, Kulik and Yaklich (1982) reported that tetrazolium and accelerated-aging-normal seedling tests produced quite consistent regression equations at two planting years, i.e. 1975 and 1976. In this study, field emergence data of each planting year were obtained from mean data of field emergence at 2 planting sites and 3 planting dates. For the tetrazolium test, regression equations with field emergence in those planting years were $Y = 0.832 X + 7.2$, $R^2 = 55\%$ and $Y = 0.780 X + 7.4$, $R^2 = 45\%$, respectively, whereas for accelerated aging-normal seedling those were $Y = 0.503 X + 56.6$, $R^2 = 39\%$ and $Y = 0.469 X + 57.7$, $R^2 = 65\%$, respectively. Although the pairs equations were very similar, it should be noted that two of the equations had R^2 values of less than 50%. In addition, no information was provided concerning the significance or confidence interval of the r values.

5.2.3 The effect of seed coat condition on germination performance and the conductivity test result

5.2.3.1. The effect of seed coat condition on germination performance

The condition of the seed coat is likely to be altered during aging treatment. For example, the coat might become softer, so that aged seeds imbibe water in a faster

rate which can lead to either reduced or improved germination performance depending on the degree of imbibition stress in germination environment.
In the germination environment where there was less or no imbibtion barrier aged seeds (especially lot C) generally performed less well than unaged ones. Examples of this are to be found in the results for:

i) the prechilled seedling growth test (compare lot C to lot A in Figures 4.4.A, B and C),

ii) the controlled deterioration test (compare lot B and C to lot A, and lot E to lot D in Figure 4.5),

iii) the complex stressing vigour test (compare lot B and C to lot A, and lot E to lot D in Figure 4.6),

iv) the glasshouse test (compare lot C to lot A in Figures 4.2.A, B and C), and

v) the winter field test (compare lot C to lot A in Figures 4.3.A, B, C and D).

However, where there was an imbibition barrier or stress resulting from the presence of low temperature and/or osmotica during germination, aged seeds generally performed better than unaged ones: for example in:

i) the germination test at 10°C, -5 bar (compare lot B and C to lot A, and lot E to lot D in Figure 4.7.A and B), and

ii) germination test at 15°C either at -5 or 0 bar with respect to T_{50} radicle emergence (compare lot B and C to lot A, and lot E to lot D in Figures 4.7.B and C).

Note that measurement of different parameters reveals different aspects of the relative germination performance pattern between aged and unaged seeds. This can be seen in the low temperature germination test at 10°C, 0 bars, where, if percentage of radicle emergence was used as a measurement criterion (Figure 4.7.A), aged seeds (lot C) showed lower performance than unaged seeds (lot A). However, if T_{50} radicle emergence was used, lot C showed better performance than lot A (Figure 4.7.B).

An important point to be noticed was that there was actually an imbibition stress due to low temperature imposed in prechilling treatment in the prechilled seedling growth test. However, this stress seems to be much less than that in low temperature germination test either at 10 or 15°C, as prechilling treatment was only imposed for 7 days at 5°C prior to germination period at 20°C, whereas low temperature was imposed for the entire germination period in the low temperature germination test.

The hypothesis that low temperature can act as an imbibitional barrier was supported by the work of Tully *et al* (1981) in soybean and pea seeds which indicated that the rate of water uptake was much lower at 2°C than that at 25°C. This phenomenon occurred either in split or intact seed coat of both seeds. Whereas, the hypothesis that omostica can act as an imbibitional barrier was supported by Bradford's (1986) study in lettuce seeds which pointed out the lower the osmotic potential, the lower was the rate of water uptake.

The lower performance of seeds with softened coats in conditions where there was less or no imbibitional barrier might be related to some hindrances to the reorganisation of membranes during water entry, so that membrane reorganisation did not keep up with the rate of imbibition. In the environment where there was an imbibitional barrier, the rate of water uptake in aged seed was slower so that allowed membranes time for better reorganisation, resulting in improved emergence. In this environment, however, the rate of water uptake in unaged seeds became too slow so that radicle emergence was not promoted. Similar phenomenon to the result of this study was reported by Vertucci (1989) in soybean seeds. In this case, seed coat removal produced a better germination index (percentage of germination x radicle length) than the intact ones at osmotic potential of -3.0 MPa (61.0 compared to 50.4). At germination media of 0 MPA, however, intact seedcoat seeds produced a better performance than the decoated ones (germination index was 93.5 compared to 70). Woodstock and Tao (1981, cited in Vertucci, 1989) and Woodstock and Taylorson (1981, cited in Vertucci, 1989) also reported that aged soybean seeds performed better than unaged ones under osmotic stress conditions.

5.2.3.2. The effect of the condition of the seed coat on the conductivity test results

The inconsistencies of the conductivity tests (Appendix 6.A) may be related to effects of seed coat condition on the conductivity test result. Three factors can be identified:

1. surface contamination during seed development,

2. surface contamination due to fungal infection during aging treatment, and

3. the hard integument of the seed coat which is typical of gymnosperms (Baldwin, 1942).

Surface contamination of organic or inorganic material might occur during seed development as *P. radiata* belong to gymnosperm class in which the seeds were developed from naked ovules without enclosing ovaries (Baldwin, 1942), whereas contamination due to fungal infection might also happen during aging treatment (see section 2.2). Fungal contamination, however, was greatly reduced as all seeds visibly infected with fungi were removed from all seed lots prior to conductivity measurements (see section 3.2.1.).

Surface contamination might increase conductivity reading of soaked water so that the results might be higher than the actual value, whereas hard integument of the seed coat might inhibit leachates from the inner part of the seeds so that the result might become less than the actual value. Nevertheless, the results indicate that the same part of the leachates may come from the inner part of the seed as the longer the imbibition time the higher was the conductivity. The pattern was the same for 5 seed lots measured.

The test results also showed that, in general, aged seeds had a tendency to produce higher conductivity readings than unaged one (Appendix 6.A), an indication that aged seeds might suffer from impaired membrane integrity. In addition, some of the test results gave significant correlations with some results of the glasshouse and the winter field tests. As shown in Appendix 7.B, the test result using 50 seeds after 8, 10 and 24 hours of imbibition correlated significantly with T_{50} normal seedling in glasshouse with r value of 0.97**, 0.94* and 0.98**, respectively, whereas those using 100 seeds after 4 and 9 hours of imbibition correlated

significantly with cumulative emerge seedling in the winter field test (r = -0.94* and -0.93*, respectively). Furthermore, that using 100 seeds after 10 hours of imbibition also gave significant correlation with normal seedling shoot dry weight in the winter field test (r = -0.89*).

To overcome problems of seed condition, it is probably necessary to surface sterilise or to rinse the seeds prior to conductivity measurement or to use only the embryo instead of the whole seeds. However, the success of these suggested methods still need to be investigated. There are some reports in tree seeds concerning these approaches, like the use of surface sterilisation with 0.1% Calcium hypochlorite applied to *Pinus pallustris*, *P. glabra*, and *P. taeda* (Barnett, 1985), seed rinsing with water for 30 minutes applied in scotch pine, slash pine, sand pine and lobbolly pine (Vozzo, 1984), and excised embryos applied in *P. lambertiana* (Murphy and Noland, 1982). However, there was no correlation analysis with other vigour measurements nor with field emergence was conducted in those studies. Clearly, conductivity testing of tree seeds is an area which requires further development.

5.2.4 The relationship between seed weight and seedling performance

It seems likely that there was a contrast between the results of section 4.2 and section 4.3 concerning the relationship between seed weight and seedling performance.

In section 4.2 where 5 seed lots which varied according to seed weight and aging treatment were used, seed weight gave significant effect on seedling dry weight and T_{50} radicle emergence. The effect on seedling dry weight was detected in the seedling growth test (section 4.3.2, Figure 4.4.D), the glasshouse test (section

4.2.1, Figure 4.2.E), and the winter field test (section 4.2.2., Figure 4.3.D). Also the effect on T_{50} radicle emergence was detected in the germination tests at 10°C, either at -5 or 0 bar, and at 15°C at -5 bar (section 4.2.9, Figure 4.7.A and C). Under each test, generally heavier seeds had better performance than the lighter ones.

In section 4.3 where 16 seed lots which varied according to mother tree types and collection date were used, however, seed weight did not correlate (Appendix 17) with shoot and root dry weight ($r = -0.23^{ns}$ and -0.31^{ns}, respectively) nor with T_{50} field germination ($r = -0.05^{ns}$). High and significant correlation was only given in relation to seedling height ($r = 0.82****$). Another significant correlation was also given in relation to seedling diameter, but the r value was quite low ($r = 0.56*$).

This contrast might be caused by the difference in the degree of seed weight variation between the two studies. In the section 4.2 studies, two clearly different seed weight classes were used, i.e. heavier seed which had 20-25,000 seeds/kg or 100 seed weight = 4-5 grams, and the lighter ones which had 25-30,000 seeds/kg or 100 seed weight = 3.3-4 grams (see section 3.2). In section 4.3 studies,however, there were only small variations in overall seed weight among the 16 seed lots and great variations among clones within individual lots (see section 4.3.2, Table 4.1 and Figure 4.18). Therefore, it is hypothesised that important seed weight effects were masked in the FRI nursery studies. This hypothesis is supported by reports of other studies below which generally indicate that heavier and longer seeds have better seedling performance than lighter and smaller ones.

FRI (1985) reported that seed weight had a great effect on field germination and seedling height of *P. radiata* in the nursery. Field germination (five weeks after sowing) of grade A (0.04 to 0.049 grams/seed), B (0.03 to 0.039 grams/seed), and C (0.02 to 0.029 grams/seed) were 84, 81 and 70%, respectively, whereas their seedling heights (one year after sowing) were 34, 31 and 31 cm, respectively. Griffin (1972) also found that in *P. radiata* larger (and heavier) seeds after 32 weeks in nursery produced seedlings which were taller (19.2 compared to 15.7 cm), bigger in collar diameter (0.19 compared to 0.14 cm), and heavier in dry weight (0.83 to 0.46 gram) compared to the seedlings grown from smaller (and lighter) seeds. In addition, the larger seeds also produced higher germinability (98% compared to 83%). Seed size variation in *P. radiata* may occur at several levels, i.e. between sites, between trees within sites, between cones within trees or clones (Griffin, 1972) and between scales within cones (Fielding, 1964).

Better performance of larger seeds was also found in other tree species like *Pinus thunbergii* (Kim *et al.*, 1989), *Pseudotsuga menziesii* (Sorenson and Campbell, 1985), *Pinus elliotii* (Langdon, 1958; Belcher *et al.*, 1984), and *Pinus taeda* (Dunlap and Barnett, 1983).

The reason for the superiority of larger seeds in seedling production may be related to the numbers of cells in the embryo meristems or due to more subtle differences like the higher quantity of mitochondrial protein which leads to higher respiratory rate and greater amount of energy (ATP) production (McDaniel, 1969, working with barley). Certainly, seedlings produced by larger seeds seem to have a greater growth potential.

VI. CONCLUSION

6.1. Deterioration in *P. radiata* seeds

The pattern of deterioration of *P. radiata* seeds follows the general pattern for orthodox seeds in which the higher the temperature and relative humidity of the storage environment (and thus seed moisture content), the faster is the rate of deterioration. In this case, conditions of 45°C, 100% RH had a greater effect on seed vigour than those of 40°C, 100% RH, as in these conditions vigour was reduced after 6 days compared to after 8 days in those of 40°C, 100% RH.

The fall in vigour (measured here by increasing T_{50} radicle emergence or T_{50} normal seedlings) preceding the fall in viability has a similarity to the general pattern of deterioration suggested by Delouche and Caldwell (1960) which showed that vigour will tend to reduce steeply before there is any great reduction of viability. In the initial stages of deterioration, differences between vigour and viability may not be very pronounced, but at the later stages the difference becomes large.

6.2. Promising suitable vigour tests for *P. radiata*

Based on the sensitivity of the tests in differentiating quality differences among seed lots and the predictivity of the tests to assess field emergence (especially numbers of surviving normal seedlings in the winter field test), it is suggested that some promising suitable vigour tests for *P. radiata* include:

(i) the controlled deteriorating test with two days aging treatment (CD2d test),

(ii) the prechilled seedling growth test (SG + pr test),

(iii) the complex stressing vigour test (CSV test), and

(iv) the low temperature germination test at 10°C, 0 bar.

Except for the low temperature germination test at 10°C, 0 bar, which requires a relatively long germination period (up to 24 weeks), all of these tests seem to have met most of the AOSA's (1983) criteria for a practical vigour test as these methods are simple and can be done in a relatively short period of time. Therefore, the possibilities for wider application of the methods in standard seed testing laboratories is quite promising.

6.3. Suitable vigour tests for assessing seedling establishment of *P. radiata* at Rotorua (FRI) nursery

Based on the correlation analyses with the results of the Rotorua nursery trial, it is concluded that three of the vigour tests in this study, i.e.

(i) CD2d test

(ii) SG + pr test, and

(iii) CSV test

are quite suitable for predicting seedling establishment at the Rotorua nursery, as all gave good correlations with some of the nursery test parameters, especially percentage of plantable seedlings.

For application purposes, it is suggested that vigour tests parameters which gave the highest correlation coefficient (r) value with percentage of plantable seedlings in the nursery should be used as a reliable test parameter. Therefore, percentage of normal seedlings should be used either in the CD2d or the CSV tests, whereas T_{50} radicle emergence or T_{50} normal seedlings should be used in the SG+pr test. An important point to be noticed is that pre-sowing treatment (by soaking the seeds in water for 48 hours at 10°C) in the nursery seems to have an important influence on the results, especially in relation to the predictivity of the SG+pr and CSV tests. Therefore, removing or altering this pre-sowing treatment may alter the effectiveness of these tests. In addition, this treatment might also influence the predictive effectiveness of different parameters in the SG+pr test. In the previous study, when there was no pre-sowing treatment applied (i.e. the glasshouse and winter field tests) the best correlation coefficient (r) was shown by percentage radicle emergence and percentage of normal seedlings. However, when the seeds had been given pre-sowing treatment (i.e. at the FRI nursery), the best r value was shown by T_{50} values for radicle emergence and normal seedlings.

For application in other nurseries, those three tests may still be valid, especially if the pre-sowing treatment and nursery conditions are about the same as in the Rotorua nursery. If there are some differences, however, the CD2d and SG+pr tests are more likely to be useful than the CSV test. This hypothesis is based on the fact that the CD2d and SG+pr tests, apart from their good correlations with the result of the FRI nursery trial, also gave good correlation with the glasshouse

(optimum conditions) and winter field tests (sub-optimum conditions). In contrast, there was no significant correlation given by the CSV test in relation to the glasshouse and winter field tests.

6.4. The relationship between seed weight and seedling performance

In the earlier study (where 5 seed lots which varied according to seed weight and age were used), seed weight gave a significant effect on seedling dry weight and T_{50} radicle emergence. In this case, generally heavier seeds had better performance than the lighter ones. In the later study (where 16 seed lots which varied according to type of mother tree and collection date were used), however, seed weight did not correlate with shoot and root dry weight nor with T_{50} field germination. This contrast results from the difference in the degree of seed weight variation between the two studies. In the earlier study, two clearly different seed weight classes were used. In the later study, however, there was only small variation in overall seed weight among the 16 seed lots and great variation among clones within individual lots, so that important seed weight effects were masked.

6.5. Scope for further studies

The direction for further studies would seem to be to evaluate the reproducibility of r value and regression equations given by the CD2d, SG+pr and CSV tests in the same nursery site over several sowings. A consistent regression equation with high r and R^2 produced by any of those tests could then be used for assessing

planting value or plantable seedlings. In later stages, the evaluation of reproducibility of those vigour tests results within and between laboratories would also seem to be important.

Another important thing which needs to be further investigated would seem to be vigour tests evaluation using seed lots which come from individual clones, as it was shown that each clone had a great effect on seed weight which might mean that it also had a great effect on seed vigour.

BIBLIOGRAPHY

Allen, G.S. (1958a). Factors affecting the viability and germination behaviour of coniferous seed. Part I. Cone and seed maturity, *Tsuga heterophylla* (Rafn.). Sarg. For. Chron. 34: 266-274. (Cited in Edwards, 1980).

Allen, G.S. (1958b). Factors affecting the viability and germination behaviour of coniferous seed. Part II. Cone and seed maturity, *Pseudotsuga menziesii* (Mirb.) Franco. For. Chron. 34: 275-282. (Cited in Edwards, 1980).

Anderson, J.D. (1970). Physiological and biochemical differences in deteriorating barley seed. Crop Sci. 10: 36-39.

AOSA. (1983). Seed vigor testing handbook. Contrib. no. 32 to the handbook of seed testing. Publ. Assoc. Off. Seed Anal., U.S.A.

Arisman, H. and Powell, G.R. (1986). Effects of cone colour and seed extraction methods on yield and quality of seeds of *Pinus merkusii* in Indonesia. Seed Sci. and Technol. 14(1): 177-190.

Asakawa, S. (1976). Further report on seed storage of three coniferous species. Proc. 2nd Internatl Symp. IUFRO 52. 01. 06, 1976.

Baldwin, H.I. (1942). "Forest Tree Seed of the North Temperate Region with Special Reference to North America". Publ. Chron. Bot. Co., Waltham, Mass. U.S.A. 240 pp.

Bannister, M.H. (1973). The origins of radiata pine in cultivation. What's New in For. Res. No. 2. Publ. N.Z. For. Res. Inst., Rotorua.

Barla-Szabo, G. and Dolinka, B. (1988). Complex stressing vigour test: a new method for wheat and maize seeds. Seed Sci. and Technol. 16: 63-73.

Barnett, J.P. (1979). Storing spruce pine seeds. Tree Planters' Notes 30(4): 20-21.

Barnett, J.P. (1985). Estimating seed vigor by sugar exudates and radicle elongation. Tree Planters' Notes 36(3): 16-19.

Baskin, C.C. (1981). Accelerated aging test. In "Handbook of Vigour Test Methods". (D.A. Perry ed.). Publ. ISTA, Zurich. pp: 43-56.

Belcher, E.W., Leach, G.N. and Gresham, H.H. (1984). Sizing slash pine seeds as a nursery procedures. Tree Planters' Notes 35(2): 5-10.

Bergsten, U. (1988). Invigoration and IDS-sedimentation of *Pinus sylvestris* seeds from Northern Finland. Silva Fenn. 22(4): 323-327.

Berjak, P., Dini, M. and Pammenter, N.W. (1984). Possible mechanisms underlying the differing dehydration responses in recalcitrant and orthodox seeds: desiccation-associated subcellular changes in propagules of *Avicennia marina*. Seed Sci. and Technol. 12: 365-384.

Bewley, G.D. (1986). Membrane changes in seeds as related to germination and the pertubations resulting from deterioration in storage. In "Physiology of Seed Deterioration". (M.B. McDonald and C.J. Nelson, ed.). Publ. CSSA Spec. Pub. No. 11., Madison, U.S.A. pp. 27-45.

Bhumibhamon, S. (1976). Genetic variation in seed respiration of Norway spruce. Proc. 2nd Internatl Symp. IUFRO S2.01.06, 1976.

Bonner, F.T. (1974). Tests for vigor in cherrybark oak acorns. Proc. AOSA 64: 109-114.

Bradford, K.J. (1986). Manipulation of seed water relations via osmotic priming to improve germination under stress conditions. Hort. Sci. 21(5): 1105-1112.

Burris, J.S., Edje, O.T. and Wahab, A.H. (1969). Evaluation of various indices of seed and seedling vigor in soybeans [*Glycine max* (L.) Merr.]. Proc. AOSA 59: 73-81.

Carver, M.F.F. and Matthews, S. (1975). Respiratory measurements as indicators of field emergence ability in peas. Seed Sci. and Technol. 3: 871-879. (Cited in Matthews and Powell, 1981).

Cherry, J.P. (1983). Protein degradation during seed deterioration. Hort. Sci. 73(2): 317-321.

Ching, T.M. (1972). Metabolism of germinating seeds. In "Seed Biology". (T.T. Kozlowski, ed.). Publ. Acad. Pres., N.Y. pp. 103-218.

Ching, T.M. (1973). Adenosine triphosphate content and seed vigor. Plant Physiol. 51: 400-402.

Ching, T.M. (1982). Adenosine triphosphate and seed vigor. In "The Physiology and Biochemistry of Seed Development, Dormancy and Germination". (A.A. Khan, ed.). Publ. Elsevier Biomedical Press, Amsterdam. pp. 487-506.

Ching, T.M. and Ching, K.K. (1962). Physical and physiological changes in maturing douglas-fir cones and seed. For. Sci. 8(1): 21-31.

Ching, T.M. and Ching, K.K. (1972). Content of adenosine phsophates and adenylate energy charge in germinating ponderosa pine seeds. Plant Physiol. 50: 536-540.

Ching, T.M. and Ching, K.K. (1973). In: International Symposium on Dormancy in Trees, Polish Academy of Sciences pp. 13-19. (Cited in Ching, 1982).

Ching, T.M. and Fang, S.C. (1963). Utilization of labelled glucosa in developing douglas-fir seed cones. Plant Physiol. 38: 551-554.

Christensen, C.M. and Kaufmann, H.H. (1974). Microflora. In "Storage of Cereal Grains and Their Products". (C.M. Christensen, ed.). Publ. Amer. Assoc. Cer. Chem. Inc., St Paul, Minnesota. pp. 158-192.

Clifton, N.C. (1985). New Zealand radiata pine. Publ. N.Z. For. Service, Wellington. 27 pp.

Coolbear, P. (1988). Seed deterioration during ageing. In lecture note of Advance Seed Physiology. Seed Technology Centre, Massey Unversity, Palmerston North, N.Z.

Coolbear, P., Grerson, D. and Heydecker, W. (1980). Osmotic pre-sowing treatments and nucleic acid accumulation in tomato seeds (*Lycopersicon lycoperiscum*). Seed Sci. and Technol. 8: 289-303. (Cited in Rimbawanto, 1987).

Czabator, F.J. (1962). Germination value: an index combining speed and completeness of pine seed germination. For. Sci. 8: 386-396. (Cited in Bonner, 1974).

Delouche, J.C. and Caldwell, W.P. (1960). Seed vigor and vigor tests. Proc. AOSA. 50: 124-129.

Djavanshir, K. and Pourbeik, H. (1976). Germination value - a new formula. Salvae Genetica 25(2): 79-83. (Cited in Arisman and Powell, 1986).

Dunlap, J.R. and Barnett, J.P. (1983). Influence of seed size on germination and early development of loblolly pine (*Pinus taeda* L.) germinants. Can. J. For. Res. 13(1): 40-44.

Edwards, D.G.W. (1980). Maturity and quality of tree seeds - a state-of-the art review. Seed Sci. and Technol. 8: 625-557.

Fiala, F. (1981). Cold test. In "Handbook of Vigour Test Methods". (D.A. Perry, ed.). Publ. ISTA, Zurich. pp. 28-36.

Fiala, F. (1987). Report of the vigour test committee 1983-1986. Seed Sci. and Technol. 15: 507-522.

Fielding, J.M. (1964). Some characteristics of the cones and seed of *Pinus radiata*. Leaflet No. 89. Publ. Dep. Nat. Dev. For. Timb. Bur., Canberra.

FRI (1974). Radiata pine seed orchards. What's New in For. Res. No. 16. Publ. N.Z. For. Res. Inst., Rotorua.

FRI (1984). Report of Forest Research Institute for 1 January to 31 December 1983. Publ. N.Z. For. Serv., Wellington.

FRI (1985). Forest Research Institute Report 1985. Publ. N.Z. For. Serv., Wellington.

FRI (1987). New Zealand pine one of the world's most versatile forest species. Publ. For. Res. Inst., Rotorua, N.Z.

FRI (1988). Seedling quality and seedling specifications of radiata pine. What's New in For. Res. No. 171. Publ. N.Z. For. Res. Inst., Rotorua.

Fuchs, H. (1981). Hiltner test. In "Handbook of Vigour Test Methods". (D.A. Perry, ed.). Publ. ISTA, Zurich. pp. 21-27.

Gelmond, H., Luria, I., Woodstock, L.W. and Perl, M. (1978). The effect of accelerated aging of sorghum seeds on seedling vigour. J. Exp. Bot. 29(109): 489-495.

Germ, H. and Kietreiber, M. (1953). Vitalität des Endosperms und Feldaufgang von *Zea mays*. Bodenkultur 4. Sonderheft, 14-16. (Cited in Fiala, 1981).

Germ, H. and Kietreiber, M. (1954). Die Prüfung der Vitalität des Maiskornes. Bodenkultur 5. Sonderheft, 29-47. (Cited in Fiala, 1981).

Gorecki, R.J. and Harman, G.E. (1987). Effects of antioxidants on viability and vigour of aging pea seeds. Seed Sci. and Technol. 15: 109-117.

Grabe, D.F. (1965). Prediction of relative storability of corn seed lots. Proc. AOSA 55: 92-96. (Cited in AOSA, 1983).

Griffin, A.R. (1972). The effects of seed size, germination time and sowing density on seedling development in radiata pine. Aust. For. Res. 5(4): 25-28.

Halloin, J.M. (1983). Deterioration resistance mechanism in seeds. Phyto pathol. 73(2): 335-338.

Halloin, J.M. (1986). Microorganism and seed deterioration. In "Physiology of Deterioration". (M.B. McDonald and C.J. Nelson, ed.). Publ. CSSA Spec. Pub. No. 11., Madison, U.S.A. pp. 89-99.

Hampton, J.G. (1985). Seed vigour. A quality factor. Aglink. FPP 834: 1/3000/5/85. N.Z. MAF.

Hampton, J.G. and Coolbear, P. (1990). Potential versus actual seed performance - can vigour testing provide an answer? Seed Sci and Technol. 18: (in press).

Harman, G.E. and Granett, A.L. (1972). Deterioration of stored pea seed: changes in germination, membrane permeability and ultrastructure resulting from infection by *Aspergillus ruber* and from aging. Physiol Plant Pathol. 2: 271-278.

Harman, G.E. and Mattick, L.R. (1976). Association of lipid oxidation weith seed ageing and death. Nature 260: 323-324.

Harrington, J.F. (1959). Drying, storing and packaging seeds to maintain germination and vigor. U.S.D.A. Agric. Handb. 354: 1-55. (Cited in Harrington, 1972).

Harrington, J.F. (1972). Seed storage and longevity. In "Seed Biology". (T.T. Kozlowski, ed.) vol. 3. Publ. Acad. Press, N.Y. pp. 145-245.

Heit, C.E. (1967). Propagation from seed. X. Storage methods for conifer seeds. Amer. Nurseryman 126: 14-15, 38, 40, 42-44, 46, 48, 50, 52 and 54. (Cited in Harrington, 1972).

Huang, U.G. (1989). Evaluating lodgepole pine and white spruce seed vigour by seedling uniformity. Seed Sci. and Technol. 17: 543-553.

Hocking, D. and Etter, H.M. (1969). Rapid germinability test for white spruce. Can. J. Plant Sci. 49: 527-528.

I.S.T.A. (1966). International Rules for Seed Testing 1966. Proc. ISTA 31(1): 1-152.

I.S.T.A. (1976). International Rules for Seed Testing 1976. Seed Sci and Technol. 4: 3-177.

I.S.T.A. (1985). International Rules for Seed Testing 1985. Seed Sci and Technol. 13(2): 338-341.

Johnston, W.J., Yusuf, H.A., Konzak, C.F. and Maquire, J.D. (1986). Tetrazolium chloride test for spring wheat seedling vigor at low temperatures. Crop Sci. 26: 167-169.

Kamra, S.K. (1971). The x-ray contrast method for testing germinability of *Picea abies* (L.) Karst. seed. Stud. For. Suec. 90: 1-29.

Kim, K.S., Kwan, H.M., Choi, S.K. and Tak, W.S. (1989). Effects of seed size of two pine species on early seedling growth. For. Abs. 50(3): 112.

King, M.W. and Roberts, E.H. (1980). Maintenance of recalcitrant seed in storage. In "Recalcitrant Crop Seed". (H.F. Chin and E.H. Roberts, ed.). Publ. Trop. Press SDN. BHD., Kualalumpur. pp. 53-89.

Kittock, D.L. and Law, A.G. (1968). Relationship of seedling vigour to respiration and tetrazolium chloride reduction by germinating wheat seeds. Agron. J. 60: 286-288.

Kozlowski, T.T. (1971). "Growth and Development of Trees. Vol. I. Seed Germination, Ontogency, and Shoot Growth". Publ. Acad. Press, N.Y. 443 pp. (Cited in Tasimin, 1980).

Kulik, M.M. and Yaklich, R.W. (1982). Evaluation of vigor tests in soybean seeds: relationship of accelerated aging, cold, sand bench, and speed of germination tets to field performance. Crop Sci. 22: 766-770.

Kuznetsova (1939). In Lesnoe Khozyaystvo, Moskow. (Cited in Baldwin, 1942).

Langdon, O.G. (1958). Cone and seed size of South Florida slash pine and their effects on seelding size and survival. J. For. 56(2): 122-127.

Larson, M.M. and Schubert, G.H. (1969). Effect of osmotic water stress on germination and initial development of ponderosa pine seedlings. For. Sci. 15(1): 30-36.

Lin, S.S. and Pearce, R.S. (1990). Changes in lipids of bean seeds (*Phaseolus vulgaris*) and corn caryopses (*Zea mays*) aged in contrasting environments. Ann. Bot. 65: 451-456.

Matthews, S. (1980). Controlled deterioration: a new vigour test for crop seeds. In "Seed Production". (P.D. Hebblethwaite, ed.). Publ. Butterworths, London. pp. 647-660.

Matthews, S. and Powell, A.A. (1981a). Electrical conductivity test. In "Handbook of Vigour Test Methods". (D.A. Perry, ed.). Publ. ISTA, Zurich. pp. 37-42.

Matthews, S. and Powell, A.A. (1981b). Controlled deterioration test. In "Handbook of Vigour Test Methods". (D.A. Perry, ed.). Publ. ISTA, Zurich. pp. 49-60.

McDaniel, R.G. (1969). Relationships of seed weight, seedling vigour and mitochondrial metabolism in barley. Crop Sci. 9: 823-827.

McDonald, M.B. (1977). The influence of seed moisture on the accelerated aging seed vigor test for soybean. J. Seed Technol. 2: 18-28.

McDonald, M.B. (1980). Assessment of seed quality. Hort. Sci. 15(6): 784-788.

McGee, D.C. (1983). Introduction. Symposium: Deterioration mechanism in seeds. Phytopathol. 72(2): 314-315.

Michel, B.E. and Kaufmann, M.R. (1973). The osmotic potential of polyethylene glycol 6000. Plant Physiol. 51: 914-916.

Mirov, N.T. (1946). Viability of pine seed after prolonged cold storage. J. For. 44(3): 193-195. (Cited in Schubert, 1952).

Moore, M.B. and Kidd, F.A. (1982). Seed source variation in induced moisture stress germination of ponderosa pine. Tree Planters' Notes 33(1): 12-14.

Muchena, S.C. and Grogan, C.O. (1977). Effects of seed size on germination of corn (*Zea mays*) under simulated water stress conditions. Can J. Plant Sci. 57: 921-923.

Mugnisjah, W.Q. and Nakamura, S. (1986). Methanol and ethanol stress for seed vigour evaluation in soybean. Seed Sci. and Technol. 14: 95-103.

Murphy, J.B. and Noland, T.L. (1982). Temperature effects on seed imbibition and leakage mediated by viscosity and membranes. Plant Physiol. 69: 428-431.

Osborne, D.J. (1980). Senescence in seeds. In "Senescence in Plant". (K.V. Thimann, ed.). Publ. CRC Press, Boca Raton, Florida. pp. 13-37.

Pelkonen, P. and Luukkanen, O. (1974). Gas exchange in three populations of Norway spruce. Silvae Gen. 23: 160-164. (Cited in Bhumibhamon, 1976).

Perry, D.A. (1976). Seed vigour and seedling establishment. Adv. in Res. and Technol. Seeds 2: 62-85.

Perry, D.A. (1978). Report of the Vigour Test Committee 1974-1977. Seed Sci. and Technol. 6: 159-181. (Cited in Perry, 1981b).

Perry, D.A. (1981a). Methodology and application of vigour tests. In "Handbook of Vigour Test Methods". (D.A. Perry, ed.). Publ. ISTA, Zurich. pp. 8-9.

Perry, D.A. (1981b). Seedling growth and seedling evaluation tests. In "Handbook of Vigour Test Methods". (D.A. Perry, ed.). Publ. ISTA, Zurich. pp. 10-20.

Priestley, D.A. (1986). Morphological, structural and biochemical changes associated with seed aging. In "Seed Aging - Implications for Seed Storage and Persistence in the Soil". (D.A. Priestley, ed.). Publ. Comstock Publ. Assoc., Ithaca and London. pp. 125-194.

Priestley, D.A. and Leopold, A.C. (1983). Lipid changes during natural aging of soybean seeds. Physiol. Plant 59: 467-470. (Cited in Lin and Pearce, 1990).

Rimbawanto, A. (1987). "Morphological and physiological changes in developing *Pinus radiata* D. Don seed and the effect of early cone collection and post-harvest treatment on seed quality". MAgrSc Thesis, Massey University, Palmerston North, New Zealand.

Rimbawanto, A., Coolbear, P., Dourado, A.M. and Firth, A. (1988a). Seed maturation precedes cone ripening in New Zealand *Pinus radiata*. N.Z. J. For. Sci. 18(2): 139-148.

Rimbawanto, A., Coolbear, P. and Firth, A. (1988b). Artificial ripening of prematurely harvested cones of New Zealand *Pinus radiata* and its effect on seed quality. N.Z. J. For. Sci. 18(2): 149-160.

Rimbawanto, A., Coolbear, P. and Firth, A. (1989). Morphological and physiological changes associated with the onset of germinability in *Pinus radiata* (D. Don) seeds. Seed Sci. and Technol. 17: 399-411.

Roberts, E.H. and King, M.W. (1980). The characteristics of recalcitrant seeds. In "Recalcitrant Crop Seed". (H.F. Chin and E.H. Roberts, ed.). Publ. Trop. Press SDN. BHD., Kualalumpur. pp. 1-5.

Schmidt, W. (1940). Die Bedeutung der Keimschnelligkeit für den Aussaatwert des Samens. (Zeit. f. Forst - u. Jagdw. 72: 35-47). (Cited in Baldwin, 1942).

Schubert, G.H. (1952). Germination of various coniferous seeds after cold storage. For. Res. Notes No. 83. Calfornia Forest and Range Ex. Stat.

Scott, D.J. and Close, R.C. (1976). An assessment of seed factors affecting field emergence of garden pea seed lots. Seed Sci. and Technol. 4:: 287-300.

Shelbourne, C.J.A. (1986). Historical introduction. In "Development Plant for Radiata Pine Breeding". Publ. For. Res. Inst., Rotorua, N.Z. pp. 1-6.

Simak, M. and Karma, S.K. (1963). Comparative studies on scots pine seed germinability with tetrazolium and x-ray contrast methods. Proc. Int. Seed Test. Ass. 28(1): 3-18.

Smith, A.J. and Grabe, D.F. (1985). Radiographic density measurements for determination of viability and vigour in corn (*Zea mays*). Seed Sci. and Technol. 13: 759-768.

Smith, O.E., Welch, N.C. and McCay, O.D. (1973). Studies on lettuce seed quality: II Relationship of seed vigor to emergence, seedling weight and yield. J. Amer. Soc. Hor. Sci. 98: 552-556. (Cited in Perry, 1981b).

Sorenson, F.C. and Campbell, R.K. (1985). Effect of seed weight on height growth of douglas-fir (*Pseudotsuga menziesii* (Mirb.) Franco var. menziesii) seedlings in a nursery. Can. J. For. Res. 15(6): 1109-1115.

St Angelo, A.J. and Ory, R.L. (1983). Lipid degradation during seed deterioration. Phytopathol. 73(2): 315-317.

Stewart, R.R.C. and Bewley, J.D. (1980). Lipid peroxidation associated with acclerated aging of soybean axes. Plant Phusiol. 65: 245-248.

Stormonth, D.A. (1978). Cereal vigour. In "Developments in the Business and Practice of Cereal Seed Trading and Technology". (P.R. Hayward, ed.). Publ. The Gavin Press, London. pp. 49-74.

Styer, R.C., Cantliffe, D.J. and Hall, C.B. (1980). The relationship of ATP concentration to germination and seedling vigor of vegetable seeds stored under various conditions. J. Amer. Soc. Hort. Sci. 105: 298-303.

Tamari, C. (1976). Phenology and seed storage trials of Dipterocarps. Res. Pamphlet No. 69. Publ. For. Res. Inst., Kepong, Malaysia. (Cited in King and Roberts, 1980).

Tasimin, Y.F. (1980). "The effects of tree age, light quality, stratification and chemical treatments on seed germination and seedling growth of *Pinus merkusii*". MSc Thesis, Univ. The Philippines, Los Banos.

Tilden, R.L. and West, S.H. (1985). Reversal of the effects of aging in soybean seeds. Plant Physiol. 77: 584-586.

Tully, R.E., Musgrave, M.E. and Leopold, A.C. (1981). The seed coat as a control of imbibitional chilling injury. Crop Sci. 21: 312-317.

Vertucci, C.W. (1989). The kinetics of seed imbibition: controlling factors and relevance to seedling vigor. In "Seed Moisture". (P.C. Stanwood and M.B. McDonald, ed.). Publ. CSSA Spec. Pub. No. 14., Madison, U.S.A. pp. 93-115.

Villiers, T.A. (1973). Ageing and longevity of seeds in field conditions. In "Seed Ecology". (W. Heydecker, ed.). Publ. Butterworths, Lond. pp. 265-288.

Vincent, T.G. (1986). Seed orchards. In "Development Plan for Radiata Pine Breeding". Publ. For. Res. Inst., Rotorua, N.Z. pp. 82-96.

Vincent, T.G. (1987). Certification system for forest tree seed and planting stock. (N.Z.) FRI Bull. No. 134.

Vozzo, J.A. (1984). Evaluating seed leachate measurements relative to imbibition time and solute volume. J. Seed Technol. 9(1): 54-59.

Wang, B.S.P. (1973). Laboratory germination criteria for red pine (*Pinus resinosa* AIT) seed. Proc. AOSA 63: 94-101.

Wilson, J.O. and McDonald, M.B. (1986). A convenient volatile aldehyde assay for measuring soybean seed vigour. Seed Sci. and Technol. 14: 259-268.

Woodstock, L.W. (1969). Biochemical test for seed vigour. Proc. ISTA 34: 253-263. (Cited in AOSA, 1983).

Woodstock, L.W. and Pollock, B.M. (1965). Physiological predetermination: imbibition, respiration and growth of lima bean seeds. Science 150: 1031-1032. (Cited in AOSA, 1983).

Woodstock, L.W. and Tao, K.L.J. (1981). Prevention of imbibition injury in low vigor soybean embryonic axes by osmotic control of water uptake. Physiol. Plant 5: 133-139. (Cited in Vertucci, 1989)

Woodstock, L.W. and Taylorson, R.B. (1981). Soaking injury and its reversal with polyethylene glycol in relation to respiratory metabolism in high and low vigor soybean seeds. Physiol. Plant 53: 263-268. (Cited in Vertucci, 1989).

Yaklich, R.W., Kulik, M.M. and Anderson, J.D. (1979). Evaluation of vigour tests in soybean seeds: relationship of ATP, conductivity, and radioactive tracer multiple criteria laboratory tests to field performance. Crop Sci. 19: 806-810.

APPENDICES

APPENDIX 1 Quantitative tetrazolium test results (All procedures using lot A)

A. Without preimbibition and surface sterilisation

Time of incubation in 0.7% TZ at 35°C (h)	OD_{510}[a] per gram seed	Standard error	Remarks
≤ 24	-	0	up to 24 h TZ incubation, only very light red colour was formed
36	0.914	0.103	-
38	0.836	0.110	fungi developed in the replication which had mid OD_{510} value
42	0.934	0.095	fungi developed in all replications
48	2.027	1.109	fungi developed in all replications

[a] optical density at 510 nm

B. With preimbibition in water for 24 hours and drying back at 35°C for 4 hours, but without surface sterilisation

Time of incubation in 0.7% TZ at 35°C (h)	OD_{510} per gram seed	Standard error	Remarks
≤ 24	-	-	Up to 24 hr TZ incubation, only very light red colour was formed
36	1.025	0.269	fungi developed in the replication which had the highest value of OD_{510}
38	1.248	0.293	fungi developed in the replication which had the highest value of OD_{510}
42	1.434	0.301	fungi developed in the replication which had the highest value of OD_{510}
48	1.372	0.305	-

Appendix 1 (continued)

C. With preimbibition in water for 1 day (without drying back) and with surface sterilisation[1]

Time of incubation in 0.7% TZ at 35°C (h)	OD_{510} per gram seed	Remarks
44	-	light red colour was formed, and no fungal development, but pellet and supernatant cannot be separated well

D. Without preimbibition, but with surface sterilisation[1] and drying back at 35°C for 2 hours

Time of incubation in 0.7% TZ at 35°C (h)	OD_{510} per gram seed	Remarks
72	-	no red colour formation and no fungal development. After 6 days imbibition light red colour was formed in fungi mycelium

[1] Method: 15 minutes in 1% NaOCl, then seeds were washed by water. Next, 10 minutes in 0.01 N HCl, then 6 washes with deionised water.

APPENDIX 2 Nursery and standard germination test procedure (carried out by FRI)

1. Nursery Test

In each lot, seeds of all clones were bulked to form one seed lot. All seed lots were treated by a thiram fungicide and soaked at 10°C for 48 hours. Next, they were sown at 6 cm spacing and then the beds were sprayed with pre-emergence weedicide (propazine/chlorthal) and covered with bird netting. A randomised complete block design with 12 replications was used in this experiment. All seedlings were conditioned following normal FRI nursery procedures.

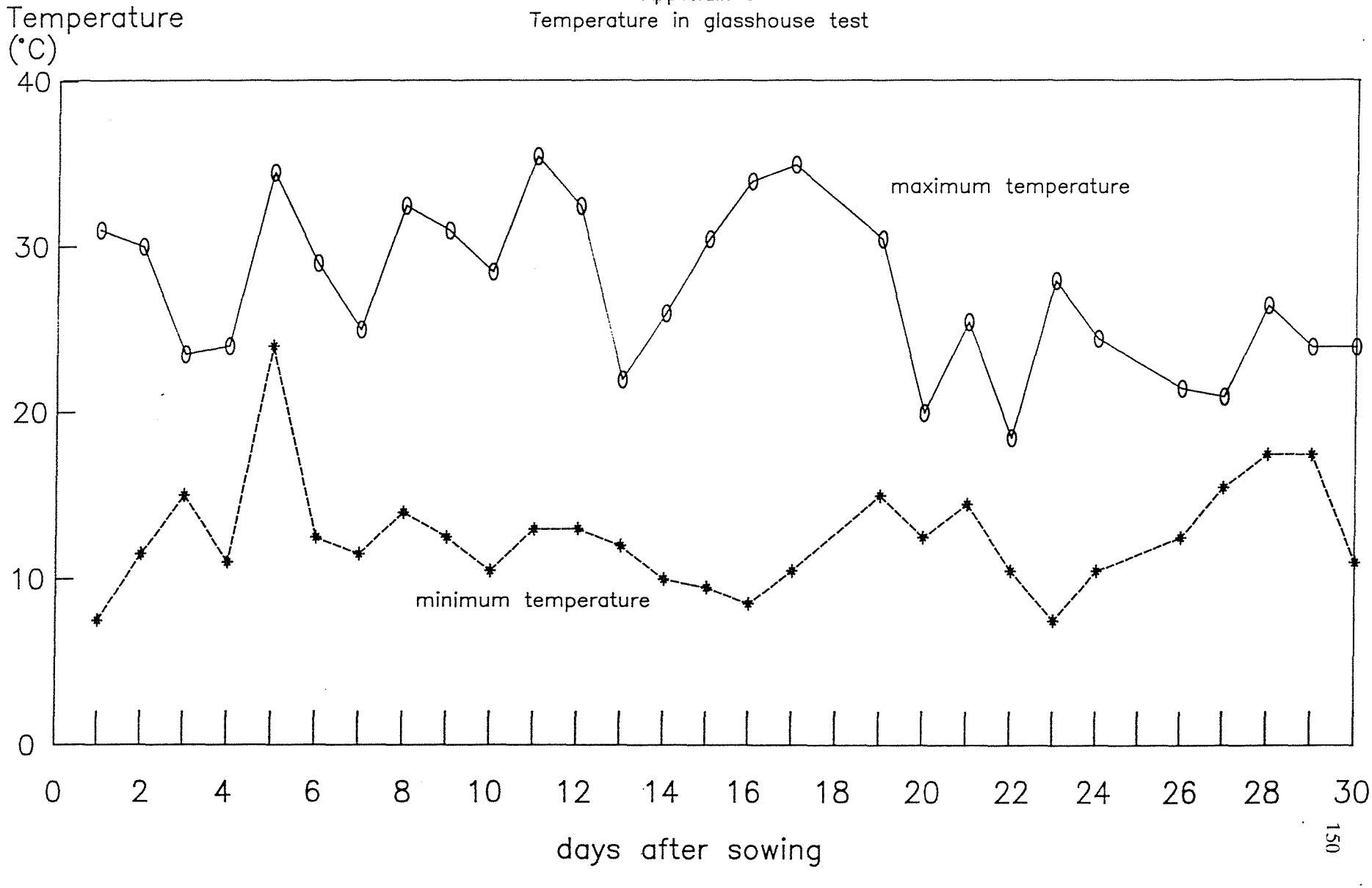
Germination was assessed daily after sowing, recording emergence until no further emergence occurred. Seedling health was visually assessed at two and three months after sowing. Seedling height was measured manually two months after sowing and then monthly until lifting when seedling height was recorded and root collar diameter measurements recorded with callipers. Dry weight measurement (after 72 h at 85°C) was divided into roots and tops. Plantable seedlings were those which had a root collar diameter of greater than 5 mm, and a sturdiness (height/diameter ratio) of less than 80. Dry weight distribution was based on 12 pooled samples of nine seedlings per seed lot divided into roots and tops.

Appendix 2 (continued)

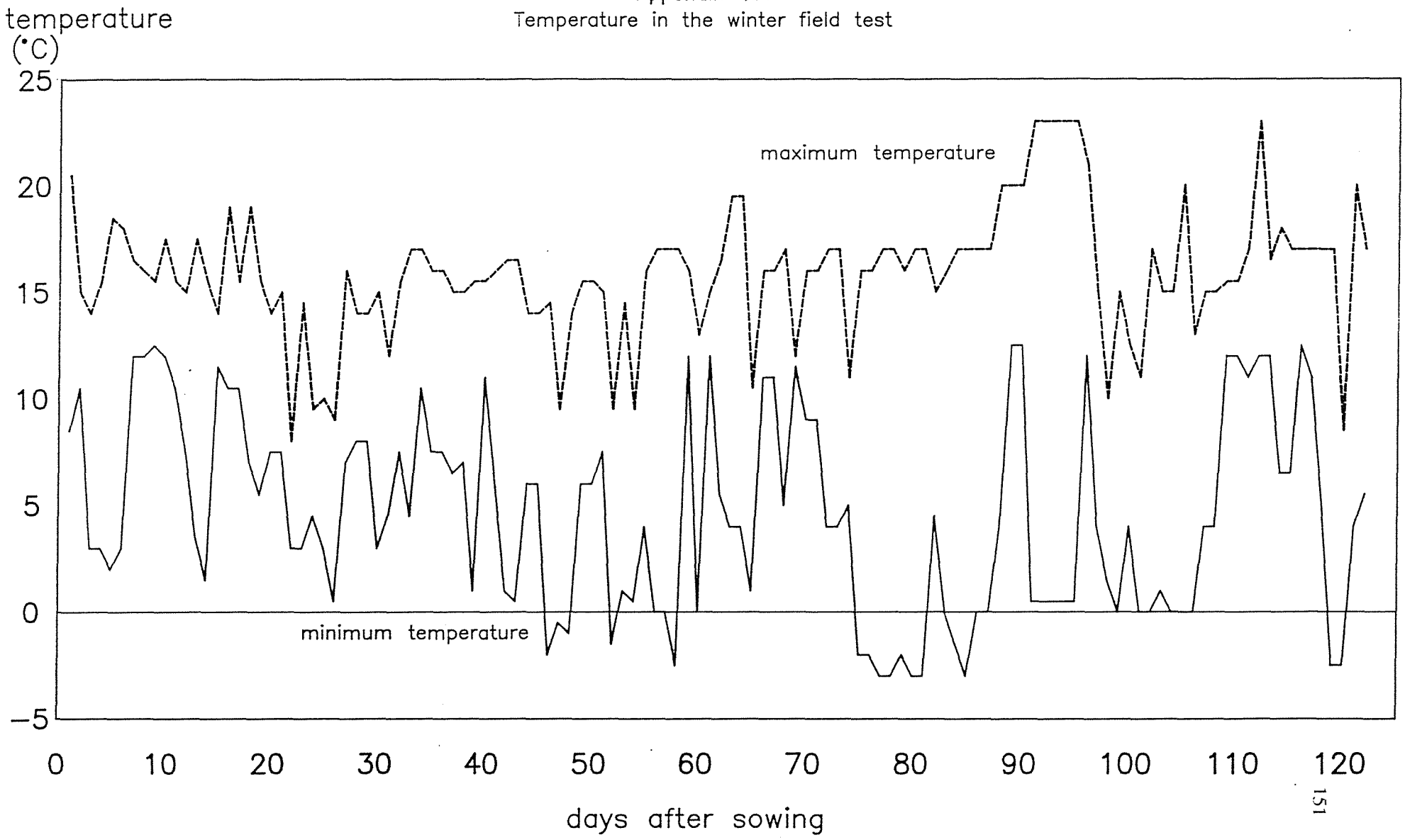
2. Standard Germination Test

In each seed lot, seeds of all clones were bulked to form one lot. For replications of 100 seeds from each seed lot were tested in a germination cabinet. Filter paper was used as the substrate and the cabinet was kept at approximately 20°C. A score was kept of the numbers germinated from over a period of 28 days. An important point to be noticed was that this method faced watering problem as seeds were not held in airtight boxes.

Appendix 3
Temperature in glasshouse test

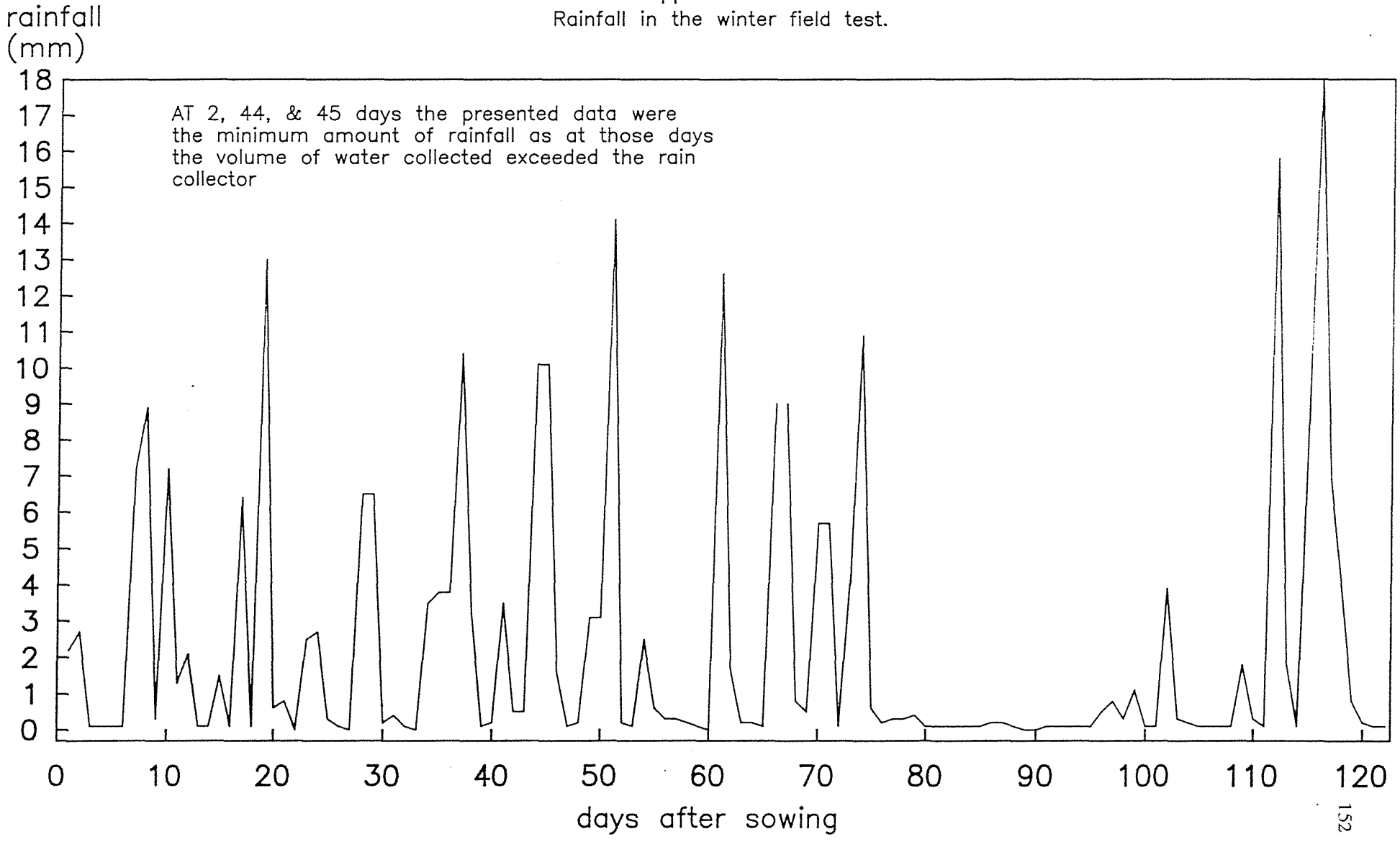


Appendix 4.A
Temperature in the winter field test



Appendix 4.B

Rainfall in the winter field test.



APPENDIX 5 **Seed lot performance in glasshouse and winter field tests. Within the same line, figures with the same letter are not significantly different.**

Tests		Seed lots					
Name	Parameter	A (heavy seeds unaged)	B (heavy seeds aged for 8 days)	C (heavy seeds aged for 10 days)	D (light seeds unaged)	E (light seeds aged for 8 days)	$lsd_{0.05}$
Glass house test	% normal seedling	89[a]	85[a]	72[b]	87[a]	87[a]	6.5203
	T_{50} normal seedling (days)	23.5[bc]	23.8[b]	24.9[a]	23.0[c]	23.7[bc]	0.78639
	norml seedl drywts (mg/normal seedl)	20.96[a]	20.43[ab]	19.74[b]	16.94[c]	17.35[c]	1.0352
Winter field test	cumltv emerg seedl (%)	72[b]	83[a]	69[b]	67[b]	69[b]	7.2155
	cumltv norml seedl (%)	65[a]	67[a]	55[b]	59[ab]	60[ab]	9.5245
	survive emerg seedl (%)	66[a]	65[ab]	55[b]	62[ab]	57[ab]	10.342
	survive norml seedl (%)	61[a]	60[a]	51[a]	57[a]	55[a]	10.479
	T_{50} emerg seedl (days)	48.3[c]	51.8[c]	61.8[a]	53.6[bc]	58.1[ab]	5.8152
	T_{50} norml seedl (days)	65.1[b]	64.6[b]	76.0[a]	69.4[b]	67.0[b]	5.1841
	norml seedl drywt (mg/norml seedl)	43.54[a]	41.17[a]	32.19[b]	30.13[b]	29.27[b]	3.5926

APPENDIX 6 Seed lot performance in vigour tests

A. In seedling growth, radiographic, topographical tetrazolium, and conductivity tests. Within the same line (except for seedling growth and low temperature/osmotic stress tests), figures with the same letter are not significantly different.

		Seed lots					
	Tests	A (heavy seeds unaged)	B (heavy seeds aged for 8 days)	C (heavy seeds aged for 10 days)	D (light seeds unaged)	E (light seeds aged for 8 days)	$lsd_{0.05}$
Unchilled seedling growth test with parameter of	% radicle emerg	96^{ab}	89^{abcd}	84^{cd}	91^{abc}	91^{abc}	9.4623[1]
	% norml seedl	85^{ab}	88^{ab}	82^{b}	80^{b}	90^{ab}	10.184[1]
	T_{50} radicle emerg (days)	9.0^{cd}	9.4^{bc}	10.6^{a}	8.6^{d}	9.9^{b}	0.5508[1]
	T_{50} norml seedl (days)	19.6^{ab}	19.9^{a}	19.9^{a}	18.5^{bc}	19.2^{ab}	1.1778[1]
	norml seedl drywt (mg/norml seedl)	18.83^{c}	20.55^{a}	19.72^{abc}	14.35^{e}	16.44^{d}	1.3081[1]
Prechilled seedling growth test with parameter of	% radicle emerg	97^{a}	90^{abcd}	66^{e}	87^{bcd}	81^{d}	
	% norml seedl	95^{a}	90^{ab}	64^{c}	85^{ab}	80^{b}	
	T_{50} radicle emerg (days)	6.8^{f}	7.4^{e}	8.6^{d}	6.9^{ef}	7.1^{ef}	
	T_{50} norml seedl (days)	16.9^{de}	16.0^{ef}	17.4^{cd}	16.3^{def}	15.6^{f}	
	norml seedl drywt (mg/norml seedl)	18.79^{c}	20.18^{ab}	18.96^{bc}	14.50^{e}	14.79^{e}	
Radiographic test with parameter of	% vigorous seeds	94^{a}	95^{a}	94^{a}	95^{a}	95^{a}	6.2748
Topographical tetrazolium test with parameter of	% vigorous seeds	100^{a}	97^{ab}	95^{b}	99^{ab}	98^{ab}	4.1912

[1] used for seed lot comparison in both unchilled and prechilled tests

Appendix 6 (continued)

	Tests	Seed lots A (heavy seeds unaged)	B (heavy seeds aged for 8 days)	C (heavy seeds aged for 10 days)	D (light seeds unaged)	E (light seeds aged for 8 days)	$lsd_{0.05}$
Conductivity test using 50 seeds (US seeds) with hours of imbibition	2 hours	2.47^{ab}	2.17^{b}	2.85^{a}	2.48^{ab}	2.32^{b}	0.40897
	4 hours	3.48^{ab}	3.02^{b}	4.02^{a}	3.07^{b}	3.41^{ab}	0.81639
	8 hours	5.04^{b}	4.83^{b}	6.60^{a}	4.01^{b}	4.76^{b}	1.4206
	10 hours	5.98^{ab}	5.46^{ab}	7.56^{a}	4.51^{b}	5.62^{ab}	2.3671
	24 hours	7.82^{ab}	7.92^{ab}	10.34^{a}	5.99^{b}	7.6^{ab}	4.2311
Conductivity test using 100 seeds with hours of imbibition	2 hours	3.32^{ab}	2.67^{b}	2.88^{ab}	3.40^{a}	2.88^{ab}	0.71006
	4 hours	3.89^{ab}	3.28^{b}	3.73^{ab}	3.96^{a}	3.85^{ab}	0.65763
	8 hours	4.58^{ab}	4.13^{b}	4.60^{ab}	4.97^{a}	4.65^{ab}	0.75281
	10 hours	5.03^{ab}	5.08^{b}	5.46^{ab}	5.49^{ab}	6.02^{a}	0.96206
	24 hours	6.27^{b}	7.13^{ab}	7.51^{ab}	6.78^{b}	10.62^{a}	3.7334

Appendix 6 (continued)

B. In stress tests

Tests			Seed lots					
Name		Parameter	A (heavy seeds unaged)	B (heavy seeds aged for 8 days)	C (heavy seeds aged for 10 days)	D (light seeds unaged)	E (light seeds aged for 8 days)	$lsd_{0.05}$
Controlled Deterioration test with 2 days aging		% norml seedl	89^{a}	91^{a}	56^{b}	89^{a}	80^{a}	11.465
Controlled Deterioration test with 4 days aging		% norml seedl	89^{a}	38^{c}	34^{c}	88^{a}	52^{b}	11.089
Complex stressing vigour test		% norml seedl	82^{a}	73^{ab}	56^{b}	83^{a}	56^{b}	19.473
Low temperature/ osmotic stress test with								
temp	osmotic potential							
10°C	-5 bar	% radicle emerg	61^{ef}	85^{abc}	79^{cd}	51^{f}	72^{de}	11.191^{2}
		T_{50} radicle emerg (days)	127.8^{b}	90.1^{de}	86.1^{de}	144.1^{a}	121.0^{bc}	13.455^{2}
		% fresh ungerm seeds	31^{b}	11^{de}	13^{d}	44^{a}	21^{c}	6.4883^{2}
10°C	0 bar (control)	% radicle emerg	92^{ab}	95^{ab}	69^{de}	87^{abc}	84^{bc}	
		T_{50} radicle emerg (days)	79.9^{e}	57.1^{f}	62.6^{f}	93.5^{d}	63.3^{f}	
		% fresh ungerm seeds	3^{fg}	0^{g}	0^{g}	1^{fg}	0^{g}	
15°C	-5 bar	% radicle emerg	91^{ab}	93^{ab}	88^{abc}	88^{abc}	79^{cd}	
		T_{50} radicle emerg (days)	78.4^{e}	35.2^{g}	55.0^{f}	109.3^{c}	52.3^{f}	
		% fresh ungerm seeds	7^{def}	3^{fg}	5^{efg}	3^{fg}	5^{efg}	
15°C	0 bar (control)	% radicle emerg	95^{ab}	92^{ab}	88^{abc}	87^{abc}	95^{ab}	
		T_{50} radicle emerg (days)	58.2^{f}	22.8^{ghij}	25.4^{ghi}	55.6^{f}	28.6^{gh}	
		% fresh ungerm seeds	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	
20°C (control)	-5 bar	% radicle emerg	87^{abc}	93^{ab}	87^{abc}	87^{abc}	93^{ab}	
		T_{50} radicle emerg (days)	19.4^{hij}	17.7^{hij}	20.7^{hij}	25.9^{ghi}	19.0^{hij}	
		% fresh ungerm seeds	4^{fg}	0^{g}	0^{g}	3^{fg}	0^{g}	
20°C	0 bar (control)	% radicle emerg	89^{abc}	89^{abc}	88^{abc}	96^{a}	92^{ab}	
		T_{50} radicle emerg (days)	14.9^{ij}	11.0^{j}	13.2^{ij}	13.2^{ij}	11.2^{j}	
		% fresh ungerm seeds	0^{g}	0^{g}	0^{g}	0^{g}	0^{g}	

[2] used for seed lot comparisons in all temperature/osmotic combinations

APPENDIX 7 Correlation (r) between vigour tests and glass house/winter field test of 5 lots of *P. radiata* seeds.

A. Seedling Growth Test

Tests		r with glass house test			r with winter field test						
Name	Parameter	r_1 (with % normal seedl)	r_2 (with T_{50} normal seedl)	r_3 (with nrml seedl drywt)	r_1 (with cumultv emerg seedl)	r_2 (with cumltv normal seedl)	r_3 (with survive emerg seedl)	r_4 (with survive normal seedl)	r_5 (with T_{50} emerg seedl)	r_6 (with T_{50} normal seedl)	r_7 (with normal seedl drywt)
Seedling Growth Test non Prechilling	% radicle emergence	0.90*	-0.77	0.07	-0.04	0.60	0.71	0.76	-0.83	-0.77	0.45
	% normal seedling	0.35	0.02	0.13	0.49	0.53	0.08	0.30	-0.60	-0.60	0.20
	T_{50} radicle emergence	-0.81	0.94*	0.16	-0.04	-0.50	-0.81	-0.74	0.47	0.62	-0.28
	T_{50} normal seedling	-0.48	0.75	0.85	0.60	0.27	-0.04	0.01	-0.16	0.06	0.57
	normal seedling drywt	-0.42	0.67	0.89*	0.70	0.37	0.11	0.13	-0.21	-0.02	0.66
Seedling Growth Test + Prechilling	% radicle emergence	0.90*	-0.79	0.23	0.36	0.85	0.94*	0.97**	-0.87	-0.90*	0.65
	% normal seedling	0.90*	-0.78	0.23	0.41	0.87*	0.95*	0.98**	-0.88*	-0.93*	0.65
	T_{50} radicle emergence	-0.99**	0.95*	0.24	0.01	-0.57	-0.69	-0.75	0.71	0.82	-0.22
	T_{50} normal seedling	-0.64	0.56	0.51	-0.26	-0.39	-0.18	-0.33	0.36	0.63	0.21
	normal seedling drywt	-0.36	0.53	0.95*	0.71	0.44	0.29	0.26	-0.24	-0.05	0.77

* significant at $P \leq 0.05$
** significant at $P \leq 0.01$

Appendix 7 (continued)

B. Test not involving germination

Tests/Parameter		r with glass house test			r with winter field test						
		r_1 (with % normal seedl)	r_2 (with T_{50} normal seedl)	r_3 (with nrml seedl drywt)	r_1 (with cumultv emerg seedl)	r_2 (with cumltv normal seedl)	r_3 (with survive emerg seedl)	r_4 (with survive normal seedl)	r_5 (with T_{50} emerg seedl)	r_6 (with T_{50} normal seedl)	r_7 (with normal seedl drywt)
Radiographic Test (% vigorous seeds)		0.47	-0.55	-0.64	0.23	0.22	0.13	0.20	-0.05	-0.42	-0.36
Topographical Tetrazolium Test (% vigorous seeds)		0.91*	-0.88*	-0.12	-0.17	0.48	0.68	0.69	-0.68	-0.69	0.29
Conductivity Test using 50 seeds (μ s/cm/g) with hours of imbibition	2 hours	-0.76	0.60	0.05	-0.62	-0.80	-0.61	-0.74	0.70	0.90*	-0.31
	4 hours	-0.77	0.82	0.26	-0.43	-0.64	-0.70	-0.71	0.45	0.75	-0.17
	8 hours	-0.88	0.97**	0.50	-0.04	-0.43	-0.58	-0.60	0.42	0.68	0.04
	10 hours	-0.82	0.94*	0.51	-0.07	-0.41	-0.57	-0.57	0.34	0.63	0.06
	24 hours	-0.85	0.98**	0.54	0.09	-0.34	-0.55	-0.55	0.35	0.60	0.09
Conductivity Test using 100 seeds (μ s/cm/g) with hours of imbibition	2 hours	0.41	-0.61	-0.25	-0.63	-0.13	0.31	0.19	-0.07	-0.03	-0.03
	4 hours	0.19	-0.32	-0.50	-0.94*	-0.52	-0.23	-0.27	0.16	0.25	-0.45
	8 hours	0.08	-0.35	-0.70	-0.93*	-0.65	-0.30	-0.39	0.42	0.39	-0.65
	10 hours	-0.07	0.04	-0.81	-0.57	-0.60	-0.74	-0.63	0.40	0.29	-0.89*
	24 hours	0.01	0.14	-0.53	-0.11	-0.20	-0.59	-0.39	0.02	-0.00	-0.60

* significant at $P \leq 0.05$
** significant at $P \leq 0.01$

Appendix 7 (continued)

C. Stress Test

Tests/Parameter	r with glass house test			r with winter field test						
	r_1 (with % normal seedl)	r_2 (with T_{50} normal seedl)	r_3 (with nrml seedl drywt)	r_1 (with cumultv emerg seedl)	r_2 (with cumltv normal seedl)	r_3 (with survive emerg seedl)	r_4 (with survive normal seedl)	r_5 (with T_{50} emerg seedl)	r_6 (with T_{50} normal seedl)	r_7 (with normal seedl drywt)
Controlled Deterioration Test with 2 days aging (% normal seedling)	0.93*	-0.89*	-0.03	0.38	0.79	0.87*	0.91*	-0.74	-0.91*	0.43
Controlled Deterioration Test with 4 days aging (% normal seedling)	0.68	-0.79	-0.19	-0.43	0.16	0.53	0.46	-0.36	-0.35	0.13
Complex Stressing Vigour Test (% normal seedling)	0.62	-0.75	0.14	0.13	0.52	0.87	0.75	-0.44	-0.51	0.50

* significant at $P \leq 0.05$

Appendix 7 (continued)

C. Stress Test

Low Temperature/Osmotic Stress Tests			r with glass house test			r with winter field test						
Temp.	Osmotic Potential	Parameter	r_1 (with % normal seedl)	r_2 (with T_{50} normal seedl)	r_3 (with nrml seedl drywt)	r_1 (with cumultv emerg seedl)	r_2 (with cumltv normal seedl)	r_3 (with survive emerg seedl)	r_4 (with survive normal seedl)	r_5 (with T_{50} emerg seedl)	r_6 (with T_{50} normal seedl)	r_7 (with normal seedl drywt)
10°C	-5 bar	% radicle emergence	-0.50	0.72	0.46	0.65	0.14	-0.25	-0.22	0.04	0.08	0.20
		T_{50} radicle emergence	0.72	-0.86	-0.55	-0.55	0.03	0.30	0.34	-0.23	-0.32	-0.21
		% fresh ungerminated seeds	0.55	-0.78	-0.49	-0.57	-0.08	0.31	0.28	-0.05	-0.13	-0.20
	0 bar (control)	% radicle emergence	0.87	-0.75	0.19	0.58	0.93*	0.90*	0.96**	-0.84	-0.96*	0.61
		T_{50} radicle emergence	0.43	-0.69	-0.39	-0.56	-0.12	0.32	0.25	0.03	-0.03	-0.14
		% fresh ungerminated seeds	0.50	-0.45	0.38	-0.15	0.37	0.63	0.61	-0.57	-0.37	0.57
15°C	-5 bar	% radicle emergence	-0.05	0.00	0.70	0.60	0.50	0.68	0.52	-0.21	-0.16	0.76
		T_{50} radicle emergence	0.32	-0.61	-0.45	-0.66	-0.26	0.19	0.11	0.15	0.10	-0.25
		% fresh ungerminated seeds	0.04	0.21	0.45	-0.28	0.01	0.00	0.06	-0.39	-0.03	0.34
	0 bar (control)	% radicle emergence	0.55	-0.16	0.33	0.29	0.60	0.30	0.48	-0.86	-0.71	0.45
		T_{50} radicle emergence	0.55	-0.69	-0.09	-0.43	0.12	0.51	0.47	-0.29	-0.23	0.18
		% fresh ungerminated seeds[1]	-	-	-	-	-	-	-	-	-	-
20°C (control)	-5 bar	% radicle emergence	0.27	-0.04	-0.09	0.57	0.44	0.00	0.16	-0.35	-0.52	0.00
		T_{50} radicle emergence	0.01	-0.41	-0.64	-0.66	-0.52	-0.11	-0.21	0.54	0.40	-0.54
		% fresh ungerminated seeds	0.54	-0.64	0.08	-0.30	0.23	0.61	0.55	-0.38	-0.29	0.34
	0 bar (control)	% radicle emergence	0.49	-0.75	-0.87	-0.49	-0.20	0.02	0.03	0.19	0.08	-0.59
		T_{50} radicle emergence	0.01	-0.08	0.31	-0.44	-0.13	0.25	0.14	-0.07	0.19	0.30
		% fresh ungerminated seeds[1]	-	-	-	-	-	-	-	-	-	-

* significant at $P \leq 0.05$

** significant at $P \leq 0.01$

[1] % fresh ungerminated seeds = 0, therefore there is no data for correlation coefficient

APPENDIX 8 **Seed lot performance in standard germination and nursery tests (carried out by FRI). Within same line, and same section, figures with the same letter are not significantly different.**

Test	Parameter	Seed lots 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Standard germination	germinated seed (%)	80^{a}	79^{a}	79^{a}	83	85	90	90	74	52	43	46	34	55	45	75.5^{b}	86.0^{a}
Nursery	% field germination at 28 das	88.7^{a}	84.1^{a}	85.6^{a}	87.2^{a}	80.0^{a}	85.4^{a}	84.2^{a}	83.2^{a}	80.3^{ab}	68.1^{c}	71.0^{bc}	67.4^{c}	80.0^{ab}	82.8^{a}	88.4^{a}	93.1^{a}
	T_{50} field germination (days)[1]	18.4	19.8	18.5	17.7	18.8	18.3	19.0	17.4	20.8	22.5	20.5	22.8	18.8	18.9	17.9	17.6
	% healthy seedling at 38 das[1]	72.4	58.9	64.1	69.2	64.8	71.5	70.7	62.6	57.4	49.8	51.6	47.7	57.2	56.3	66.9	71.6
	% healthy seedling at 66 das[1]	70.8	57.4	59.7	64.0	61.8	68.1	68.5	58.5	56.0	49.3	50.5	45.8	56.5	57.6	67.8	71.6
	seedling height (cm) at 275 das	39.3^{a}	37.9^{a}	37.7^{a}	39.3^{a}	40.3^{a}	41.6^{a}	38.8^{a}	39.4^{a}	36.1^{a}	35.8^{a}	35.3^{a}	35.7^{a}	38.0^{a}	38.0^{a}	30.0^{b}	35.0^{a}
	seedling diameter (mm)	6.2^{a}	6.1^{a}	5.9^{a}	6.3^{a}	6.5^{a}	6.6^{a}	6.2^{a}	6.5^{a}	6.5^{a}	6.2^{a}	6.0^{a}	6.1^{a}	6.4^{a}	6.4^{a}	5.85^{b}	6.44^{a}
	% plantable seedling[1]	64.8	51.2	53.9	60	60	62	61	54	54	46	45	43	51	54	55.0	63.0
	shoot dry weight (gram)	88.0^{a}	93.0^{a}	87.7^{a}	81.7^{a}	86.4^{a}	92.8^{a}	85.7^{a}	81.1^{a}	91.1^{ab}	90.8^{b}	92.7^{ab}	84.7^{b}	102.8^{a}	92.4^{b}	112.3^{b}	158.8^{a}
	root dry weight (gram)	12.0^{a}	13.8^{a}	12.9^{a}	12.17^{a}	13.13^{a}	14.47^{a}	12.44^{a}	12.81^{a}	15.0^{abc}	13.1^{c}	15.4^{ab}	14.3^{bc}	16.7^{a}	16.3^{ab}	21.1^{b}	29.7^{a}
		group 1			group 2					A		group 3		B		group 4	

[1] no lsd value available

APPENDIX 9 Seed lot ranking in standard germination and nursery test according to relative vigour score.

Test	Parameter	Seed lot rank according to relative vigour score[1] lower vigour 1	2	3	4	5	6	7	8	9	10	11	12	higher vigour 13	14	15	16
Standard germination	germinated seed (%)	12	10	14	11	9	13	8	15	3	2	1	4	5	16	6,7[2]	-
Nursery	% field germination at 28 das	12	10	11	13,5[2]	9	14	8	2	7	6	3	4	15	1	16	-
	T_{50} field germination	12	10	9	11	2	7	14	13,5[2]	3	1	6	15	4	16	8	-
	% healthy seedling at 38 das	12	10	11	14	13	9	2	8	3	5	15	4	7	6	16	1
	% healthy seedling at 66 das	12	10	11	9	13	2	14	8	3	5	4	15	6	7	1	16
	seedling height at 275 das	15	16	11	12	10	9	3	2	14,13[2]	7	4,1[2]	8	5	6	-	-
	seedling diameter	15	3	11	2,12	10,7,1[2]	4	14,13[2]	16	5,8,9[2]	6	-	-	-	-	-	-
	% plantable seedling	12	11	10	13	2	3	14,9,8[2]	15	4,5[2]	7	6	16	1	-	-	-
	shoot dry weight	7	8	4	12	5	3	1	10	9	14	11	6	2	13	15	16
	root dry weight	1	4	7	8	3	10	5	2	12	6	9	11	14	13	15	16

[1] relative vigour scores (rvs) are arranged according to mean value of parameter measured. For all parameters except T_{50} field germination, the higher the value, the higher also the rvs. But for T_0 field germination, the higher the value the lower the rvs.

[2] they have the same mean value.

APPENDIX 10 Seed lot ranking in the overall seed lots in standard germination and nursery tests according to relative vigour score

Standard Germination Test	RVS	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
	Seed lot no	12	10	14	11	9	13	8	15	3	2	1	4	5	16	6,7
Nursery Test	Mean RVS[1]	2.9	3.9	4.9	6.7	7.1	7.8	7.9	8.0	8.3	8.8	9.8	10.2	11.7	12.7	
	Seed lot no	12	10	11	9	3	13,14	7	2,8	5	4	15	1	6	16	

lower vigour → higher vigour

[1] Calculation to obtain mean RVS can be seen in Appendix 11

APPENDIX 11 Calculation of mean relative vigour score in nursery test

	relative vigour score (rvs) on parameter of									
Seed lot no.	% field germination at 28 das (RVS_1)	T_{50} field germination (RVS_2)	% healthy seedling at 38 das (RVS_3)	% healthy seedling at 66 das (RVS_4)	height at 275 das (RVS_5)	seedling seedling diameter (RVS_6)	% plantable seedling (RVS_7)	shoot dry weight (RVS_8)	root dry weight (RVS_9)	Mean RVS $\frac{\Sigma rvs}{g}$
1	14	10	16	15	11	5	13	7	1	10.2
2	8	5	7	14	8	4	5	13	8	8.0
3	11	9	9	9	7	2	6	6	5	7.1
4	12	13	12	11	11	6	9	3	2	8.8
5	4	8	10	10	13	9	9	5	7	8.3
6	10	11	14	13	14	10	11	12	10	11.7
7	9	6	13	14	10	5	10	1	3	7.9
8	7	15	8	8	12	9	7	2	4	8.0
9	5	3	6	4	6	9	7	9	11	6.7
10	2	2	2	2	5	5	3	8	6	3.9
11	3	4	3	3	3	3	2	11	12	4.9
12	1	1	1	1	4	4	1	4	9	2.9
13	4	8	5	5	9	7	4	14	14	7.8
14	6	7	4	7	9	7	7	10	13	7.8
15	13	12	11	12	1	1	8	15	15	9.8
16	15	14	15	16	2	8	12	16	16	12.7

APPENDIX 12 Seed lot performance in vigour tests done at Seed Technology Centre. Within the same line, figures with the same letters are not significantly different

Tests	Parameter	Seed lots 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	$lsd_{0.05}$
Seedling Growth + Prechilling	% radicle emergence	87^{cd}	96^{ab}	97^{a}	95^{abc}	92^{abcd}	95^{abc}	96^{ab}	88^{bcd}	93^{abc}	88^{bcd}	77^{e}	84^{de}	92^{abcd}	96^{ab}	88^{bcd}	93^{abc}	8.9591
	% normal seedling	81^{cde}	96^{a}	96^{a}	95^{ab}	89^{abc}	93^{ab}	96^{a}	85^{bcd}	72^{e}	85^{bcd}	72^{e}	77^{de}	89^{abc}	95^{ab}	87^{abcd}	92^{ab}	9.9032
	% fresh ungerminated seeds	5^{a}	3^{a}	1^{a}	3^{a}	1^{a}	1^{a}	3^{a}	8^{a}	4^{a}	9^{a}	1^{a}	9^{a}	5^{a}	0^{a}	4^{a}	5^{a}	-[2]
	T_{50} radicle emergence (days)	6.3^{de}	6.0^{ef}	5.4^{ef}	5.2^{f}	5.8^{ef}	5.8^{ef}	5.8^{ef}	5.2^{ef}	7.2^{cd}	7.7^{bc}	8.6^{ab}	9.0^{a}	7.4^{cd}	7.3^{cd}	5.7^{ef}	5.3^{ef}	1.0902
	T_{50} normal seedling (days)	17.0^{cd}	17.0^{cd}	16.9^{cd}	16.6^{d}	16.7^{cd}	17.1^{cd}	17.2^{cd}	16.8^{cd}	17.8^{bc}	18.4^{ab}	19.4^{a}	18.5^{ab}	17.7^{bcd}	17.6^{bcd}	16.7^{d}	16.8^{cd}	1.1324
	dry weight (mg/normal seedl)	13.67^{bcd}	13.73^{bcd}	15.47^{ab}	14.90^{bcd}	15.7^{bc}	17.00^{a}	15.00^{bcd}	14.73^{bcd}	12.27^{cde}	13.76^{bcd}	14.83^{bcd}	13.93^{bcd}	13.10^{de}	14.20^{bcd}	11.40^{e}	13.57^{bcd}	1.9309[3]
Controlled Deterioration (with 2 days aging treatment)	% radicle emergence	89^{bcd}	95^{ab}	89^{bcd}	93^{ab}	99^{a}	93^{ab}	92^{abc}	88^{bcd}	92^{abcd}	77^{ef}	73^{f}	83^{de}	87^{bcd}	84^{cde}	89^{bcd}	95^{ab}	8.3744
	% normal seedling	88^{abcd}	92^{abc}	88^{abcd}	92^{abc}	97^{a}	90^{abcd}	92^{abc}	85^{bcd}	84^{cd}	72^{ef}	71^{f}	*81^{de}	81^{de}	85^{bcd}	89^{abcd}	95^{ab}	9.5269
	% fresh ungerminated seeds	5^{a}	1^{a}	4^{a}	3^{a}	0^{a}	4^{a}	7^{a}	10^{a}	4^{a}	9^{a}	8^{a}	11^{a}	4^{a}	4^{a}	5^{a}	1^{a}	-[2]
	T_{50} radicle emergence (days)	9.4^{cde}	9.1^{de}	8.7^{ef}	7.9^{f}	8.5^{ef}	7.7^{f}	9.2^{de}	8.4^{ef}	10.4^{abc}	10.5^{abc}	10.0^{bcd}	11.3^{a}	10.6^{ab}	9.3^{cde}	8.7^{ef}	8.5^{ef}	1.1993
	T_{50} normal seedling (days)	19.8^{abc}	20.0^{ab}	19.3^{bcde}	18.8^{def}	19.4^{bcd}	19.2^{bcde}	19.1^{cde}	18.6^{def}	20.6^{a}	20.3^{a}	19.3^{bcde}	20.5^{a}	20.0^{abc}	19.4^{bcd}	18.4^{ef}	18.2^{f}	0.8833
	dry weight (mg/normal seedl)	13.93^{cde}	14.40^{bcde}	14.10^{bcde}	14.30^{bcde}	13.87^{cde}	16.97^{a}	14.90^{bcd}	13.73^{de}	16.73^{a}	13.83^{cde}	13.10^{ef}	13.13^{ef}	15.77ab	13.57^{de}	11.60^{f}	15.47^{abc}	1.6778
Complex Stressing Vigour	% radicle emergence	88^{ab}	88^{ab}	81^{abcd}	83^{abcd}	93^{a}	87^{ab}	85^{abc}	83^{abcd}	69^{d}	70^{cd}	77^{bcd}	36^{e}	68^{d}	75^{bcd}	75^{bcd}	89^{ab}	16.253
	% normal seedling	87^{ab}	85^{ab}	75^{abcde}	83^{abcd}	87^{ab}	85^{ab}	84^{abc}	75^{abcd}	47^{g}	57^{fg}	68^{cdef}	23^{h}	59^{efg}	72^{bcdef}	67^{def}	88^{a}	16.814
	% fresh ungerminated seeds	4^{e}	4^{e}	9^{de}	8^{de}	5^{e}	5^{e}	11^{de}	5^{de}	18^{bcd}	9^{de}	7^{de}	53^{a}	28^{b}	13^{cde}	23^{bc}	4^{e}	11.947
	T_{50} radicle emergence (days)	7.1^{cdef}	7.3^{cdef}	8.1^{cdef}	6.8^{ef}	6.9^{def}	6.6^{f}	6.4^{f}	6.8^{ef}	12.0^{b}	9.4^{bcde}	9.7^{bc}	16.5^{a}	11.3^{b}	9.6^{bcd}	7.2^{cdef}	6.6^{ef}	2.7659
	T_{50} normal seedling (days)	18.8^{efgh}	18.6^{fghi}	19.3^{defg}	18.1^{ghi}	18.8^{efgh}	17.8^{hi}	17.4^{i}	17.4^{i}	22.5^{ab}	19.7^{cdef}	20.0^{cde}	22.8^{a}	21.0^{bc}	20.5^{bcd}	17.8^{hi}	18.2^{ghi}	1.3308[3]
	dry weight (mg/normal seedl)	12.40^{a}	14.20^{a}	15.20^{a}	14.07^{a}	15.83^{a}	16.30^{a}	14.63^{a}	12.87^{a}	14.97^{a}	15.27^{a}	15.17^{a}	9.73^{a}	12.53^{a}	15.57^{a}	12.97^{a}	14.77^{a}	-[2]

[1] No lsd value available for comparing all seed lots

[2] No significant difference according to F test

[3] Due to missing data harmonic mean of cell size = 2.909091 was used to compute lsd value

* Originally (before reducing decimal figure), the mean value was slightly higher than lot 13

APPENDIX 13 Seed lot ranking in vigour tests according to relative vigour score.[1] Figures with the same letter are not significantly different.

Test	Parameter	Seed lot number according to relative vigour score (RVS)															
		lower vigour												higher vigour			
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Seedling Growth + Prechilling	% radicle emergence	11^{e}	12^{de}	1^{cd}	10^{bcd}	8^{bcd}	15^{bcd}	13^{abcd}	5^{abcd}	16^{abc}	9^{abc}	6^{abc}	4^{abc}	14^{ab}	7^{ab}	2^{ab}	3^{a}
	% normal seedling	11^{e}	9^{e}	12^{de}	1^{cde}	10^{bcd}	8^{bcd}	15^{abcd}	13^{abc}	5^{abc}	16^{ab}	6^{ab}	14^{ab}	4^{ab}	7^{a}	2^{a}	3^{a}
	T_{50} radicle emergence	12^{a}	11^{ab}	10^{bc}	13^{cd}	14^{cd}	9^{cd}	1^{de}	2^{ef}	7^{ef}	6^{ef}	15^{ef}	5^{ef}	3^{ef}	16^{ef}	8^{ef}	4^{f}
	T_{50} normal seedling	11^{a}	12^{ab}	10^{ab}	9^{bc}	13^{bcd}	14^{bcd}	7^{cd}	6^{cd}	2^{cd}	1^{cd}	3^{cd}	16^{cd}	8^{cd}	5^{cd}	15^{d}	4^{d}
	normal seedling dry weight	15^{e}	13^{de}	9^{cde}	16^{bcd}	1^{bcd}	10^{bcd}	2^{bcd}	12^{bcd}	14^{bcd}	8^{bcd}	11^{bcd}	4^{bcd}	7^{bcd}	5^{bc}	3^{ab}	6^{a}
Controlled Deterioration (with 2 days aging treatment)	% radicle emergence	11^{f}	10^{ef}	12^{de}	14^{cde}	13^{bcd}	8^{bcd}	15^{bcd}	1^{bcd}	3^{bcd}	9^{abcd}	7^{abc}	4^{ab}	6^{ab}	16^{ab}	2^{ab}	5^{a}
	% normal seedling	11^{f}	10^{ef}	12^{de}	13^{de}	9^{cd}	14^{cd}	8^{bcd}	3^{abcd}	1^{abcd}	15^{abcd}	6^{abcd}	7^{abc}	4^{abc}	2^{abc}	16^{ab}	5^{a}
	T_{50} radicle emergence	12^{a}	13^{ab}	10^{abc}	9^{abc}	11^{bcd}	1^{cde}	14^{cde}	7^{de}	2^{de}	3^{ef}	15^{ef}	16^{ef}	5^{ef}	8^{ef}	4^{f}	6^{f}
	T_{50} normal seedling	9^{a}	12^{a}	10^{a}	2^{ab}	13^{abc}	1^{abc}	14^{bcd}	5^{bcd}	3^{bcde}	11^{bcde}	6^{bcde}	7^{cde}	4^{def}	8^{def}	15^{ef}	16^{f}
	normal seedling dry weight	15^{f}	11^{ef}	12^{ef}	14^{de}	8^{de}	10^{cde}	5^{cde}	1^{cde}	3^{bcde}	4^{bcde}	2^{bcde}	7^{bcd}	16^{abc}	12^{ab}	9^{a}	6^{a}
Complex Stressing Vigour	% radicle emergence	12^{e}	13^{d}	9^{d}	10^{cd}	14^{bcd}	15^{bcd}	11^{bcd}	3^{abcd}	8^{abcd}	4^{abcd}	7^{abc}	6^{ab}	2^{ab}	1^{ab}	16^{ab}	5^{a}
	% normal seedling	12^{h}	9^{g}	10^{fg}	13^{efg}	15^{def}	11^{cdef}	14^{bcdef}	3^{abcde}	8^{abcd}	4^{abcd}	7^{abc}	6^{ab}	2^{ab}	5^{ab}	1^{ab}	16^{a}
	T_{50} radicle emergence	12^{a}	9^{b}	13^{b}	11^{bc}	14^{bcd}	10^{bcde}	3^{cdef}	2^{cdef}	15^{cdef}	1^{cdef}	5^{def}	8^{ef}	4^{ef}	16^{ef}	6^{f}	7^{f}
	T_{50} normal seedling	12^{a}	9^{ab}	13^{bc}	14^{bcd}	11^{cde}	10^{cdef}	3^{defg}	5^{efgh}	1^{efgh}	2^{fghi}	16^{ghi}	4^{ghi}	6^{hi}	15^{hi}	8^{i}	7^{i}
	normal seedling dry weight	12^{a}	1^{a}	13^{a}	15^{a}	8^{a}	4^{a}	2^{a}	7^{a}	16^{a}	9^{a}	11^{a}	3^{a}	10^{a}	14^{a}	5^{a}	6^{a}

[1] RVS is arranged according to mean value of each parameter. For % radicle emergence, % normal seedling and seedling dry weight, the higher the value, the higher also the RVS. But for T_{50} radicle and T_{50} normal seedling, the higher the value of parameters, the lower the RVS.

APPENDIX 14 Seed lot ranking (within groups) in vigour test according to mean relative vigour score (rvs). The higher is the rvs, the higher the vigour.

Tests	Mean rvs/seed lots	Group 1				2				3 A				B			4
SG+pr	mean rvs	5.8	9.4	14.2	9.8	11.2	11.4	13.8	-	3.2	4.2	5	3.2	5.2	9	8	9.8
	seed lots	1	2	3	8	6	5,7	4	-	11	10	9	12	13	14	15	16
CD2d	mean rvs	7.4	9	10.6	9.2	11	12	12.6	13.4	3.2	3.8	7	2.4	5.6	6	8.8	14
	seed lots	1	3	2	8	7	5	4	6	10	11	9	12	14	13	15	16
CSVT	mean rvs	8.4	10	10.2	10	10.2	12.4	12.8	13.6	4	6.6	6.8	1	2.6	6.6	7.8	13
	seed lots	3	1	2	8	4	7	5	6	9	10	11	12	13	14	15	16

Calculation to obtain mean rvs can be seen in Appendix 16

APPENDIX 15 Seed lot ranking in vigour test according to mean rvs[1]

Seedling Growth Test + Prechilling	mean RVS	3.2	4.2	5	5.2	5.8	8	9	9.4	9.8	11.2	11.4	13.8	14.2	-	-	-
	seed lot no	11,12	10	9	13	1	15	14	2	8,16	6	5,7	4	3	-	-	-
Controlled Deterioration Test (with 2 days aging treatment)	mean RVS	2.4	3.2	3.8	5.6	6	7	7.4	8.8	9	9.2	10.6	11	12	12.6	13.4	14
	seed lot no	12	10	11	14	13	9	1	15	3	8	2	7	5	4	6	16
Complex Stressing Vigour Test	mean RVS	1	2.6	4	6.6	6.8	7.8	8.4	10	10.2	12.4	12.8	13	13.6	-	-	-
	seed lot no.	12	13	9	10,14	11	15	3	1,8	2,4	7	5	16	6	-	-	-

lower vigour — higher vigour

[1] calculation to obtain mean rvs can be seen in Appendix 16

APPENDIX 16 Calculation to obtain mean relative vigour score (rvs) in vigour tests.

Test	Parameter	Relative vigour score (RVS) of seed lot no.															
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Seedling Growth + Prechilling	% radicle emergence	3	15	16	12	8	11	14	5	10	4	1	2	7	13	6	9
	% normal seedling	4	15	16	13	9	11	14	6	2	5	1	3	8	12	7	10
	T_{50} radicle emergence	7	8	13	16	12	10	9	15	6	3	2	1	4	5	11	14
	T_{50} normal seedling	10	2	11	16	14	8	7	13	4	3	1	2	5	6	15	12
	normal seedling dry weight	5	7	15	12	14	16	13	10	3	6	11	8	2	9	1	4
	mean RVS	5.8	9.4	14.2	13.8	11.4	11.2	11.4	9.8	5	4.2	3.2	3.2	5.2	9	8	9.8
Controlled Deterioration (with 2 days aging treatment)	% radicle emergence	8	15	9	12	16	13	11	6	10	2	1	3	5	4	7	14
	% normal seedling	9	14	8	13	16	11	12	7	5	2	1	3	4	6	10	15
	T_{50} radicle emergence	6	9	10	15	13	16	8	14	4	3	5	1	2	7	11	12
	T_{50} normal seedling	6	4	9	13	8	11	12	14	1	3	10	2	5	7	15	16
	normal seedling dry weight	8	11	9	10	7	16	12	5	15	6	2	3	14	4	1	13
	mean RVS	7.4	10.6	9	12.6	12	13.4	11	9.2	7	3.2	3.8	2.4	6	5.6	8.8	14
Complex Stressing Viigour	% radicle emergence	14	13	8	10	16	12	11	9	3	4	7	1	2	5	6	15
	% normal seedling	15	13	8	10	14	12	11	9	3	4	7	1	2	5	6	16
	T_{50} radicle emergence	10	8	7	13	11	15	16	12	2	6	4	1	3	5	9	14
	T_{50} normal seedling	9	10	7	12	8	13	16	15	2	6	5	1	3	4	14	11
	normal seedling dry weight	2	7	12	6	15	16	8	5	10	13	11	1	3	14	4	9
	mean RVS	10	10.2	8.4	10.2	12.8	13.6	12.4	10	4	6.6	6.8	1	2.6	6.6	7.8	13

APPENDIX 17 Correlation of seed weight, standard germination, and vigour tests with nursery test.

Test	Parameter	r with nursery test								
		r_1 (with % field germination at 28 d)	r_2 (with T_{50} field germination)	r_3 (with % healthy seedling at 38 das)	r_4 (with % healthy seedling at 66 das)	r_5 (with seedling height at 275 das)	r_6 (with seedling diameter)	r_7 (with % plantable seedling)	r_8 (with shoot dry weight)	r_9 (with root dry weight)
Seed Weight	100 seed wt	0.09	-0.05	0.22	0.11	0.82****	0.56*	0.33	-0.23	-0.31
Standard Germination (by FRI)	% normal seedling	0.80***	-0.76***	0.93****	0.87****	0.36	0.16	0.84****	0.16	0.08
Seedling Growth + Prechilling	% radicle emergence	0.58*	-0.41	0.47	0.44	0.40	0.31	0.51*	0.04	-0.31
	% normal seedling	0.56*	-0.54*	0.50*	0.48	0.34	0.06	0.45	0.10	0.05
	% fresh ungerminated seeds	-0.37	0.41	-0.33	-0.33	-0.26	-0.02	-0.35	0.05	0.03
	T_{50} radicle emergence	-0.83****	0.82****	-0.84****	-0.78***	-0.29	-0.20	-0.77***	-0.16	-0.10
	T_{50} normal seedling	-0.85****	0.79***	-0.81***	-0.77***	-0.27	-0.22	-0.77***	-0.15	-0.11
	normal seedling dry weight	-0.02	-0.14	0.23	0.08	0.78***	0.34	0.25	-0.38	-0.43
Controlled Deterioration (With 2 days aging treatment)	% radicle emergence	0.72**	-0.59*	0.72**	0.68**	0.37	0.39	0.74***	0.18	0.13
	% normal seedling	0.78***	-0.65**	0.78***	0.75***	0.30	0.25	0.78***	0.21	0.19
	% fresh ungerminated seeds	-0.63**	0.49	-0.50	-0.52*	-0.19	-0.24	-0.57*	-0.39	-0.31
	T_{50} radicle emergence	-0.74***	0.82****	-0.79***	-0.72***	-0.35	-0.23	-0.71**	-0.12	-0.10
	T_{50} normal seedling	-0.65**	0.80***	-0.65**	-0.65**	0.10	0.00	-0.51*	-0.43	-0.49
	normal seedling dry weight	0.22	-0.10	0.26	0.22	0.51*	0.67**	0.36	0.14	0.05
Complex Stressing	% radicle emergence	0.68**	-0.71**	0.73**	0.71**	0.40	0.23	0.73**	0.15	0.05
	% normal seedling	0.72**	-0.77***	0.78***	0.75***	0.44	0.19	0.76***	0.15	0.06
	% fresh ungerminated seeds	-0.48	0.50*	-0.53*	-0.50*	-0.38	-0.20	-0.55*	-0.09	0.03
	T_{50} radicle emergence	-0.71**	0.76***	-0.79***	-0.77***	-0.28	-0.12	-0.72**	-0.15	-0.07
	T_{50} normal seedling	-0.66**	0.72**	-0.78***	-0.74***	-0.20	-0.06	-0.65**	-0.12	-0.07
	normal seedling dry weight	0.20	-0.25	0.23	0.22	0.30	0.33	0.30	0.05	0.01

* $P \leq 0.05$ ** $P \leq 0.01$ *** $P \leq 0.001$ **** $P \leq 0.0001$

APPENDIX 18 **Summary of weather during seedling growth of *P. radiata* in the nursery at the FRI, Rotorua in 1987/1988.**

(Data from Rotorua Branch of the New Zealand Meteorological Service).

Month	air temperature (°C)			air frost number (days)	ground frost number (days)	rainfall (mm)	
	mean max	mean min	mean			total	maximum daily
OCT 1987	16.7	9.1	12.9	0	0	97	27
NOV 1987	19.0	9.7	14.4	0	0	89	22
DEC 1987	20.5	12.2	16.4	0	0	160	44
JAN 1988	24.0	12.1	18.1	0	0	10	9
FEB 1988	22.7	14.4	18.6	0	0	303	112
MAR 1988	20.4	11.0	15.7	0	0	167	46
APR 1988	17.9	7.6	12.8	0	1	25	9
MAY 1988	15.8	7.2	11.5	2	8	90	23
JUN 1988	13.2	5.3	9.3	5	10	71	21
JUL 1988	12.6	4.8	8.7	4	12	166	85
AUG 1988	13.3	4.8	9.1	4	14	223	101
mean	17.8	8.9	13.4	-	-	-	-