A STUDY OF SEED VIGOUR TEST METHODOLOGY VARIABLES

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TAO ZHANG

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ABSTRACT

Further research of some variables and procedures for promising seed vigour tests, such as the conductivity, Accelerated Ageing (AA) and Controlled Deterioration (CD) tests, is needed for their wider application and standardisation. Experiments to determine the effects of fungicide and insecticide seed treatments, and breaking hard seed using concentrated sulphuric acid (H_2SO_4) and mechanical scarification on results of the conductivity and AA tests; determining tolerances for the conductivity test; and a comparison of the efficiency of two methods of raising seed moisture content (SMC), i.e., the water added method (WAM) and the filter paper method (FPM) for the CD test; were carried out using a number of seed species and methodology according to the procedures recommended by the International Seed Testing Association (ISTA) and its Vigour Test Committee.

At the recommended application rate, protectant and systemic fungicide seed treatments had little effect on seed conductivity of the large seeded legumes garden pea (*Pisum sativum* L.), soybean (*Glycine max.* (L.) Merril), French bean (*Phaseolus vulgaris* L.) and broad bean (*Vicia faba* L.), and the cereals maize, sweet corn (*Zea mays* L.) and wheat (*Triticum aestivum* L.) both immediately after treatment and after two months storage. However, at double the application rate, systemic fungicide seed treatments significantly increased conductivity, but not necessarily for all the species, cultivars and chemicals used. Seed treated at the recommended fungicide application rate can be directly tested for conductivity without removal of the chemicals. Seed treatment chemicals, particularly insecticides, tended to increase conductivity of the small seeded legume white clover (*Trifolium repens* L.). However, the reliability of the conductivity test for small seeded legumes needs further investigation as the method currently recommended produced variable results. There was no clear trend for the effects of seed treatment chemicals on AA test results because different chemicals, particularly systemic ones, had different phytotoxicity, even at the recommended rate. However, when comparing the vigour of seed lots treated with the same fungicide or insecticide at a similar application rate, the seed lots can be directly AA tested. The effects of the chemicals on seeds are modified by their phytotoxicity, and their

beneficial effects that are determined by chemical application rate, physical condition of the seed lot, vigour status of the seed lot, fungal infection and storage time.

The tolerances for differences between the highest and lowest conductivity result among four replicates of a seed lot for garden pea cv. Bolero were calculated as 4.77 and 5.56μS/cm/g at the 5 and 1% significance level respectively. The present tolerance of 5μS/cm/g recommended by the Vigour Test Committee of ISTA is appropriate for pea and other large seeded legumes. However it may be not suitable for cereals and small seeded legumes because of large differences in conductivity value among them.

Both the WAM and the FPM provided a SMC for large seeded species of garden pea and maize very near the desired SMC for the CD test. Variability was small, and ranking of seed lot vigour did not differ between the two methods of raising SMC. The WAM provided a reasonably acceptable SMC in terms of mean and variance for the small seeded species onion (*Allium cepa* L.) and swede (*Brassica napus* var. *napobrassica* L.), but was very dependent on the accurate operation of the micro-pipette and improvement of SMC determination methodology after the CD test. Therefore the WAM, after further refinement, will be able to be used for the CD test, superseding the FPM.

Artificial deterioration conditions i.e., high temperature of 40^0C for 48h and 45^0C for 24h at near 20% SMC induced high seed dormancy in swede seed lots of cultivars received from the United Kingdom and New Zealand, but the extent varied with cultivar and initial SMC. Pre-chilling and 20-30^0C germination temperature broke the dormancy. Caution should be used when swede and its close species e.g., rape (*Brassica napus* L.) and other *Brassica* spp. are artificial ageing tested.

H_2SO_4 treatment had little negative effect on germination of white clover and lotus (*Lotus uliginosus* Schk.), but significantly increased conductivity and reduced AA germination because of seed coat degradation and fungal invasion.

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CHAPTER 1: INTRODUCTION

Is it not reasonable to expect that the condition of storage or age, which has proved fatal to one-third of the seed, has left its degenerating influence upon many of the remaining seeds? In other words, the vigour of the 62% which germinated, has been impaired.

-------W. L. Goss (1933)

A seed quality test must be repeatable and reliable. The laboratory germination test, which indicates the potential of a seed lot to produce normal seedlings under optimum conditions, is an important and standardised test for seed quality (ISTA 1996). However, for seed lots with a high germination percentage, a germination test may fail to detect differences among seed lots in field planting value and storage ability (e.g., Helmer *et al.* 1962; Wang & Hampton 1991), because it cannot detect difference in seed vigour. Differences in seed vigour exist among high germination seed lots (Delouche & Baskin 1973) and may determine the potential performance of the seed lot under realistic field and storage conditions. In this situation, a more sensitive indicator of seed quality, i.e., seed vigour testing, is required.

Although many vigour tests have been developed (AOSA 1983; Anonymous 1988; Hampton & TeKrony 1995), no test has yet been standardised and therefore accepted as a part of the International Seed Testing Association Rules (ISTA 1996), because of some inconsistencies in results. This was attributed to extraneous sources of variables and unclear assumptions behind each test (Hampton & Coolbear 1990), although many of them have been eliminated (e.g. McDonald 1977; Tao 1978a; Loeffler *et al.* 1988; Tomes *et al.* 1988; Hampton *et al.* 1992a, 1992b).

The basic requirements of a vigour test are that it should be a more sensitive index of seed performance than the germination test, and provide a consistent ranking of seed lots in terms of their potential performance (Isely 1958; McDonald 1980b; Powell & Matthews 1981; Perry 1984b). The conductivity test and artificial ageing tests, (i.e. Accelerated Ageing (AA) and Controlled Deterioration (CD) tests) have shown

promise in meeting the basic requirements of a vigour test and are able to reasonably consistently predict seed lot potential performance (Hampton & Coolbear 1990; Hampton & TeKrony 1995).

Seed treatments, such as fungicides and insecticides, are extensively used in the seed industry to enhance seed performance by preventing fungi and insect attacks. Both protectant and systemic products are available. Much research on the mechanisms (e.g., Lyr 1995) and effects of chemical seed treatment on seed germination, seedling growth rate and emergence have been reported (e.g. Van Toai *et al.* 1986; Lewis *et al.* 1991; Saraswthi *et al.* 1995), but little is known as to what effects they may have on vigour testing. A few reports concluded that protectant fungicides, such as seed treatment with *captan* and *thiram,* and the systemic fungicide *carboxin* had no effects on soybean (*Glycine max.* (L.) Merril) conductivity (McDonald & Wilson 1979; Loeffler *et al.* 1988; Eua-umpon 1991), but conductivity was reduced after long storage (Van Toai *et al.* 1986; Saraswthi *et al.* 1995). AA germination of soybean seeds treated with *carboxin* and *thiram* after six months storage was higher than that of the control because the fungicide delayed and reduced the invasion of fungi during storage and AA testing (Van Toai *et al.* 1986). However, the effects of systemic chemical seed treatments other than *carboxin* on electrical conductivity and AA tests have not been reported, and therefore, there are no guidelines in the vigour test procedures (Hampton & TeKrony 1995).

The reliability of seed test results is measured by tolerances. The amount of allowable deviation from a standard or the allowable difference between test results is called a tolerance. These tolerances were obtained by comparing the observed results with the calculated distribution of results assuming random sampling variation only (Justice 1972; Thomson 1979; Bould 1986). For the conductivity test, tolerances of less than 4 or 5 μS/cm/g between two replicates for readings in excess of 30 μS/cm/g were suggested (Matthews & Powell 1981, 1987). Hampton & TeKrony (1995) proposed no more than 5 μS/cm/g differences among four replicates. However, these tolerances are based on the actual variation found in tests, rather than on theoretical calculation (Hampton, pers. comm.).

The CD test is considered to have an advantage over the AA test in that the control of seed moisture is more accurate, and thus deterioration is controlled more readily (Matthews 1980). It can consistently predict seed field emergence (Matthews 1980; Powell & Matthews 1981), and storage ability (Powell & Matthews 1984a, 1984b) in small-seeded vegetable species, and also emergence of some larger seed species- e.g. garden pea (*Pisum sativum* L.) (Bustamante *et al.* 1984). However, raising seed moisture content using the filter paper method (Matthews 1980), and the high relative humidity method for large seeds, such as pea (Don *et al.* 1984; Bustamante *et al.* 1984) or maize (*Zea mays* L.)(Bruggink 1989), requires frequent weighing and is time and labour consuming, particular for large seeded species. The high RH method is considered impractical because it takes a few days to achieve the desired SMC (Powell 1995). The water added method is an easier way (Wang & Hampton 1991) and was reliable and repeatable in red clover (*Trifolium pratense* L.) (Wang & Hampton 1991) and mungbean (*Phaseolus mungo* L.) (Hampton *et al.* 1992a). However large variance was also reported in French bean (*Phaseolus vulgaris* L.) (Hampton *et al.* 1992a). In rape (*Brassica napus* L.) seed, the method resulted in bigger variance than the filter paper method because of inexpert use of micro-pipette and evaporation of water drops when sealing bags (Powell 1995). Which method of raising SMC to the desired level for the CD test is more accurate is still to be determined.

Hard seed in small-seeded legumes is common, and has ecological and economic significance. However, it causes problem in seedling establishment and seed testing. Hard seed breaking methods, such as sulphuric acid (H_2SO_4) and mechanical scarification, which are appropriate for small seed lots and large seed lots respectively (Hare & Rolston 1985), can effectively break hard seed and not affect viability and germination if used at suitable concentrations, duration and speeds of the machines (Brant *et al.* 1971; Viado 1989; Fu *et al.* 1996). Seed vigour is a more sensitive parameter of seed quality. However, the effect of hard seed breaking method on seed vigour testing has scarcely been reported.

The objectives of this research, therefore, were:

1) To determine the effects of protectant but especially, some systemic fungicide and insecticide seed treatments on conductivity and AA testing using a variety of species;

2) To determine the tolerance for conductivity test results among four replicates of a seed lot using garden pea;

3) To compare the advantages and disadvantages of two methods (filter paper and water added methods) of raising seed moisture content (SMC) in the CD test using both large, i.e., garden pea, maize and small i.e., onion (*Allium cepa* L.), swede (*Brassica napus* var. *napobrassica* L.) seeded species, and to investigate the possibility of superseding the former with the latter method, thus widening the use of the CD test;

4) To determine the effects of H_2SO_4 and sandpaper methods of breaking hard seed on conductivity and AA testing of the small seeded legumes white clover (*Trifolium repens* L.) and lotus (*Lotus uliginosus* Schk.).

CHAPTER 2: LITERATURE REVIEW

2.1 Seed quality

2.1.1 Seed structure

The angiosperm seed usually comprises the embryo, endosperm, perisperm and seed coat. The embryo itself includes the embryonic axis and one or more cotyledons. The axis incorporates the embryonic root (radicle), the hypocotyl to which the cotyledons are attached, and the shoot apex with the first true leaves (plumule). It is easy to identify these components in dicot embryos. However, in monocots, especially *Gramineae*, the single cotyledon is reduced considerably and modified to form the scutellum (Bewley & Black 1994). The seed coat provides a protection against deterioration of the living seed by pathogens, toxic chemicals and adverse environmental conditions (e.g., Hurd 1921). Different species, even cultivars, have different structures, such as the size of the embryo and cotyledon, components and thickness of seed coat, etc. These may affect seed quality.

2.1.2 Seed quality

Even under favourable standard germination conditions, not all seeds can germinate or develop into a normal seedling in the absence of dormancy. It has long been realised that inside the seed are properties that determine seed quality (Hampton 1994).

Seed quality has been defined as "a collection of seed properties which are considered to be of importance for the value of seed for sowing purpose" (Esbo 1980). These properties are analytical purity, freedom from weeds, germination capacity, size, health, species purity, cultivar purity, vigour, uniformity and moisture content (Thomson 1979). However, from the point of view of the seedsman, there are four aspects that are most related to seed quality: purity, germination, seed vigour and seed health. Of these, seed germination and vigour are determined by the seed itself (Powell 1986).

2.2 Seed testing

Quality seed is essential for the rapid emergence of uniform, healthy, vigorous seedlings and thus, the capacity to produce an abundant crop of the required cultivar. Seed testing has been developed to determine the value of seed for planting by assessing seed quality (ISTA 1996). In the ISTA Rules (ISTA 1996), the standardised definitions and methods for many tests, such as purity, germination, moisture content and seed health are listed for the purpose of international trade and also, national business. However, vigour tests are not yet included in the Rules.

2.2.1 Germination test

2.2.1.1 Definition

There are different concepts between seed physiologists and seed technologists. From the seed physiology point of view, germination is the sum total of the processes preceding and including the protrusion of the radicle through the surrounding structures. In this sense, viability equals germination in the absence of dormancy (Hampton 1995; Hilhorst & Toorop 1997). Germination of a seed in a laboratory test is the emergence and development of the seedling to a stage where the aspect of its essential structures indicates whether or not it is able to develop further into a satisfactory plant under favourable conditions in soil. It is reported by the percentage of normal seedlings, abnormal seedlings, and ungerminated seeds including hard seed, fresh seed and dead seed (ISTA 1996). In this sense, a seed may be viable, but not contribute to the germination because it has produced an abnormal seedling. Although the germination test is partly based on the subjective assessment of germinated seeds, it is successful in two aspects: it is repeatable, and reliable in that it can provide information about the seed's potential performance under optimum conditions (Matthews 1981). This is because artificial, standardised, essentially sterile media and humidified temperature-controlled germinators are used (ISTA 1996). The germination test is an internationally recognised method for determining seed planting value and storage ability.

2.2.1.2 Limitations

According to the ISTA Rules (1996), the objectives of seed testing including the germination test are to determine the value of seed for planting. Unfortunately, the standard germination test often fails to predict field emergence, especially for high germinating seed lots (Tables 2.1 and 2.2; Scott & Close 1976; Castillo *et al.* 1993). Seed carry-over for the next season is an important and essential practice in the seed industry. However, the germination test also provides limited information for the storage ability of high germinating seed lots (Tables 2.1 and 2.2; Delouche & Baskin 1973; Wang & Hampton 1991). These imply that a high initial germination percentage provides no assurance that the seed lot will emerge or store as well as another lot of the same cultivar with the same or even lower germination. The limitations of the germination test arise from the nature of seed deterioration and the philosophy of the germination test (Goss 1933; Delouche & Baskin 1973; McDonald 1980b; 1993).

Loss of germination is the last event in seed deterioration. Before that, many degenerating activities occur (Delouche & Baskin 1973). Death in populations of seeds is normally distributed (Roberts 1972). High germinating seed lies in the prolonged initial periods of the seed survive curve which is the negative slope of the normal distribution. Thus a small difference in percentage germination of high germinating seeds may represent a large difference in the progress of deterioration (Roberts 1972; Powell & Matthews 1981).

Germination testing refers to the ability of a seed to produce a normal plant under "favourable" conditions: optimum temperature and moisture, sterile substrate and adequate testing time, etc., for the purpose of producing maximum germination percentage and standardisation. These conditions are so optimum and synthetic that they are seldom related to field conditions that seeds are likely to encounter. Therefore, it is not surprising that field emergence is often overestimated by the germination test. The germination test fails to consider the progressive nature of seed deterioration revealed by the uneven, slow, weak seedlings, which are categorised into the normal seedlings of the germination test. These aged seeds, though they may have high germination percentages, may not store well or emerge well under stress

conditions because they have deteriorated to a certain level that was not detected by the germination test (McDonald 1980b, 1993; AOSA 1983).

Because of the limitations of the germination test, a supplementary test is essential to solve the practical problems.

Table 2.1: Germination and emergence percentages of crimson clover seed lots in various conditions and treatments (Helmer *et al.* 1962)

	Seed lot							
Treatment	1	2	3	4	5	6	7	8
Standard germination	88.0	86.8	80.8	83.2	86.2	83.2	77.8	82.2
Field emergence	73.4	68.0	67.0	74.0	43.0	32.4	29.6	23.6
5 months storage (20^0 C, 75% RH)	84.0	84.0	83.0	67.0	35.5	30.0	31.5	26.0
40^0 C, 100% RH 5 days	92.0	84.5	77.5	73.0	39.5	31.5	16.5	6.5

Table 2.2: Correlation of responses of crimson clover seed under various conditions with field emergence and viability after 5 months storage (Helmer *et al.* 1962)

Conditions	r
With field emergence	
Standard germination	0.491
40^0 C, 100% RH 5 days	0.976 **
With 5 months storage (20^0 C, 75% RH)	
Standard germination	0.432
40^0 C, 100% RH 5 days	0.967**

** Differs significantly at $P< 0.01$.

2.3 Seed vigour

2.3.1 Definition of seed vigour

Defining seed vigour and developing standardised test methods were the two objectives when the Biochemical and Seedling Vigour Committee, later the Seed Vigour Committee of ISTA was first established in 1950. Before a vigour test can be devised and standardised, the manifestations of vigour and the factors affecting vigour should be known (Heydecker 1965). After lengthy debate and fruitful works (Isely 1957; Delouche & Caldwell 1960; Heydecker 1965; Woodstock 1969; Perry 1973; McDonald *et al.* 1978), seed vigour was defined in 1977 by ISTA (Perry 1978) and in 1979 by the Association of Official Seed Analysts (AOSA) (McDonald 1980a), respectively.

The definition of seed vigour adopted by ISTA is:

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

The following different aspects ranging from physiological and biochemical activities in the laboratory to observations on seedling emergence and growth in the field were included in the concept:

> "The aspects of performance which may show variations associated with differences in seed vigour include: (1) biochemical processes and reactions during germination such as enzyme reactions and respiratory activity, (2) rate and uniformity of seed germination and seedling growth, (3) rate and uniformity of seedling emergence and growth in the field, (4) emergence ability of seedlings under unfavourable environmental conditions" (Perry 1978).

The definition of seed vigour accepted by AOSA is: "Seed vigor comprises those seed properties which determine the potential for rapid uniform emergence and development of normal seedlings under a wide range of field conditions" (McDonald 1980a).

The ISTA definition is considered more "academic", and the AOSA one more "operational", because the former discuses what seed vigour *is*, whereas, the latter, focuses on what seed vigour *does*. However, they both relate seed vigour to seed emergence in field conditions (McDonald 1980b). This is logical because the ultimate goal of seed quality assessment is to determine planting value. However, seed storage is an important and essential parameter in the seed industry; thus, the concept of seed vigour should include storage ability (Hampton & Coolbear 1990). Also, seed vigour remains a vague qualitative concept which cannot be measured (Ellis & Roberts 1980). There has been a change of emphasis in the approach of studying vigour in the last two decades, i.e., from physical characteristics of seed (Perry 1980) to the physiological and biochemical mechanisms of seed vigour (Perry 1988). With accumulated knowledge and better understanding of seed vigour, the definition is expected to be modified.

2.3.2 Factors affecting seed vigour

The main factors influencing seed vigour were initially listed as genetic constitution, environment and nutrition of the mother plant, stage of maturity at harvest, deterioration and ageing, mechanical integrity, pathogens, and seed size, weight or specific gravity (Perry 1978). However, seed deterioration and ageing have come to be recognised as the major reason for reduced seed vigour (Ellis & Roberts 1980). The other factors originally listed actually influence the seed deterioration rate to some extent (Hampton & TeKrony 1995).

2.3.2.1 Genetics

Different species or cultivars may have different vigour levels, as demonstrated by different field emergence and storage ability. Soybean seed deteriorates faster than that of cereal crops because of a higher content of protein and lipid (Van Toai *et al.* 1986). Sweet corn normally has lower vigour than that of maize due to the different physiological and biochemical processes controlled by genetics (Parera *et al.* 1996). The same situation exists in wrinkle pea compared to round pea (Padrit 1996). Different cultivars have different storage ability (Van Toai *et al.* 1986). For instance, different soybean cultivars have different coat permeability (permeable, delayed-permeable and hard seed) affecting seed storability and imbibition damage (Kuo 1989).

It is a certainty that genetics influence seed vigour, but when concluding this based on experiments, the history and maturity of the cultivars should be the same, because production and storage conditions have a greater effect on seed quality, including seed vigour. In other words, seed lots of the same cultivar can differ significantly in their vigour.

2.3.2.2 Seed deterioration

Seed, like other organisms, goes through many degenerative processes, which culminate in the loss of viability (Delouche & Baskin 1973; AOSA 1983). Seed deterioration can be described as loss of seed quality with time (Coolbear 1995). It is a matrix of interrelated events, and involves a considerable number of biochemical and physiological changes (Coolbear *et al.* 1984). Therefore, it is difficult to separate the changes which are the primary causes of seed deterioration from secondary effects (AOSA 1983; Coolbear 1995). In each event, the factors that lead to seed deterioration are influenced by the seed's own capacity of repair and detoxification (Coolbear 1995). For example, seed vigour can be improved by the repair mechanism when hydration occurs (Burgass & Powell 1984).

Deterioration within a lot is on an individual basis. In the same seed lot, although the seeds have identical genetics, and often but not always the same environment during development, they have different capacities to germinate. This is because the same seed lot is composed of the following seeds: 1) Seeds that never attained high vigour; 2) seeds that attained and maintained high vigour; 3) seeds that attained vigour and then lost it partly or totally (Abdul-Baki 1980). This suggests that the different maturity of seeds interacting with environmental deteriorating effects is a main reason why the same chronologically aged seeds may have different degrees of deterioration.

Few general conclusions have been made about the exact mechanisms of seed deterioration, because of conflicting data (Coolbear 1995), e.g., Gidrol *et al.* (1989) and Powell (1986) in regard to the membrane integrity by lipid peroxidation and leachate conductivity relationship. However, the following general statements seem reasonable.

Seed deterioration is an inevitable and a progressive process, but the pace varies considerably depending on environmental conditions (AOSA 1983). It is, often, a sequential process (Delouche & Baskin 1973; AOSA 1983), but does not always follow this pattern (Coolbear 1995). Primary deterioration takes place at cellular level. It is related to membrane integrity (Powell 1986), enzyme activity (Roos 1980;

Basavaraajappa *et al.* 1991), impaired DNA, RNA and (therefore) protein synthesis (Blowers *et al.* 1980), respiration changes (Woodstock *et al.* 1984; Ferguson *et al.* 1990a), genetic damage (Cheah & Osborne 1978) and accumulation of toxic metabolites (Woodstock & Taylorson 1981). As a result, at the seed response level, the seeds show reduced germination rate and uniformity, reduced tolerance to environment stress revealed by lower, slower and more uneven seedling emergence in sub-optimum conditions, and narrowed germination environmental requirements, e.g. temperature (Heydecker 1969a; Roos 1980; Woodstock *et al.* 1985; Ferguson *et al.* 1990a; Nath *et al.* 1991; Alsadon *et al.* 1995). Thus, seed deterioration and vigour are reciprocal, i.e., when deterioration increases, seed vigour decreases (AOSA 1983).

However, exactly what biochemical reactions occur during the deterioration process has not been fully understood. Fortunately, as Roberts (1986) pointed out, it is not essential for a seed technologist to know. But this field needs essentially, further research from seed physiologists for a better understanding of seed deterioration, control of vigour loss and refinement and development of seed vigour tests. Anderson & Gupta (1986), Ferguson *et al.* (1990a) and Coolbear (1995) have suggested some strategies.

2.3.2.3 Pathogens

Field and storage fungi can have a great effect on seed deterioration by the mechanism of producing enzymes and mycotoxins that are detrimental to seed (Hallion 1986), although intrinsic deteriorative processes enhanced by moisture and temperature is the main reason (see Section 2.3.2.2).

Field fungi can be an important factor impairing seed vigour during seed development on the plant in the field or between periods of physiological maturity and harvest maturity. They grow on seeds with a high SMC e.g. 33% for cereals. Storage fungi, almost exclusively of the genera *Aspergillus* and *Penicillium*, infest seeds only under storage conditions above SMC that are in equilibrium with an ambient RH over 68%. Each species of storage fungi has a defined minimum SMC below which it will not grow (Harrington 1972). The moisture requirements for *Aspergillus* development are

less than those for *Penicillium* (Hurd 1921). For example, *Aspergillus glaucus* developed on red clover seeds at 20^{0}C and 75% RH, but at 90% RH, *Penicillium* are the dominating species that infect red clover seeds (Wang 1989). Their invasion reduced seed germinability of soybean (Dorworth & Christensen 1968) and maize (Moreno *et al.* 1994). Seed field emergence is primarily a function of seed vigour and micro- organism interaction (Isely 1957). Also, when imbibing, low vigour seeds have more leakage which stimulates the growth of pathogens which in turn deteriorates the seed (Mohamed-Yasseen *et al.* 1990). Therefore, it is also difficult to separate which aspects of deterioration are micro- organism induced from those self-inflicted by the seeds (Halloin 1986).

2.4 Seed vigour testing

Increased requirements for vigour information by the international seed trade demand repeatable and reliable vigour results. Vigour tests are supplements to standard germination tests for high germinating seed lots (Isely 1957; Perry 1978; Matthews 1980) because of the limitations of the germination test (see Section 2.2.1.2). They should be reproducible laboratory methods, which distinguish seed lots of different levels of vigour (Perry 1978). When seed lots differ considerably in germination capacity, the germination test itself often shows a high correlation with field emergence because viability, in this case, dominates the performance of the seeds. However, results from vigour tests of similar high germinating seed lots show a superior relation to field establishment compared with germination (Perry 1978; Castillo *et al.* 1993). Seed vigour, if appropriately evaluated, is not only a better measurement of seedling emergence under stress conditions, but also a better predictor of storage ability (e.g. Tables 2.1 and 2.2; Wang & Hampton 1991). Its application can avoid the risk of planting or storing seed lots of acceptable high germination but low vigour that can result in poor performance.

2.4.1 Requirements of seed vigour tests

Relevant criteria must set up before developing a test. After much research (Isely 1958; McDonald 1975, 1980b; Perry 1978; Matthews 1980; Powell & Matthews 1981; Perry 1984b), the requirements of a vigour test were established as: a good theoretical background rather than just based on an empirical relationship; a more sensitive index of seed performance than the germination test; a consistent ranking of seed lots in terms of their potential performance; objective, rapid, simple and inexpensive; interpretable-a good relationship between tests result and practical expression.

2.4.2 Development and future of vigour testing

Because a vigour test is a more sensitive index of seed potential performance than the germination test (Isely 1957), any event that precedes loss of germination could serve as a basis for vigour testing. The farther the event is from loss of germination, the more sensitive the measure of seed vigour (AOSA 1983). For example, according to the probable sequence of changes in seed during deterioration (Delouche & Baskin 1973), measurement of membrane integrity is a promising test among the large number of vigour tests available.

Although it has been difficult, great progress has been achieved in developing seed vigour testing. Isely (1958) revealed that only one crop, maize, was regularly subjected to a vigour test (cold test), and this was rarely conducted by official laboratories in 1958. However, in 1978, 52% of seed testing stations in US conducted one or more vigour tests. The percentage had increased to 63% in 1983 and 75% in 1990 (McDonald 1993).

The first step of the Vigour Committees of ISTA and AOSA was to assess and select existing tests and devise new vigour tests (Heydecker 1962, 1969b; McDonald *et al.* 1978). This resulted in the development of the conductivity (Matthews & Bradnock 1967), AA (Delouche and Baskin 1973), and CD tests (Matthews 1980). After that, improving the reproducibility and standardising the promising tests of AA, CD, conductivity test for large seeded legumes, and cold test for maize, became the main

objective of the Committees. After a series of referee programmes organised by ISTA (Perry 1984a; Fiala 1987; Hampton 1992a) and AOSA (Tao 1980a, 1980b; TeKrony 1988 etc.) and other research (McDonald 1977; Tao 1978a, 1978b; Loeffler *et al.* 1988; Tomes *et al.* 1988; Hampton *et al.* 1992a, 1992b), the procedures were improved, most variables identified and eliminated. The conductivity test for pea and soybean, and AA test for soybean became "recommended" methods instead of "suggested" ones in the ISTA (Hampton & TeKrony 1995) and AOSA (AOSA 1983; Anonymous 1988;) vigour testing handbooks, reflecting the perception that these tests are nearly standardised (McDonald 1993).

Besides recommended methods, six other suggested methods i.e. cold, cool germination, controlled deterioration, complex stressing vigour, Hiltner, seedling growth and tetrazolium tests are also published for reference (Hampton & TeKrony 1995). The vigour tests can be grouped into three categories: single tests based on some aspect of germination behaviour, attempts to develop physiological or biochemical indices of vigour, and multiple testing procedures (Hampton & Coolbear 1990).

Seed vigour is an integrated concept. Because many vigour tests detect only one aspect of seed vigour, some authors have investigated the possibility of multiple seed vigour indices to predict field emergence. Expected field emergence for pea in New Zealand is a successful example (Scott & Close 1976). This approach was attempted in other crops, such as barley (*Hordeum vulgare* L.) by Kim (Hampton 1992; Kim *et al.* 1994) and wheat (*Triticum aestivum* L.) by TeKrony (Hampton 1992). Van de Venter suggested that multiple tests, rather than multiple stress in a single test might be the approach to take (Hampton 1992). McDonald (1995) argued that it is difficult and, maybe, subjective to decide how much weight each single test contributes to the index. However, the weight of each single test can be determined by multiple regression analysis (Scott & Close 1976).

Although progress has been considerable, and many test methods proposed, only a few of them are internationally employed. There have been major problems with vigour test

reproducibility, sensitivity, relevance, and a lack of understanding of the variables and assumptions involved (Hampton & Coolbear 1990). Therefore, they are not yet included in the ISTA Rules (ISTA 1996).

Because of the increasing importance of seed vigour information, standardisation of vigour tests is a necessity (Ferguson 1993). There will be an increasing market demand for better assessment of planting value and storage potential of seed lots both internationally and nationally or locally. Therefore, besides developing internationally standard methods, methods useful under anticipated local conditions should be emphasised (Hampton 1995). The research should focus on the extraneous sources of variation and hidden assumptions behind each test (Hampton & Coolbear 1990), such as the physiological and biochemical changes during ageing conditions in the AA and CD tests for different species.

New vigour methods are continually being developed, such as ethylene production converted by ACC (1-aminocyclopropane-1-carboxylic acid) (Khan 1994; Chojnowski *et al.* 1997); Sinapine (fluorescent compound: choline ester of sinapic acid) leakage concentration measured by light absorption at specific wavelengths (Hill *et al.* 1988; Huang *et al.* 1995); seed nucleic acid RNA: DNA ratio (Redfearn 1996), as indices of seed vigour. But their reliability needs further evaluation.

2.5 Recommended vigour tests

2.5.1 Electrical conductivity test

2.5.1.1 Current status

Increased leaching of inorganic salts and organic compounds from mechanically damaged and naturally aged seed was suggested as an index of seed viability or field performance (Hibbard and Miller 1928). The electrical conductivity test was developed into a routine vigour test to predict field emergence of garden pea by Matthews & Bradnock (1967) and Matthews & Whitbread (1968).

As seeds dry to harvest maturity, the membrane structures change. Upon rehydration, the seeds need time to reorganise and repair the membranes. In the process of becoming intact, the membrane allows leakage of solutes including sugars, amino acids and electrolytes. Both ageing and imbibition damage lead to the impaired membrane integrity that is considered a fundamental cause of difference in seed vigour, and thus higher leakage (Powell 1986). The promise of this vigour test lies in that the higher the vigour of seeds, the more rapid reorganisation of cellular membranes and the greater extent of damage-repair during imbibition, means that seeds have less leakage of electrolytes and a lower conductivity value compared to lower vigour seeds (AOSA 1983; Hampton & TeKrony 1995). The conductivity test has the characteristics of simplicity, objectivity, rapidity and accuracy and can be readily applied (Heydecker 1972; McDonald 1980b; Hampton & TeKrony 1995).

Many variables, such as seed size (Tao 1978a; McDonald & Wilson 1979), damaged seeds (Loeffler *et al.* 1988), seed number and replicates (Loeffler *et al.* 1988; Hampton *et al.* 1992b), initial SMC (see Section 2.5.1.2) and soaking temperature (Tao 1978a; McDonald & Wilson 1979; Loeffler *et al.* 1988; Hampton *et al.* 1992b), etc. (see Hampton 1995) have been detected and removed or standardised. The conductivity test is regarded as a reliable, standardised method in large seeded legumes because it can consistently rank seed lots regarding their potential performance; e.g. for pea (Matthews & Bradnock 1967; Heydecker 1969b; Perry 1981; 1984a; Fiala 1987), and soybean (Tao 1980a, 1980b; Fiala 1987; Loeffler *et al.* 1988). The reports also indicated that the conductivity test has potential use in other species, such as maize (Tao 1980a, 1980b; Chen & Burris 1990; TeKrony & Hunter 1995), Brussels sprout (Thornton *et al.* 1990) and *Rudbeckia fulgida*-an ornamental and herbaceous perennial (Fay *et al.* 1993).

For attaining results more quickly, a few workers have reported consistent results between less than 24h and the recommended 24h soaking time; for example 4h soaking of *Phaseolus vulgaris* L. (Brouwer & Mulder 1982), 6h soaking in soybean (Loeffler *et al.* 1988), and 8h in red clover (Wang 1989).

2.5.1.2 SMC effects

Many reports have shown that the initial SMC can affect conductivity results (Table 2.3).

Table 2.3: The effect of initial SMC on conductivity test results

Species	SMC (%) at which seed conductivity significantly			References
	Increased	No change	Decreased	
soybean	7.2 & 8.8	13-19.5		Tao (1978a)
	<10	11-18		Loeffler *et al.* (1988)
	<10	10-22		Hampton *et al.* (1992b)
mungbean	<10	10-14	>14	Hampton *et al.* (1992b)
French bean	<10 >14	18-22	<6 (hard seed)	Hampton *et al.* (1992b)
birdsfoot trefoil	<11	11-17		Hampton *et al.* (1994)
greater birdsfoot trefoil	8-11	11-17	<8	Hampton *et al.* (1994)

Generally, the lower the SMC, the higher the conductivity value (Pollock 1969; Simon & Wiebe 1975; McDonald & Wilson 1979). When dry seed takes up water, cell reorganisation occurs (Abdul-Baki 1980). This is mainly the organisation of cell membranes to form the bilayer conformation to slow down and avoid leakage (Simon & Wiebe 1975). The lower the SMC, the more rapid is the uptake of water by seeds (Pollock *et al.* 1969), the more imbibition damage occurs (Ellis *et al.* 1990) resulting in more disruption of membrane reorganisation (Powell 1986), and subsequent cracking (Pollock *et al.* 1969; Tao 1978a). This is demonstrated by the faster electrolyte leakage (Ismail *et al.* 1997) and increased electrical conductivity reading.

The phenomenon that some species decrease their conductivity at very low SMC because of the formation of hard seed (Ellis *et al.* 1990; Hampton *et al.* 1992b; Lungwangwa 1993) is an exception. This may be attributed to some seeds that are in the semi-hardseededness or delayed-permeable status (Rolston 1978; AOSA 1983; Holubowicz *et al.* 1988; Kuo 1989), and thus much less solute is leaked out. The fact that the weight of hard seed was not subtracted before the conductivity calculation may also account for this.

Retarding imbibition rate by coating seeds with polymeric films (Taylor *et al.* 1992), or humidification to raise SMC before testing (Coolbear *et al.* 1991; Ellis *et al.* 1990, 1995) can alleviate or avoid imbibition injury, indicating that fast water uptake is the main reason of soaking injury.

Lower temperature interacting with SMC can worsen the soaking damage (Pollock 1969; Wolk *et al.* 1989; Demir 1996). One important reason is that low temperature slows the re-organisation and repair process (Bewley & Black 1994).

The SMC range over which conductivity results do not differ is not the same for all species (Table 2.3). According to Hampton & TeKrony (1995), for garden pea the initial SMC should be adjusted to 10-14% before the conductivity test. However, from the above reports, there is an interaction between SMC and species affecting conductivity value. Some species differed in conductivity in this SMC range (e.g. significant differences were recorded at 10 and 14% SMC for French bean (Hampton *et al.* 1992b)). Some unexplained variability in ISTA referee tests may be partly attributed to initial SMC (Perry 1984b). Furthermore, if the test broadens its utilisation, does this SMC range suit new species? Therefore, it is better, and easier to standardise this method, to adjust SMC to a certain level for a specific species before the test; or, if possible, develop a correction factor to adjust conductivity measurements to a standard moisture level (McDonald 1977; Loeffler *et al.* 1988).

2.5.1.3 Seed treatment effects

According to a few reports, it is suggested that fungicide seed treatment should be removed before the conductivity test because of its influence on the results (McDonald 1980b; Tao 1980c; AOSA 1983). Tao (1980c) and AOSA (1983) recommended removal of seed treatment by methanol washing without changing conductivity result. However, the methanol washing did influence the conductivity of maize (Tao 1980a).
Some reports concluded that protectant fungicides, such as *captan* and *thiram* (McDonald & Wilson 1979; Loeffler *et al.* 1988; Eua-umpon 1991) and the systemic fungicide *carboxin* (McDonald & Wilson 1979; Loeffler *et al.* 1988) had little effect

on soybean conductivity, though both *captan* and *carboxin* increased the conductivity of deioned water (Tao 1980c).

However, few reports have studied the effects of systemic chemicals other than *carboxin* on seed conductivity. Conflicting results in two pea cultivars after six months storage of seed treated with *benomyl* were reported by Nascimento & Cicero (1991).

The conductivity test effectively detected the vigour status of soybean seeds treated with fungicides as supported by a germination vigour index (Van Toai *et al.* 1986; Saraswthi *et al.* 1995).

2.5.1.4 Limitations

Species other than large seeded legumes

It was believed that the conductivity test was only reliable for large seeded legumes (Perry 1984b). Matthews (1981) indicated that the species for which the conductivity test showed potential have large living cotyledons, whereas other species such as wheat (Matthews 1981) and maize (Bruggink *et al.* 1991) have only a small living portion of the seed, i.e. the embryo, which is a main source of leakage. The conductivity test may not be appropriate because of the presence of dead, readily leached tissue in aged seeds that may only slightly elevate the conductivity compared with high vigour seeds. Furthermore, the pericarp of maize seed, which is another major source of electrolyte leakage, is cultivar dependent but not related to kernel quality, and therefore may interfere with conductivity testing (Bruggink *et al.* 1991). The conclusion was supported by AOSA wheat referee tests (TeKrony 1988). There are also many depressing reports about its validity in small seeded vegetables, such as tomato (*Lycopersicon lycopersicum* L.) (Coolbear *et al.* 1984; Argerich & Bradford 1989), pepper (Sundstorm *et al.* 1986), sunflower (*Helianthus annuus* L.) (Gidrol *et*

al. 1989; Chojnowski *et al.* 1997) and lettuce (*Cactuca sativa* L.) (McDonald *et al.* 1978) and in small seeded legumes, such as lotus (Rowarth & Sanders 1996) and red clover (Wang & Hampton 1989).

Pathogens

The conductivity test can not detect differences in intact soybean seed with different pathogen infections that may be related to lower seed germination (Loeffler *et al.* 1988) or the influence of soil micro-organisms (Copeland & McDonald 1995), although the latter is also true for the germination test.

Dormancy and hardseededness

Induction of thermo-dormancy in lettuce seeds is accompanied by increased leakage of amino acid (Hendricks & Taylorson 1979). Although, it is not sure whether ion leakage could change, caution should be used. Hard seeds (Rolston 1978; AOSA 1983; Holubowicz *et al.* 1988), and delayed-permeability coats of some soybean cultivars (Kuo 1989), have no or reduced imbibition damage, and therefore less solute leaked out, which may bias the test. Aged radish, broccoli and cabbage seeds produced more leakage than control seed; however, onion and carrot did not (Min 1995). This may be attributed to the semi-permeable layer of the onion seed coat that retards leakage (Beresniewicz *et al.* 1995).

Component of electrolytes

The electrolytes from aged seeds contained higher concentration of K^+ compared to the control (Min 1995). Based on a similar study, Woodstock *et al.* (1985) suggested that leaching of individual minerals (e.g. K^+ and Ca^{2+}) was a better indicator of seed quality than was the total release of electrolytes.

Predicting seed germinability

An approach to predict seed germinability of Brussels sprout by the single seed conductivity test was unsuccessful (Thornton *et al.* 1990).

In conclusion, whether or not the electrical conductivity test applies to a species needs thorough study prior to its utilisation.

2.5.1.5 Tolerances for seed vigour tests

General

It is practically impossible to obtain a perfectly uniform seed lot. In addition, as random sampling variation (experimental error, interpretation variation and lapse of time in some cases) will always occur, the precise percentage germination or other quality factors cannot be determined, i.e. repeat tests will not necessarily produce exactly the same result, and within a test, replicates may differ (Thomson 1979). However, a reasonably uniform-homogeneous seed lot is essential for the purpose of sampling to detect characteristics present in the population. The amount of allowable deviation from a standard or the allowable difference between test results is called a tolerance. Tolerances are expressed in terms of a probability (e.g. 5% level) and the amount of tolerance. The expression means that the result of a test has a chance of about 5 out 100 of exceeding the tolerance. Normally, a commonly used tolerance is the least difference to be expected in 5 out of a 100 tests, or in other words, the greatest difference in 95 from a 100 tests (Thomson 1979).

The basis for the calculation of tolerances is the mathematical description of the underlying pattern. It can be obtained by comparing the observed results with the calculated distribution of results assuming random sampling variation only. It is possible when the tests are made in accordance with ISTA rules and the seed lot is a homogeneous one. The application of statistical methods to analyse test data gives the estimation of the quality of seed lots within calculated ranges (Justice 1972; Bould 1986).

The reliability of test results is measured by tolerances and tolerances themselves are based on observations about the way that the results of samples conform to some predictable pattern. Sampling techniques and the associated protocol ensure that this pattern is preserved and by preserving the pattern tolerances remain relevant (Bould 1986). If a test result is out of tolerance, in other words, variation is bigger than random sampling variance, and the test should be repeated to test if there are experimental errors, such as sampling method or seed lot uniformity.

However, seed quality and sample size affect random sampling variance. The lower the seed quality and, the smaller the sample size, the larger the tolerance (e.g. tolerance of germination test) is (Thomson 1979; Bould 1986; ISTA 1996).

Conductivity test

Tolerances in some ISTA rules are based on the actual variation found in tests, rather than on theoretical calculation (Thomson 1979), such as that used for the conductivity test (Hampton, pers. comm.). In the first and second edition of the Vigour Test Handbook, the tolerance for two replicates were less than 5μS/cm/g when readings were in excess of 30μS/cm/g (Matthews & Powell 1981, 1987). Now, in the third edition, if the difference among the four replicates is more than 5μs/cm/g, the tests should be repeated (Hampton & TeKrony 1995).

2.5.2 Accelerated ageing test

2.5.2.1 Current status

The AA test was first developed by Delouche (1965), and popularised by a comprehensive report to predict storability of many species of seeds (Delouche and Baskin 1973). The AA test exposes seed to the two greatest variables, high temperature and humidity, which cause rapid seed deterioration (Hampton & TeKrony 1995). A basic assumption of the AA test is that the processes of deterioration under AA conditions are similar to those under natural conditions- only the rate of

deterioration is greatly increased (Delouche & Baskin 1973). The principle applies to most species. Sufficient reports support this assumption in the seed response level both in field emergence and storage practices (e.g. Nath *et al.* 1991). But caution in extrapolating the biochemical changes to natural ageing is needed (see Section 2.5.2.5).

Variable results were reported initially in referee test programs (Tao 1978b). After refining techniques and assessing variables (Tao 1978b; Tomes *et al.* 1988), ISTA and AOSA referees and other research often, but not always, revealed uniformity among laboratories, which resulted in differentiating vigour level of seed lots and a close relationship between AA germination and field emergence (Tables 2.1 and 2.2; Tao 1980a, 1980b; TeKrony 1985; Fiala 1989).

2.5.2.2 SMC effects

The initial and final SMC after ageing should be within a certain range (Hampton & TeKrony 1995). Tomes *et al.* (1988) reported that varying the initial SMC of two seed lots of soybean from 8.0, 10.5 and 13.5% at any of three ageing times, did not significantly affect final seed SMC. Although the authors argued that it was not necessary to adjust the initial SMC to a standard level when SMC is from 8 to 14% which was recommended by McDonald (1977), this was not reasonable. Firstly, final SMC in the AA test is not an ideal parameter in terms of deterioration rate (McDonald 1977); Secondly, 13.5% initial SMC, in some cases had significant lower AA germination than that of 8.0 and 10% SMC, especially in lower seed vigour lots (Tomes *et al.* 1988), indicating different deterioration among treatments with different initial SMC. AOSA (1983) believed that differences of 1-2 % SMC would not significantly bias test results. This is true in terms of data, but the result was, maybe, just a coincidence (Hampton & Coolbear 1990). However, what about other experimental results? What about other species? The philosophy is that much smaller quantitative change can become qualitative change. The most important thing is that the vigour test should have a good theoretical basis rather than be based on an empirical relationship (Powell 1995).

The AA test is often criticised because there is not an accurate initial SMC requirement. Seed lots with different SMC take up moisture from a humid atmosphere at different rates and thus undergo different times of deterioration (McDonald 1977; Tao 1978b; Matthews 1980; Powell 1995). Testa integrity and permeability which are not necessary related with vigour can affect seed moisture (Hampton & Coolbear 1990) even when seed lots have the same SMC before ageing. Indeed, seed moisture influences the rate of seed deterioration. Seeds possessing high initial SMC deteriorate more rapidly than those with low initial SMC because of different rate of SMC increase and finial SMC (McDonald 1977; Tao 1978b).

Some erratic AA test results may partly be due to variation of moisture uptake. Initial SMC is an important factor (Perry 1984b), e.g. for soybean (Perry 1984a). Also, if initial SMC is the same, SMC may become different during the time course of ageing, due to different cultivar, seed size and position of the seeds in the ageing box that affects moisture uptake rate.

Saturated salt solutions have been used, instead of water, to lower the %RH, thus retarding seed moisture uptake and improving the value of the AA test for small-seeded species. In such species a rapid SMC increase results in fast, uneven seed deterioration and encourages the growth of fungi which may influence the result when water is used (Zhang & McDonald 1997).

2.5.2.3 Seed treatment effects

Delouche & Baskin (1973) suggested that fungicide treated seeds generally are not affected quite as much by AA as untreated seeds of the same lot. This conclusion may be based on the control of fungal infection. The authors also noted that "mould growth is profuse" in the AA test. It is well documented that fungi have a great effect on seed deterioration (Halloin 1986) especially under artificial ageing conditions. Thus, AA germination of soybean seeds treated with *carboxin* and *thiram* after six months storage was higher than for seeds which had been treated but for which the fungicide was removed by methanol washing before the AA test (Van Toai *et al.* 1986).

Therefore, all lots tested should either be treated or, preferably, untreated (Baskin 1981). The results from treated and untreated seed lots should not be compared because the seed treatment could alter results (Baskin 1981; AOSA 1983).

Seed treatment may affect seed water uptake. When the seeds are soaked in a liquid medium, seed treatment had no effect. In saturated air, treatment enhanced seed moisture uptake at low application rate, and slowed it at higher rate (Schneider & Renault 1997), implying that chemical seed treatment may be a new variable in the AA test.

2.5.2.4 Micro-organism effects

Inoculation of soybean seeds with *Aspergillus* significantly reduced seed germination following AA. The reduction varied according to the levels of inoculum. Thus, both fungal and physiological deterioration are involved in AA of the seeds. However, present test parameters for AA tests do not consider the fungal factor (Gupta *et al.* 1993). This is another variable. The modified AA test, therefore, was introduced by dusting *thiram* on seeds before ageing, and was reported to have a significant correlation with field emergence of soybean under varying soil moisture regimes (r=0.82), whereas normal AA and the germination test failed to predict field emergence (r=0.21 and 0.32 respectively) (Adkins *et al.* 1996).

SMC interacting with fungal growth can affect the vigour test. Increasing SMC resulted in alterations of dominant *Aspergillus* and *Penicillium* storage fungi (Harrington 1972) and increased seed infection (Dorworth & Christensen 1968; Lokesh & Hiremath 1993).

Mould growth in AA tests is profuse (Delouche & Baskin 1973) because of favourable conditions of high humidity and temperature. The fungi hasten the seed deterioration and reduced germination of soybean compared to uninfected controls in the AA test. Conversely, with seeds infected by *Fusarium* spp., the germination after AA can be greater than that of the unaged control because the high temperature during the ageing process kills the fungus. In the control, fungal infection of seedlings produces

abnormals, which in the absence of the fungus would be normal seedlings (Kabeere 1995)

2.5.2.5 Comparison of natural and artificial ageing of seeds

Conflicting data collected from biochemical aspects for seeds aged naturally and artificially, would indicate that natural and accelerated deterioration resulted in different metabolic changes (Wilson & McDonald 1986; Ferguson *et al.* 1990b), such as phospholipid loss in tomato seeds (Francis & Coolbear 1987) and, conductivity in wheat (Nath *et al.* 1991). Different conditions, especially different SMC would contribute to this. In artificial ageing with high humidity, the seed has a high moisture content and, thus the repair mechanism is activated (Francis & Coolbear 1987). Another reason may be this: because it is difficult, if not impossible, to quantify seed ageing, seeds were subjected to different severity of ageing in artificial and natural deterioration, resulting in conflicting results. Although there are problems, the accelerated deterioration is valid as a vigour test to predict seed potential performance (Nath *et al.* 1991; Hampton & TeKrony 1995). But caution in extrapolating the biochemical changes to natural ageing is needed. Ferguson *et al.* (1990b) suspected the methodology of artificial ageing in biochemical deterioration research. There are also, many converse data among natural storage or artificial ageing conditions. One of the reasons may be, once again, seed lots of different vigour are utilised. For instances, since the loss of viability is the last events of deterioration, the biochemical reactions in this stage should be different from those of the early deterioration stage (Ferguson *et al.* 1990a). Koostra & Harrington (1969) suspected that the same oxidative changes related to membrane integrity take place in both normally or artificial aged cucumber seeds.

2.5.3 Controlled deterioration test

2.5.3.1 Current status

The CD test was initially developed for small-seeded vegetable species (Matthews 1980; Matthews & Powell 1981). Recently, it has been used in larger seed, such as pea

(Don *et al.* 1984), maize (Bruggink 1989), French bean and mungbean (Hampton *et al.* 1992a).

The principle of the CD test is similar to the AA test. The conditions under which seeds are deteriorated is determined empirically by selecting those which give a wide range of germination after treatment without deteriorating any seed lots to the extent that they fail to germinate (Matthews 1980). There has been less research and reports on the CD ageing test compared to the AA, conductivity and cold tests. However, the CD test is now, maybe, a promising vigour method because of its good theoretical background (Powell & Matthews 1981; Powell 1995) and its ability to consistently rank seed lots in terms of their potential performance (Matthews 1980; Powell & Matthews 1981, 1984a, 1984b, 1985; Wang & Hampton 1989; Hampton *et al.* 1992a).

2.5.3.2 Comparison of the CD and AA tests

The principles of the CD test are similar to the AA test (Hampton & TeKrony 1995). However, unlike the AA test, the initial SMC is raised to the same level for all seed lots prior to ageing. Thus, SMC during ageing remains constant (Matthews 1980) and seeds undergo the same deterioration (Powell 1995) contrary to the AA test.

Although when conducted correctly the final SMC does not differ significantly in the AA test (Tomes *et al.* 1988), a 2.5% SMC difference can influence the ageing result (Powell 1995). In the CD test, even a ±1% SMC difference had a major effect on results (Powell & Matthews 1981). It is well documented that different SMC can affect the ageing rate (see Section 2.5.2.2). Reports showed that the CD test could better predict the relationship between CD germination and field emergence than the AA test in birdsfoot trefoil (Kim *et al.* 1992) and red clover (Kim *et al.* 1992; Wang *et al.* 1994).

Fungi are a variable in the AA test (Gupta *et al.* 1993). The higher the SMC, the more detrimental the effect of fungi on seed quality. Final SMC after AA is higher than the

desired SMC in the CD test (Hampton & TeKrony 1995). Thus, fungi have less effect in the CD test.

There is an interaction between seed moisture, temperature and ageing time to determine the rate of deterioration (Tomes *et al.* 1988). Thus, different SMC used in ageing tests can affect temperature and time employed in the AA and the CD test to achieve the desired ageing germination to distinguish seed lot vigour. Normally, the higher the SMC is, the lower the temperature and/or time, such as in the ageing test (Hampton *et al.* 1992a).

The suggested modifications for the AA test, such as adjusting initial SMC to the same level (McDonald 1977), using saturated salt solution, instead of water to reduce RH leading to lower moisture uptake rate for small seeded species and retarded fungi development (Zhang & McDonald 1997), indicated the major disadvantages of the AA test. It is not a necessity for the small seeded species because the CD test using the filter paper method to raise SMC (not too long and do not need to determine SMC after ageing) solves these problems perfectly (e.g. Powell & Matthews 1981). The major problem for the CD test is to find a method to raise SMC, especially for larger seed, accurately and rapidly to the desired level.

2.5.3.3 Methods of raising SMC

The CD test avoids the problem of variability of rate of water uptake present in the AA test. However, it is only suited to small seeds and practical difficulties are involved in raising large seeds to a high SMC in a short time (Perry 1984b) using the filter paper method (Matthews & Powell 1981). The high relative humidity method for large seed, such as pea (20^0C and 95% RH) (Don *et al.* 1984; Bustamante *et al.* 1984) or maize (Bruggink 1989), is impracticable, because it takes a few days to achieve the desired SMC (Powell 1995). Although the method is accurate, it is time-consuming and frequent weighing of seeds is needed. How to raise SMC easily, rapidly and evenly to the desired level is a hurdle for its extensive application, especially for large seeded species.

The water added method was designed and first used in red clover (Wang & Hampton 1991), large-seeded mungbean and French bean (Hampton *et al.* 1992a) and soybean (Boersma *et al.* 1996). Although the method is reliable in red clover and mungbean (Wang & Hampton 1991; Hampton *et al.* 1992a), a reasonably large SMC variance was found in French bean (Hampton *et al.* 1992a). Furthermore the water added method resulted in larger variance of desired SMC than the filter paper method did in rape seed lots (Powell 1995). However, its advantages are that it is easy and fast and reasonably reliable according to the few available data.

2.6 Seed treatment

Seed treatment is a broad concept including any treatment of compounds, processes or various energy forms to enhance seed performance (Heydecker & Coolbear 1977; Scott 1989). The compound constituents of seed treatment include fungicides, insecticides, nutrients, rhizobia, organic compounds, enzymes, fertiliser, dyes, bird repellents and "inert" matter for seed ballistics and adhesives to bind materials to the surface of seeds. Because of different chemicals treated in a specific seed lot, the mechanisms by which the chemicals have effects on seed vary (Scott 1989).

In this thesis, seed treatment refereed to the application of finely ground solids or liquids, especially fungicides and insecticides, onto the surface of the seeds in small quantities to protect seeds and seedlings against seed-borne, soil-borne and air-borne pathogens, and insects.

Seed treatment is a relatively cheap and effective means to improve seedling establishment by preventing and curing infection or damage of pathogens and pests to seeds and seedlings. Seed treatment only require 10-15% of the amount of chemicals used for foliar spraying (Baughan &Toms 1984). Chemical treatments can be applied indoors and thus are independent of weather conditions. There is the minimum of pollution. Lastly, local application of the chemicals to the seed before sowing can not greatly influence beneficial organisms in the soil (Potter 1986).

2.6.1 Protectant and systemic chemicals and their mechanisms

In the early day, fungicides were mainly simple inorganic compounds such as sulphur, mercury and copper. They had limitations with the spectrum of activity and were sometimes toxic to plants. In the 1950s and 60s, most synthetic fungicides were protective fungicides- protectants. They lacked the ability to penetrate and translocate within the plant and thus could not kill internal fungal pathogens. However they acted against fungi in a multiple mode of actions, inhibiting several different enzymes associated with energy production, and are termed multi-site inhibitors. They had advantages in that they were broad spectrum, had improved disease control and, were non-toxic to plants; examples are *captan* and *thiram* (Cremlyn 1973; Anonymous 1995).

Systemic fungicides were first reported by Schmeling & Kulka (1966). They discovered that dusting barley seeds with *carboxin*, a systemic fungicide, controlled internal infections of *Ustilago nuda* (loose smut). Unlike the protectants, the systemic fungicides enter the seed and plant and are transported to the site of the pathogen in sufficient quantity to cure infection when the seeds imbibe water from the medium or soil (Schmeling & Kulka 1966; Kirk *et al.* 1969). The marketing of systemic chemicals makes the control of disease organisms in the seeds and air-borne diseases that infect seedlings after emergence possible (Anonymous 1996). Therefore, systemic fungicide seed treatment offers an easier, more practical and economical method to control internal infection. They have much less dependence on climate conditions for activity and even distribution than the protectants do. These fungicides frequently target a single metabolic reaction in the pathogen, which interferes with the ability of a fungal cell to reproduce; in other words they are more selective than the protectants. They are termed single site inhibitors. Although they are effective at lower doses and control pathogens inside the seed and plant, they have disadvantages in their narrow spectrum of activity and the rapidity with which fungi develop resistance towards them due to mutation (Wain & Carter 1977; Anonymous 1995, 1996) and also, they are relatively selective in their action (Davidse & Waarde 1984). The systemic chemicals are thus usually mixed with other chemicals e.g. protectants to control a wide spectrum of

pathogens. The systemic fungicides are grouped into eight families such as *benzimidazoles* and *phenylamides* etc. Fungicides in each family have a common mode of action, inhibiting the same metabolic process (Anonymous 1995).

Uptake of systemic fungicides may occur in two ways. Firstly they may be absorbed directly into the seed before or during imbibition. Secondly, the chemical may spread into the soil from which it is taken up by the root (Cremlyn 1973; Evan 1977). This accounts for the longer effect of systemic chemicals due to the reservoir role of soil, whereas, the fungicides are only short-lived in the plant (Brooks & Buckley 1977). For example, systemic fungicides can protect wheat and barley from infection by aerial spores of rusts for about three months and their effects persist on the third true leaves of pima cotton (Shtienberg 1991). The latter is perhaps the main method of uptake of systemic fungicides because the root is an absorption organ. *Chloroneb* and *DMOE* fungicides were systematically translocated in cotton seedlings whether seed, germinating seed or roots of seedlings placed into water solution which contained the fungicides was used, as detected by autoradiography of fungicides labelled by ^{14}C (Kirk *et al.* 1969).

There is no discussion in the literature about why systemic chemicals can penetrate the seed or plant. *Thiram* is regarded as a protectant. However soaking seeds in a 0.2% aqueous suspension of *thiram* for 24 hours at 30^0C was effective in controlling internal fungi. This is because *thiram* dissolves in water at 30^0C to the concentration of 20-30ppm, and thus *thiram* enters the seed. On the contrary, dusted *thiram* did not have this effect (Maude *et al.* 1969). This implied that the ability to dissolve seems to be the important factor in determining whether fungicides can penetrate the host tissue. This poses a question: what is the criterion to distinguish the protectant and systemic chemicals?

Davies (1965) showed that chemicals could easily penetrate the seed coat when the seed had a high SMC. Free water in the soil is necessary for uptake of fungicides (Cremlyn 1973). In the report of Schmeling & Kulka (1966), *carboxin* was fairly water soluble (170ppm), and appeared to be readily translocated in the transpiration stream (xylem) to the site of the pathogen, which is the most observed mode of translocation of systemic fungicides (Wain & Carter 1977). Two modes of action may be involved in

the function; direct activity, which refers to fungal toxic chemicals accumulating to an effective level at the infection site, and indirect activity, which refers to a systemic compound which reduces disease indirectly by some mechanism (Wain & Carter 1977). However, the reasons for selectivity of systemic fungicides are very poorly understood (Lyr 1995).

2.6.2 The effects of seed treatment

2.6.2.1 Beneficial effects

The ideal chemicals used for seed treatment should be toxic to the pathogens and the pests, but not damage or inhibit the growth of developing seedlings at the recommended rate, or even at double the rate. They must adhere to the seed and remain active on the seeds for long periods (Bateman *et al.* 1986; Anonymous 1996).

It is well documented that fungi, mainly storage fungi, seed or soil-borne fungi and insects may greatly impair seed storage ability and field emergence (e.g. Scott 1989). Therefore, fungicide and insecticide seed treatment, if at an appropriate application rate, can improve emergence (e.g. Lewis 1988; Lewis *et al.* 1991; Cicero *et al.* 1992) storage ability (e.g. Markova & Golyshin 1988; Van Toai *et al.* 1986; Moreno *et al.* 1994), germination rate and vigour index (Saraswthi *et al.* 1995) for a variety of species. For example, after 24 months storage, the AA germination of five soybean cultivars was highest for seeds treated with *carboxin* and thiram, lowest for untreated, and medium for seeds originally treated, but from which the fungicide was removed by methanol washing before the AA test (Van Toai *et al.* 1986). The results indicated that beyond protecting the seeds and young seedlings during imbibition and germination, the fungicide seed treatment also provides a beneficial influence in maintaining seed quality during storage. Lower conductivity values for treated seeds provide further support because the conductivity test did not subject seed to micro-organism stress (Van Toai *et al.* 1986). The chemicals normally have no toxic effect on germination (e.g. Silva & Marcos-Filho 1990).

2.6.2.2 Seed treatment phytotoxicity

"It is the dose that makes the poison" (Anonymous 1996). Dose rate is a compromise between maximum effectiveness and phytotoxicity. Different application rates can greatly affect seed vigour and/or test results. Fungicide seed treatment appears to be without phytotoxicity around the recommended application rate (Van Toai *et al.* 1985; Cane & Hampton 1989;) e.g. *carboxin* (Reinbergs & Edigington 1968), and *triadimefon* (Bunion *et al.* 1991). Seeds that have been over-treated with fungicides or insecticides commonly produce toxic effects, such as a linearly decreased emergence of seeds of Southern pines with increased fungicide application rate from two to four times the recommended rate (Bunion *et al.* 1991), decreased germination and field emergence of barley seed treated with *triadimenol+ imazalil* at twice the recommended rate after 12 months storage (Cane & Hampton 1989), decreased vigour indexes revealed by the germination, AA, cold and field emergence tests of maize seeds treated with the insecticide *carbofuran 350 FMC* with increasing rate (Nascimento *et al.* 1996). All ten insecticides tested were detrimental to some extent to broad beans (*Vicia faba* L.), depending on the applied doses. The expression of phytotoxicity of individual treatments was influenced by time of seedling evaluation (length of test period) and germination substrate. Normally, the phytotoxic effects were present during the early stage of germination and/or high doses of the chemicals and may have disappeared or been relieved at a later stage (Nijenstein & Ester 1990). Chemicals that show a slight phytotoxic effect in the laboratory may have higher yield because of control of diseases and pests (Nijenstein & Ester 1990).

2.6.3 Effect and problems in seed testing

Kashyap *et al.* (1994) found that more than 36% of seeds of wheat cv. Sonalika treated with the insecticide *Formothion 20EC* had not germinated by the day of the final count (8^{th} day), even though 88% were viable according to the tetrazolium test. This chemical may delay germination. The standard required in ISTA Rules for bare seed may not be suitable for treated seed (Cornish 1986). Test methods need to be refined when conditions change. The prescribed germination substrate, i.e. sand, for field bean may not be as optimal for treated seeds as it is for untreated ones because of more

phytotoxicity expression of insecticide seed treatment than in other germination media. Therefore, extending the test period and modifying the ISTA rules for treated seeds (e.g. substrate) is advisable (Nijenstein & Ester 1990).

Seeds treated with *thiram* and *captan* had a higher field emergence than did untreated seeds, but had little effect on standard germination (Matthews & Brodnock 1969).

Chemical seed treatments also have some effects on conductivity (see Section 2.5.1.3) and AA (see Section 2.5.2.3) tests.

2.7 Dormancy and Hardseededness

2.7.1 Dormancy

The basic characteristic of dormancy is the absence of germination of a viable seed under favourable conditions, whereas quiescent seed will germination under suitable environmental conditions. Some dormancy breaking methods (e.g. pre-chilling treatments) shift the seed from the dormant to quiescent state without inducing germination (Hilhorst & Toorop 1997).

Secondary dormancy is a dormancy where mature, non-dormant seeds are induced to the dormant state by unfavourable conditions such as unsuitable temperatures, anoxia or illumination, etc. It is different from primary dormancy. The mechanism of induction is unknown. However, in general, secondary dormancy is characterised by a loss of sensitivity to dormancy-breaking factors. e.g. high temperature induced loss of key receptors for nitrate or Pfr (a kind of *phytochrome*) in the plasma membrane (Bewley & Black 1994). *Ambrosia trifida*, *Avena sativa* and *Taraxacum megalorhizon* are examples of species which exhibit secondary dormancy when at temperatures above the maximum for germination (Bewley & Black 1994).

Dormancy controlled by genetic and environmental factors is a means by which distribution of germination in time and space can be achieved. It provides the natural

protection against seed deterioration under adverse conditions. In fact, there would be little survival value in dormancy if it did not also reduce the rate of deterioration of seeds (Delouche 1980). The dormancy of a seed is not an all-or-nothing property. Dormancy varies on a continuous scale, which is verified by continuous changes in the range of conditions suitable for germination, e.g. temperature. The temperature has a dual role in regulating both dormancy and germination. However, the temperature requirements of both processes are quite different. Some species or cultivars are temperature sensitive, and will be dormant when subject to high temperature at imbibition, for instance, lettuce, especially "Grand Rapids" cultivars (Maguine 1980). On the other hand, alternative temperatures are commonly accepted to relieve dormancy (Vleeshouwers *et al.* 1995).

2.7.2 Hardseededness

Seeds that exhibit water impermeability are known as hard seed. The seed coat is mainly composed of cuticle, macrosclerid cells and osteosclerid cells, while some waterproof chemicals, such as lignin, tannin and suberin are formed in between (Corner 1951; McKee *et al.* 1977). It is responsible for the impermeability. Hardseededness is a kind of primary physical dormancy, often with the embryo in a non-dormant state. It is typical of many families, especially *Fabaceae* (*Leguminosae*) (ISTA 1996). Species of small seeded legumes, such as in *Trifolium*, *Medicago*, *Lotus, Lens* and *Ornithopus* etc., are very nutritive, important crops for pasture (Duke 1981). However, high hard seed content after seed maturity is common.

Seed lots with a considerable hard seed content tend to delay seed deterioration and maintain viability longer than that of lower ones (Dexter 1955; Flood 1978), because the impermeable coat protects the seed against high humidity and temperature stress, machine damage and micro-organism infection during pre-harvest in the field, harvesting, conditioning, and storage (Christiansen & Justus 1963; Garay & Grabe 1983; Mohamed-Yasseen *et al.* 1994). For instance, the viability of crownvetch (*Coronilla varia* L.) hard seeds was 24% higher on average than that of naturally softened seeds (Garay & Grabe 1983).

Hardseededness is an important mechanism for germination regulation. Hard seed can carry the species through adverse seasons and cropping years (Quinlivan 1965). The sequential breakdown of hard seed ensures seedlings emerge at successive intervals from the soil seed bank, to avoid competition and stress conditions, such as emergence following autumn rain in *Medicago* spp. (Quigley & Carter 1989), especially in annual self-regenerating legumes (Carter & Lake 1985; Williams *et al.* 1987; Matches 1989; Fairbrother 1991). A positive linear relationship exists between numbers of soft seed and seedling plants/m^2 in the soil (Porqueddu *et al.* 1996).

However, hardseededness in some cases, is considered an undesirable parameter due to: the failure of germination and slow seedling establishment (Mohamed-Yasseen *et al.* 1994); contamination in seed production and the downgrading of seed quality (Shillito 1974; Hampton *et al.* 1987); cost needed to break them in some cases (Stout & Langton 1991).

2.7.2.1 Methods for breaking hardseededness

Just as the development of hard seeds in leguminous plant has been attributed to both genetic and environmental factors (Quinlivan 1965; Donnely 1970; Smith 1988), the natural breaking of hard seed is in the same situation (Taylor & Ewing 1992; Fairbrother & Pederson 1993). Fluctuating temperature and high constant temperature and humidity are the most important environmental parameters affecting the reversibility of hard seed (Quinlivan & Millington 1962; Hagon & Ballard 1970; Taylor 1981; Fairbrother 1991). Hardseededness reversibility is the function of SMC (Rolston 1978). Hard seed content increased with reduced SMC (Argel & Humphreys 1983). Hyde (1954) suggested that the hilum is a hygroscopically activated valve that prevents water uptake but permits water loss at low relative humidity. As the severity of desiccation by loss of water through the hilum increased, the reversibility of soft seed and duration of impermeable seed increased. However, this is not always the case, even at very low SMC, say, 4% (Vik 1986). The process of natural breaking takes a long time, which cannot be afforded in seed quality testing. Therefore, rapid artificial

breaking methods are widely researched. For assessing seed quality or achieving plant value, the hard seed needs to be softened.

Sulphuric acid method

Although there are a few reports of chemicals such as acetone, benzene and ethanol etc. being used for hard seed breaking in crownvetch (Brant *et al.* 1971) and in subterranean clover (Fairbrother 1991), sulphuric acid (H_2SO_4) is likely the most promising chemical to break hard seed. Concentrated sulphuric acid can effectively soften hard seed without damaging germination although the suitable treatment time differs in different species (Brant *et al.* 1971; Fu *et al.* 1996). The optimum H_2SO_4 immersion time of *Lotus uliginosus* cv. Grasslands Maku was 60-75 minutes (Hare & Rolston 1985). Also, treatment of 19 seed lots of crownvetch with H_2SO_4 (18N) for 15 minutes (with agitation) reduced hard seed from 60% in the control to 11% in H_2SO_4 treated seed, and increased readily germinable seed from 16% in the control to 75%, without injuring the already permeable seed (Brant *et al.* 1971). Concentrated H_2SO_4 soaking for 20 minutes removed hardseededness of common vetch and the acid scarified seeds had faster field emergence (Aswathiah 1987). However, unsuitable times have negative effects on seed quality (Viado, 1989; Fu *et al.* 1996). Because of different coat thickness due to different maturity, the hard seeds with a thicker coat were not broken when treatment time was short. However, the embryos of the thinner coat seeds were damaged if longer time treatments were employed (Yi *et al.* 1994). Scanning electron micrographs showed that the lumens of the macrosclereid cells were exposed after treatment and thus imbibition occurred (Brant *et al.* 1971). H_2SO_4 is mostly used in experiments for small seed lots rather than for commercial application (Rolston 1978; Hare & Rolston 1985).

Mechanical scarification

Brushing is an effective method of decreasing hard seed, without increasing abnormal seedlings and dead seed (Pedron 1978). Brushing, sandpaper and tumbling treatments reduced hard seed of 16 field-dressed seed lots of lucerne to 6.6%, 21.4% and 26.9%, respectively, compared to the control of 38.4% (Viado 1989). Emery paper inside a

hand scarifier (120 rpm) treatment for 5 seconds effectively broke hard seed of penngift crownvetch from 60% to 4% without reducing total live seed (Brant *et al.* 1971). Mechanical scarification removed the cuticle and terminal cap of macrosclerids, permitting imbibition of water (Brant *et al.* 1971) and is the most common commercial method (Rolston 1978; Hare & Rolston 1985).

Other methods

There are some other methods that have been studied. Heating, including dry heat (Rincker 1954) and hot water (Brant *et al.* 1971; Singh *et al.* 1992), were effective ways to break hard seed. Freezing, including liquid N (-196^{0}C) (Brant *et al.* 1971; Viado 1989; Wiesner *et al.* 1994) and the treatment cycle of 2h at -80°C followed by at least 2h at 20°C (Stout 1990) was highly effective in decreasing hard seed content. The treatments of liquid N in the reports were too severe, yielding damaged seeds. However, the freeze-thaw cycle did not influence the germination rate and seedling dry matter (Stout 1990; Hall *et al.* 1993). Similar to heating, freezing caused cracking in the seed coat because of different thermal expansion, thus breaking hard seed (Brant *et al.* 1971). Electrically generated radiation is also an interesting method (Ballard *et al.* 1976; Nelson *et al.* 1982; Viado 1989).

2.7.2.2 The problems in seed testing

See Section 2.5.1.4

CHAPTER 3: MATERIALS AND METHODS

3.1 General

3.1.1 Seed sources

Seed lots used for these experiments were obtained from several sources (Table 3.1). All seed lots were stored in plastic bags placed into plastic buckets with lids at 5^0C until needed for the experiments.

3.1.2 Seed sampling

A riffle divider was used to obtain working samples from the submitted samples using the method described by ISTA (1996). Quality tests were conducted on pure seed (ISTA 1996) of each species only.

3.1.3 Standard germination

Seed lots were tested using the required method as provided by the ISTA Rules for Seed Testing (ISTA 1996) i.e. top of paper or between paper at 20, 25 or 20-30^0C depending on species. Usually four replicates of 100 seeds were tested (ISTA 1996), but occasionally because of the number of seeds available, replicates of 50 or 25 seeds were used. After the prescribed germination time period (ISTA 1996), germination was recorded by counting the number of normal and abnormal seedlings, and remaining seeds.

3.1.4 Seed moisture content (SMC)

Seed moisture was determined for duplicate samples from each seed lot using the low constant temperature oven method (103^0C for 17h) or the high constant temperature oven method (130^0C for 1-4h) depending on the species (ISTA 1996).

Table 3.1: Species and seed lots used in the experiments

Species and cultivar	Seed Source	Submitted Sample size (g)
Pea cv. Pania (two lots)	Seed Technology Centre	3500 each
Pea (9 commercially treated lots. see Table 4.6)	Asgrow Seed (NZ) Ltd. Masterton	200 each
Pea cv. Bolero	Asgrow Seed (NZ) Ltd.	10,000
Soybean	Seed Technology Centre	6,000
Broad bean cv. Coles Prolific	Watkins NZ Ltd., New Plymouth	3000
Bean French cv. Dwarf Bush Top Crop	Watkins NZ Ltd., New Plymouth	3000
Maize cv. CF96005	Food & Crop Research, Palmerston North, NZ	3000
Maize cv. CF96003	Food & Crop Research, Palmerston North, NZ	3000
Sweet Corn cv. Jubilee	Wattie Frozen Foods Ltd. Gisborne, NZ	3000
Sweet Corn cv. Illini Gold	Seed Technology Centre	6600
Wheat cv. Otane	Seed Technology Centre	4800
White clover cv. Huia	Seed Technology Centre	
White clover cv. Kopu	AgResearch, Palmerston North, NZ	100
White clover cv. Pitau	Seed Technology Centre	
White clover cv. Sustain	Seed Technology Centre	
Lotus cv. Maku	Seed Technology Centre	720
Swede cv. Doon Major	Wrightson Seeds Ltd Palmerston North	1000
Onion (four lots)	ISTA Vigour committee, UK	
Swede (five lots)	ISTA Vigour committee, UK	

3.1.5 Thousand seed weight (TSW)

TSW was determined by weighing eight replicates of 100 seeds per seed lot (ISTA 1996), obtaining a mean weight, and multiplying by 10.

3.2 Conductivity Testing

3.2.1 Standard method

A distilled water supply was equilibrated at 20^{0}C overnight. From this source 250ml was measured using a 250 ± 2ml measuring cylinder and poured into a 500ml conical

flask. Four replicates of 50 seeds drawn from the working sample of a seed lot with a SMC between 10-14%, were weighed to two decimal places, and then 50 seeds were placed into a flask containing 250 ml distilled water (i.e. four flasks per seed lot). A control flask containing only distilled water (250ml) was also set up to monitor water quality. The flasks were covered with laboratory sealing film to prevent contamination and evaporation (Plate 3.2), and kept at 20°C for 24h. At the end of the 24h soak period, the flask (with seeds) was gently swirled for 10-15 seconds, the film removed, and the conductivity of the solution (μScm^{-1}) was measured at 20°C using a CDM83 conductivity meter (Radiometer, Copenhagen; Plate 3.1). The dip cell of the conductivity meter was immersed in the solution avoiding touching the seeds, and the reading recorded. Between readings, the dip cell was rinsed twice using distilled water. The conductivity of the control flask was measured and this value subtracted from the conductivity reading already recorded for each flask. The conductivity per gram of seed weight ($\mu Scm^{-1}g^{-1}$) for each replicate was calculated by dividing the conductivity (μScm^{-1}) of each flask by the weight (g) of the seed sample (Hampton & TeKrony 1995).

However, for white clover and lotus, because 50 seeds weighed very little, around 0.3g and 0.5g seeds for each replicate was used, respectively, rather than 50 seeds. The weight was recorded to four decimal places.

3.2.2 Achieving the desired SMC

The SMC of some of the seed lots was not within the 10-14% range required (Hampton & TeKrony 1995), and the following methods were used to obtain the desired SMC.

SMC of white clover cv. Kopu and Huia was raised to the desired level by placing the seeds on a wire-mesh tray inside a plastic box with a lid containing 40 ml mixture of Glycerine (55%) and distilled water (45%) which was then incubated at 25°C. The following formula was used to calculate the desired SMC:

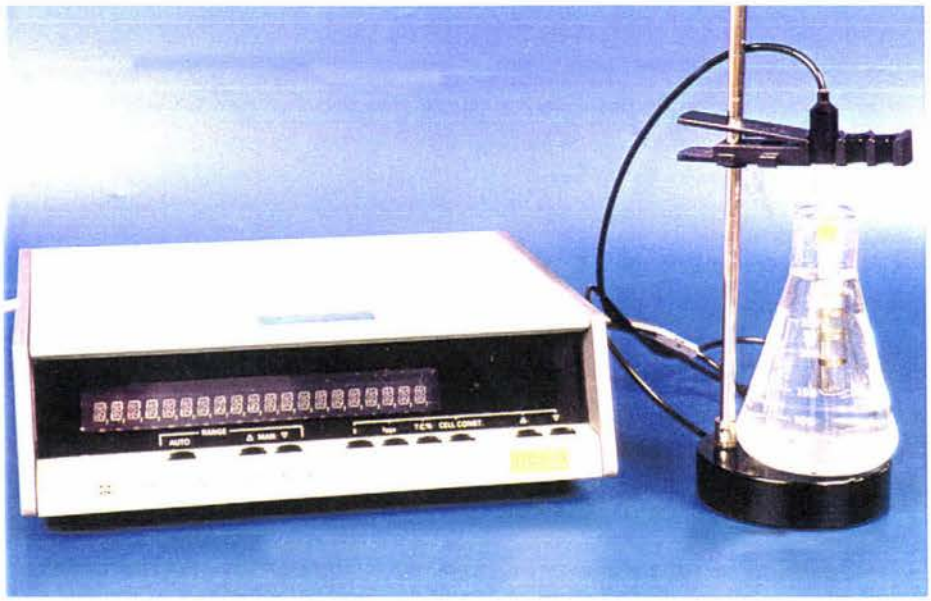


Plate 3.1: CDM83 conductivity meter (Radiometer, Copenhagen)

Plate 3.2: The flasks covered with laboratory sealing film to prevent contamination and evaporation in the conductivity test

$$B = 100 - \frac{(100 - A) \times W_1}{W_2}$$

Where B= desired SMC (wet weight basis), A= initial SMC, W_1= initial weight of sample, W_2= final weight of seed sample.

W_2 was weighed every day until the SMC was within the range of 10-14%.

Soybean seed moisture was raised to the desired level by putting the seeds on a wet blotter in a lid covered plastic box at laboratory temperature (20-25^{0}C). The samples were weighed every half an hour at the early stage. When seed moisture approached the desired SMC (i.e., W_2), the seeds were weighed every few minutes until achieving the required SMC.

3.2.3 Presence of hard seed

Any hard seeds in the white clover and lotus seed lots tested were picked out using tweezers from the solution in the flask after measuring conductivity, placed on a paper towel to dry, and weighed to four decimal places. The weight of the hard seeds was subtracted from the initial weight before the conductivity per gram was calculated.

3.2.4 Effect of seed treatment

3.2.4.1 Laboratory treated seeds

Chemicals and application rate

The chemicals used as seed treatments are listed in Table 3.2.

Application method

According to the recommended application rate (Table 3.2) and double this rate, the weight and/or volume of chemicals and distilled water was calculated for the

Table 3.2: Recommended chemical application rate (NZ Agrochemical Manual 1995 & Novachem Manual 1996)

Chemical	Active ingredient	Recommended rate
Apron 35 SD (systemic fungicide)	metalaxyl	200g / 100kg seed /500ml H_2O
Apron TZ (systemic fungicide)	metalaxyl+ thiabendazole	150g/100kg seed/enough H_2O
Aliette Super (systemic fungicide)	fosetyl aluminium + thiram+ thiabendazole	290g/100kg seed/300ml H_2O
Orthocide-80W (protectant fungicide)	captan	250g/200kg seed/1L H_2O
Vitaflo 200 (systemic fungicide)	carboxin + thiram	250ml/100 kg seed
Benlate[1] (systemic fungicide)	benomyl	100g/45kg seed/800ml H_2O
Thiram[2] (protectant fungicide)		150g/1L H_2O/ for 211kg maize, 99kg sweet corn and 214kg wheat seed. 285g / 1L H_2O/ 92kg clover seed
Gaucho (systemic insecticide)	imidacloprid	583.3g suspension / 100kg seed
Promet 365 CS (systemic insecticide)	furathiocarb	54.8 ml / 1kg seed / 66.4g absorbent TZ 10 powder[3]

[1] it is recommended that Orthocide 80W is added (100g /45kg seed) to the systemic fungicide *benlate*. However, for the purpose of studying the effects of systemic chemicals, Orthocide 80W was not applied except in maize cv. CF96005.
[2] recommended application rate of *thiram* which was not available in the manuals, was the same as Orthocide 80W (Hampton, pers. comm.).
[3] the powder is added to ease seed flow and thus avoid blocking machinery (Robert Southward, pers. comm.).

appropriate weight of the seed sample. An electronic balance or a measuring cylinder was used to obtain the required weight or volume of the chemicals. Then, the chemicals were poured into flasks (1000, 500 or 250ml flasks depending on the size and quantity of the seeds). Different scaled pipettes were used to put the required volume of distilled water into the flasks. The flasks were shaken immediately and rapidly for about 30 seconds to make an even slurry. After that, the seeds were put into the slurry, the flasks stopped with a rubber bung, and shaken until an even cover of chemical adhered to the seeds. Seeds were poured out on to a manila paper and dried at laboratory temperature (20-24^{0}C) for 1- 2 hours. Then, seed samples were packaged into paper or plastic bags and stored at 5^{0}C ready for the conductivity test following the procedures given in Hampton & TeKrony (1995) (see Section 3.2.1) and AA

testing (see Section 3.3). Conductivity was determined on the same or the next day after treatment. For soybean and sweet corn cv. Jubilee, the conductivity was also tested after two months storage (20d at 5^0C and 40d at 20^0C) in sealed moisture-proofed foil bags for the recommended application rate only. Furthermore, the effect of the chemical alone on conductivity was studied following the procedures described by Hampton & TeKrony (1995) by placing specific amount of chemical into flasks containing 250 ml distilled water.

Controls were treated with only distilled water calculated from the average amount used in the chemical treatments. When double the recommended chemical rate was applied, the weight or volume of the chemicals was doubled, but the volume of the distilled water remained the same as for the recommended application rate. Also, the absorbent *Tqlc Sa50* powder was not doubled when *Promet 365 CS* was applied.

3.2.4.2 Commercially treated seed lots

Nine seed lots of garden pea commercially treated with chemicals (Table 4.6) were supplied by Asgrow Seed (NZ) Ltd., along with untreated (control) seeds from the same seed lots. These seed lots were tested for germination, SMC and TSW following ISTA Rules (ISTA 1996) and conductivity tested according to the procedures described in Section 3.2.1.

3.2.5 Determining tolerances for conductivity testing

A garden pea cv. Bolero seed lot was sealed in moisture-proofed foil bags at 13.4 % SMC. Conductivity test runs of four replicates each were conducted every day (except for a few weekends) until 100 runs were completed. The 100 ranges (difference between the greatest and the least measurement per replicate) were tested for normal distribution, and a tolerance calculated using the probability formula for normal distribution, i.e., $Z = \frac{Y - \mu}{\sigma}$, at the 5 and 1% significance levels, where μ is the population mean, σ is standard deviation, Y is the observation value and Z is the

standardised normal variable. According to the formula, Z is a deviation from the mean, namely Y- μ, measured in units of standard deviation (Steel & Torrie 1980).

3.3 Accelerated Ageing (AA) Testing

As per the recommendation of Hampton & TeKrony (1995), the inner chambers used were boxes (a plastic box of 11.0× 11.0× 3.5cm with a lid) with screen trays (10.0× 10.0× 3.0cm wire mesh). They were washed using Janola and dried after each use to prevent fungal contamination. Distilled water (40 ml) was placed in each inner chamber. A minimum of 200 seeds of each seed lot determined on a weight basis (e.g. 60g for pea), was drawn from the working sample with a SMC range of 10-14% as required by Hampton & TeKrony (1995). If SMC was not within the range, it was raised using the methods described in Section 3.2.2. A single layer of the seeds was placed on the surface of the screen tray inside the inner chamber. After the lid was secured, the inner chambers were transported cautiously and placed in the outer ageing chambers- the Series Five Contherm incubator (Plate 3.3)- at 2.5 cm apart from each other to assure temperature uniformity. The seed weights and ageing conditions were different for different species (Table 3.3).

Table 3.3: Variables for the AA test using an inner chamber

Species	Seed weight per box (g)	No. of boxes per replicate	Ageing conditions
Garden pea	30	2	40^{0}C for 72h
Soybean	42	1	41^{0}C for 72h
Maize	40	2	45^{0}C for 72h
White clover	0.3	1	41^{0}C for 72h
Lotus	0.3	1	41^{0}C for 72h

During the ageing period, the temperature of the out ageing chamber was maintained at the desired value ± 0.3^{0}C, and the door was not opened. After ageing, a small sample was removed and weighed immediately to determine SMC using the oven method (ISTA 1996). When SMC was not in the range recommended by Hampton & TeKrony (1995), the tests were repeated. The germination test was conducted using the remaining aged seeds. The aged control seeds were separated into two parts. One was

Plate 3.3: The Series Five Contherm incubator used as the outer ageing chamber in the AA and CD tests

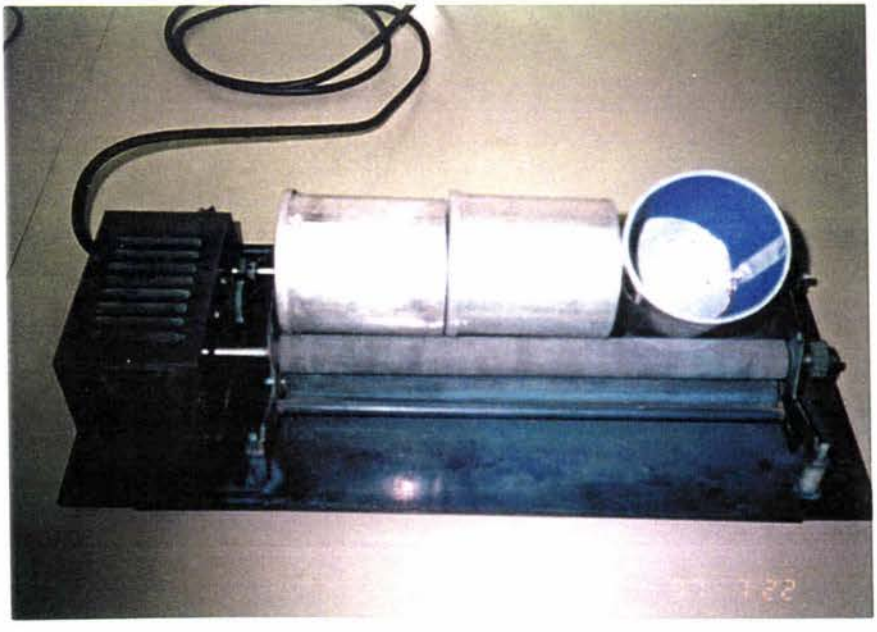


Plate 3.4: Scarifier lined with sandpaper (P150 grade) for mechanical scarification of hard seed

dusted with *thiram* (1% by weight) as control 2, another part was germination tested directly as control 1. At the same time, the standard germination testing of the non-aged seed sample was carried out (ISTA 1996) for comparison.

3.4 Controlled Deterioration (CD) Testing

3.4.1 Raising SMC

3.4.1.1 Filter paper method

Four replicates of 100 seeds of the species used (onion, swede, garden pea and maize) were each placed on a moist blotter inside a plastic box to imbibe until the required moisture content was reached. This was calculated by the formula:

$W_2 = \frac{(100 - A) \times W_1}{100 - B}$ Where A= initial SMC (wet weight basis), B= desired SMC, W_1= initial weight of sample, W_2= final weight of seed sample.

Each blotter was wetted by adding 9ml distilled water. Seed replicates were weighed regularly to 4 decimal places as they approached the weight W_2. Once the seeds had achieved the desired SMC, the seeds of each replicate were immediately placed into a moisture-proof (polyester-aluminum foil-polyethylene laminate) bag. The bags were flatted with the edge of the hand to remove air and heat sealed by a Ribbon Heat Sealer approximately 3 cm above the level of the seeds. After that, the bags were placed at 10^0C for 24 h for moisture equilibration (Matthews & Powell 1987). At the end of the 24 h moisture equilibration, the bags were placed side down in the deterioration chamber and incubated for the required time at their specific temperature (see Section 3.4.2) (Hampton & TeKrony 1995).

3.4.1.2 Water added method

A minimum of 150 seeds each for four replicates of the same species were placed into a foil bag. The amount of water required to raise SMC to that required was calculated by the following formula:

amount of water to be added (ml)= W_2 (g)-W_1 (g),

where W_1= initial weight of sample, W_2= final weight of seed sample (W_2 was calculated by the formula in Section 3.4.1.1).

The required amount of distilled water measured by micro-pipette was added to each foil bag to bring the seeds to the desired SMC. The foil bags were flattened with the edge of the hand to remove air and then immediately heat sealed about 3 cm above the seed level. Then the bags were gently shaken for 30 seconds and placed at 10^0C for 24 h in a horizontal position. The bags were turned once every hour for the first 4 h. At the end of the 24 h moisture equilibration, the bags were placed side down in the deterioration chamber and incubated for the required time at their specific temperature (see Section 3.4.2) (Hampton & TeKrony 1995). After deterioration, 50 seeds from each bag were removed immediately to determine SMC using the oven method (ISTA 1996).

The above operations were carried out in the laboratory with temperature ranging from 21-25°C. Garden pea cv. Bolero seed sub-samples were sealed in foil bags at 13.4% SMC and stored at 5, 20 and 25^0C for six months. The experiment to compare efficacy of the two methods of raising SMC was conducted.

3.4.2 Temperature and time

The Series Five Contherm incubator (Plate 3.3) was used as the deterioration chamber. The combination of 20% SMC and 24h at 45^0C deterioration temperature was used for swede, pea and maize and the same time/temperature but a SMC of 19% for onion. The conditions for CD testing for onion, swede and pea were suggested by Hampton & TeKrony (1995). After deterioration, the deteriorated seeds were dusted with *thiram* (1% by weight) to avoid fungal development prior to germination testing which used 100 seeds from each of the four replicates (ISTA 1996). A standard germination test for non-deteriorated seeds (ISTA 1996) was conducted for comparison. Top of paper germination method was used in test runs 1 and 2 for the onion, but the between paper germination method was used in run 3 because of a limitation of germination boxes which meant that the top of paper method could not conducted. For swede, 20^0C was

used for germination of run 1, but 20-30^{0}C was employed for run 2-3 because of high dormancy induced by the first method. However, all the methods used were recommended by ISTA (1996).

3.4.3 Dormancy study of swede cv. Doon Major

Because dormancy was induced in all the five swede seed lots of different but unknown cultivars from the United Kingdom (Table 3.1) in the CD test mentioned above, the following experiments were designed to investigate the reason.

Three lots of 10g of swede cv. Doon Major seeds obtained in New Zealand (Table 3.1) with an initial SMC of 7.1% were put onto moist blotters in plastic boxes until the designed SMC, i.e. 8.5, 10.0 or 11.5% was achieved (as calculated by the formula given in Section 3.2.2) under laboratory conditions. The seeds were stored at 5^{0}C for a few days before CD testing. The procedures of CD testing were the same as described in Section 3.4.1 and 3.4.2 except that 40^{0}C for 48 h was also used to deteriorate seeds. Germination tests were conducted at both 20^{0}C and 20-30^{0}C (ISTA 1996). After the CD germination, any fresh ungerminated seeds were relocated to new wet blotters and chilled at 5^{0}C for four days. After that, these seeds were transferred to 20^{0}C or 20-30^{0}C respectively, to check whether they would germinate or remained dormant. The remaining fresh seeds were pre-chilled for a second cycle and were re-germinated using the same method as for the first cycle.

3.5 Effect of hardseededness on vigour test results

3.5.1 Breaking hardseededness using sulphuric acid and sandpaper

3.5.1.1 Sulphuric acid (H_2SO_4) treatment

3 × 10g seed samples of white clover cv. Sustain and lotus cv. Maku were immersed in concentrated H_2SO_4 inside jars for 5, 10 and 15 minutes. During treatment, a glass rod was used to stir the seeds to prevent them sticking together. After completing the

treatments, the seeds were poured into a sieve immediately, rinsed under running tap water for one minute, and then put onto a paper towel. After one hour of drying, the SMC was tested and seeds sealed in moisture-proof foil bags for experiments.

3.5.1.2 Sandpaper treatment

Seed samples were tumbled for 30, 35 or 40 minutes for lotus cv. Maku and 50, 70 or 90 minutes for white clover cv. Sustain. in a small modified scarifier (Plate 3.4) lined with sandpaper (P150 grade) inside the tumbling container. The speed of the container was 9.8 rpm. After treatments, the SMC was tested and seeds sealed in foil bags ready for experiments.

3.5.2 Germination and vigour (conductivity and AA) testing

Seed lots were germination tested using the required method (ISTA 1996). Conductivity and AA testing were carried out following the methods described in Section 3.2.1 and 3.3, respectively.

3.6 Statistical analysis

The SAS computer programmes for WINDOWS 6.12 or 6.11, available on the Massey University network were used for analysis of variance; the VAREST program developed by Dr. I.L.Gordon (Plant Science, Massey University) was employed to analyse normal distribution data from the pea cv. Bolero conductivity tolerance test.

CHAPTER 4: RESULTS OF CONDUCTIVITY TESTING

4.1 Initial seed quality

Quality data for the seed lots used for the experiments are presented in Table 4.1.

Table 4.1: Quality of seed lots employed in all the experiments.

Species and Cultivar	TSW [1] (g)	SMC [2] (%)	Germination (%)	Hard seed (%)
Pea cv. Pania (lot 1)	238.2	10.9	99	*[3]
Pea cv. Pania (lot 2)	240.0	10.5	98	*
Pea cv. Bolero	222.9	13.1	99	
Soybean	163.5	10.2	89	*
Broad bean cv. Coles Prolific	1398.5	12.5	91	*
French bean cv. Dwarf Bush Top Crop	428.1	12.8	90	*
Maize cv. CF96005	287.1	11.5	98	*
Maize cv. CF96003	363.0	11.5	99	*
Sweet corn cv. Jubilee	199.1	11.6	99	*
Sweet corn cv. Illini Gold	138.9	10.4	66	*
Wheat cv. Otane	48.3	12.6	96	*
White clover cv. Huia	0.64	9.9	93	3
White clover cv. Kopu	0.69	7.1	82	14
White clover cv. Pitau	0.59	7.4	88	5
White clover cv. Sustain	0.65	9.6	74	15
Lotus cv. Maku	0.79	10.7	62	13
Swede cv. Doon Major	0.292	7.1	99	*

[1] thousand seed weight.
[2] seed moisture content.
[3] no hard seed present.

4.2 Effects of seed treatment on conductivity testing

4.2.1 Large seeded legumes (garden pea, soybean, broad bean, French bean)

At the recommended application rate, no fungicide treatments significantly increased the conductivity of soybean, broad bean or French bean (Table 4.3). But for garden pea, there was a difference in results between the two seed lots (Table 4.2), where for seed lot 2, *Apron 35 SD*, *Aliette Super*, and *orthocide* increased conductivity significantly.

Table 4.2: Effect of fungicide seed treatment at the recommended (R) and double the recommended (D) application rates on conductivity for two seed lots of pea cv. Pania

Treatment	Conductivity (µS/cm/g)		Treatment	Conductivity (µS/cm/g)	
(R)	Seed lot 1	Seed lot 2	(D)	Seed lot 1	Seed lot 2
Apron 35 SD	17.3	18.9*	Apron 35 SD	19.6*	17.5
Apron TZ	17.9	18.1	Apron TZ	17.0	18.7
Aliett Super	18.3	18.6*	Aliett Super	19.5*	19.9*
Orthocide	17.7	19.4*	Orthocide	16.2	18.9*
Control	17.1	16.4	Control	17.2	17.3
LSD(P<0.05)	ns	1.9	LSD(P<0.05)	2.1	1.5

* differ significantly from the control at P< 0.05

Table 4.3: Effect of fungicide seed treatment at the recommended (R) and double the recommended (D) application rates on conductivity for one seed lot each of soybean, broad bean and French bean

Treatment	Conductivity (µS/cm/g)					
	Soybean[1]		Broad bean[2]		French bean[3]	
	R	D	R	D	R	D
Apron 35 SD	20.3	24.0 *[4]	14.0	14.4	22.3	21.4
Apron TZ	19.5	23.6 *	13.2	14.1	22.3	21.9
Aliett Super	21.1	23.4 *	14.9	16.4 *	22.3	22.9
Orthocide	19.2	20.9	13.8	*[5]	22.1	22.8
Control	19.4	20.1	13.8	13.8	22.4	21.9
LSD (P<0.05)	ns	2.2	1.4	0.8	ns	1.14

[1] cultivar unknown. [2] cv. Coles Prolific. [3] cv. Dwarf Bush Top Crop. [4] differ significantly from the control at P< 0.05. [5] no data as insufficient seed.

Table 4.4: Conductivity of the solutions of the chemicals used for large seeded legume seed treatment

Chemicals[1]	Conductivity (µS/cm/g)	Chemicals[2]	Conductivity (µS/cm/g)
Apron 35 SD	21.5b[3]	Apron 35 SD	29.3 b
Apron TZ	6.20d	Apron TZ	7.5 d
Aliette S	84.5a	Aliette S	106.0 a
Orthocide	9.89c	Orthocide	12.8 c
control	2.63d	control	2.49 e
LSD (P<0.05)	3.16	LSD (P< 0.05)	3.13

[1] chemical weight per flask as calibrated and measured by the weight applied to 50 seeds (21.4g) of French bean according to the recommended application rate.

[2] chemical weight per flask as calibrated and measured by the weight applied to 50 seeds (29.0g) of average weight of peas, soybean, French bean and broad bean according to the recommended application rate.

[3] means with the same letter in the same column are not significantly different at P< 0.05.

At double the recommended application rate, *Aliette Super* significantly increased the conductivity of both pea seed lots, *Apron 35 SD* and *Orthocide* increased conductivity for one seed lot each, and *Apron TZ* did not alter the conductivity (Table 4.2). The three systemic products at double the application rate significantly increased soybean conductivity, but not French bean (Table 4.3), and *Aliette Super* at this rate increased broad bean conductivity (Table 4.3).

In the absence of seed, all the fungicides increased conductivity significantly (Table 4.4).

After two months storage of treated seed lots of soybean at 10.2% SMC, the fungicides had marginally increased conductivity with a significant difference for *Apron TZ* treated seed (Table 4.5). However, germination of the *Aliette Super* treated seed had also significantly increased (Table 4.5).

Conductivity of seed lots commercially treated with systemic fungicides did not differ significantly from the untreated same lot in seven out of nine cases (Table 4.6).

Table 4.5: Effects of seed treatment of soybean with systemic and protectant fungicides at the recommended application rate on conductivity and germination immediately after treatment (I) and after two months storage (T)

Treatment (I)	Conductivity (µS/cm/g)	Germination (%)	Treatment (T)	Conductivity (µS/cm/g)	Germination (%)
Apron 35 SD	20.1	91	Apron 35 SD	21.4	92
Apron TZ	20.7	90	Apron TZ	22.4 *	93
Aliett Super	21.0	89	Aliett Super	21.2	95 *
Orthocide	19.4	90	Orthocide	21.1	91
Control	20.4	90	Control	19.9	89
LSD (P<0.05)	ns	ns	LSD (P<0.05)	1.6	6

* differ significantly at P< 0.05.

Table 4.6: Effect of commercial pea seed treatment on conductivity[1]

Cultivar	Treatment	Conductivity (µS/cm/g) Untreated seed	Treated[2] seeds	LSD (P< 0.05)
Talbot	Apron 35 SD	12.3	13.5	0.8
Quantum	Apron 35 SD	18.1	19.5	ns
	Apron 35 SD	25.8	25.5	ns
Spring	Apron TZ + Peridiam[3]	19.1	19.1	ns
	Aliette Super + Peridiam	16.8	18.3	ns
Resal	Apron 35 SD	21.9	27.5	2.4
Hailey	Apron 35 SD	17.8	18.3	ns
Ravel	Apron TZ + Molybdate[4]	21.9	23.5	ns
Cabree	Apron TZ + Molybdate	17.3	17.2	ns

[1] other quality data are presented in Appendix 1.
[2] application rates not provided
[3] seed binder.
[4] trace element

4.2.2 Cereal

At the recommended application rate, systemic and protectant fungicide seed treatments had no significant effects on conductivity of seed lots of maize, sweet corn and wheat (Table 4.7-4.9). The exception was *benlate* on the maize cv. 96005 seed lot (Table 4.7).

At double the recommended rate, the fungicide seed treatment of maize, sweet corn and wheat did not increase conductivity significantly except for *Vitaflo 200* and *benlate* on maize cv. 96005 (Table 4.7-4.9).

In all cases where germination was tested, seed lots treated with chemicals at the recommended rate did nor drop in germination (Table 4.8-4.9). At double the application rate, germination of seeds of sweet corn cv. Jubilee treated with *benlate* was decreased (Table 4.8). But all fungicide treatments increased germination of sweet corn cv. Illini Gold (Table 4.8). Fungicide seed treatment had no effect on germination of wheat cv. Otane (Table 4.9). During the germination test, fungal infection was found in *benlate* treated and untreated seeds of sweet corn cv. Jubilee in

both application rate tests. Also, there was *Penicillium* and *Fusarium* spp. growth on the untreated seeds of sweet corn cv. Illini Gold.

After two months of storage, treatments at the recommended rate had no effect on conductivity of sweet corn cv. Jubilee. However, germinations of seed lots treated with chemicals, which did not differ from the control immediately after treatment, were higher than that of control (Table 4.10).

Table 4.7: Effect of fungicide seed treatment at the recommended (R) and double recommended (D) rates on the conductivity of two maize seed lots

Treatment	Conductivity (µS/cm/g)			
	Cultivar 1[1]		Cultivar 2[2]	
	R	D	R	D
Vitaflo 200	3.07	3.26 *	2.43	2.23
Benlate	3.52 *[3]	3.84 *	2.42	2.31
thiram	2.98	3.16	2.36	2.45
control	2.92	2.94	2.37	2.30
LSD (P< 0.05)	0.34	0.27	ns	0.20

[1] cv.CF96005; [2] cv. CF96003; [3] differ significantly from the control at P< 0.05

Table 4.9: Effect of fungicide seed treatment of wheat cv. Otane at the recommended application rate (R) and double that rate (D) on conductivity and germination.

Treatment (R)	Conductivity (µS/cm/g)	Germination (%)	Treatment (D)	Conductivity (µS/cm/g)	Germination (%)
Vitaflo 200	4.34	98	Vitaflo 200	3.89	98
Benlate	4.11	98	Benlate	4.59	97
thiram	4.15	99 *	thiram	4.49	96
control	4.13	95	control	4.00	97
LSD (P<0.05)	ns	3	LSD (P<0.05)	0.68	ns

* differ significantly from the control at P< 0.05.

Table 4.8: Effects of fungicide seed treatment at the recommended (R) and double recommended (D) application rates on the conductivity and germination of two sweet corn seed lots

Treatment	Cultivar Jubilee				Cultivar Illini Gold			
	R		D		R		D	
	Conductivity (µS/cm/g)	Germination (%)	Conductivity (µS/cm/g)	Germination (%)	Conductivity (µS/cm/g)	Germination (%)	Conductivity (µS/cm/g)	Germination (%)
Vitaflo 200	7.94	99 *	8.16	98	12.26	73	11.60	73 *
Benlate	7.79	94	8.17	91 *	10.67	76	9.86	75 *
thiram	7.95	97	8.36	98	10.58	75	10.02	76 *
control	7.79	94	7.92	95	9.88	70	10.43	59
LSD(P<0.05)	ns	5	ns	4	ns	ns	ns	12

* differ significantly from the control

Table 4.10: Effect of fungicide seed treatment at the recommended application rate on sweet corn cv. Jubilee conductivity and germination immediately after treatment (I) and after two months storage (T)

Treatment (I)	Conductivity (µS/cm/g)	Germination (%)	Treatment (T)	Conductivity (µS/cm/g)	Germination (%)
Vitaflo200	8.24	98	Vitaflo200	7.62	97 *
Benlate	7.69	98	Benlate	7.47	95 *
thiram	8.33	96	thiram	7.84	97 *
control	8.23	97	control	8.01	91
LSD (P<0.05)	ns	ns	LSD (P< 0.05)	ns	3

* differ significantly from the control at P< 0.05.

Table 4.11: Conductivity of the solutions of the chemicals used for maize, sweet corn and wheat seed treatment

Chemicals[1]	Conductivity (µs/cm/g)	Chemicals[2]	Conductivity (µs/cm/g)
Vitaflo 200	3.41 b[3]	Vitaflo 200	5.43 b
Benlate	2.87 c	Benlate	3.98 d
thiram	3.24 b	thiram	4.84 c
control	2.17 d	control	2.51 e
Benlate+ Orthocide	9.28 a	Benlate+ Orthocide	14.0 a
LSD (P<0.05)	0.32	LSD (P<0.05)	0.31

[1] chemical weight per flask as calibrated and measured by the weight applied to 10.0g seeds according to the recommended application rate.
[2] calculate the amount of chemicals per flask according to 20.0 g seeds.
[3] means with the same letter are not significantly different.

4.2.3 Small seeded legumes

At the recommended rate of insecticide seed treatments, *Gaucho* had no effect on conductivity of white clover cv. Pitau and Kopu, but significantly increased the value of cv. Huia (Table 4.13). *Promet 365 CS* and *thiram* significantly increased conductivity of cv. Huia and Kopu. However, at double the rate, only *Gaucho* and *Promet 365 CS* significantly increased the conductivity of cv. Huia and Pitau, respectively.

It was common that the results were out of tolerance i.e., >5µS/cm/g, when 50 seeds of white clover were used (Table 4.12). However, when 0.3g seeds were used, the values of four replicates were usually within the tolerance (Table 4.13).

The fungicide seed treatments had no effect on germination at both rates (Table 4.13).

Table 4.12: Effects of insecticide and fungicide seed treatments at the recommended application rates on the conductivity of white clover cv. Huia using 50 seeds

Treatment	Replicate	Seed weight (g)	Hard seed weight (g)	Conductivity (µS/cm/g)
Gaucho	1	0.0316	0	50.3
	2	0.0329	0.0009	66.9
	3	0.0340	0.0008	64.8
	4	0.0326	0	64.4
Promet 365cs	1	0.0355	0	65.4
	2	0.0359	0	61.6
	3	0.0363	0.0009	54.5
	4	0.0356	0.0005	48.7
thiram	1	0.0326	0	62.0
	2	0.0334	0	64.7
	3	0.0328	0.0006	74.8
	4	0.0313	0.0008	67.5
control	1	0.0320	0	54.7
	2	0.0330	0.0006	42.9
	3	0.0336	0.0013	39.0
	4	0.0359	0	50.4

[1] the conductivity reading of the distilled water was 3.04µS/cm

conductivity and germination (Germ) of three white clover seed lots using 0.3g seeds

Treatment	cv. Pitau				cv. Huia[1]			cv. Kopu	
	R		D		R	D		R	
	Conductivity (µS/cm/g)	Germ (%)	Conductivity (µS/cm/g)	Germ (%)	Conductivity (µS/cm/g)	Conductivity (µS/cm/g)	Germ (%)	Conductivity (µS/cm/g)	Germ (%)
Gaucho	38.2	90	40.7	90	44.8*	48.6 *	89	41.1	80
Promet	39.7	93	45.6*[2]	91	47.2*	46.9	86	43.7*	82
365cs thiram	40.9	90	39.8	90	47.1*	48.5	91	43.4*	84
control	39.1	93	39.1	93	42.5	45.7	91	38.2	81
LSD (P<0.05)	ns	ns	2.4	ns	2.2	2.9	ns	3.1	ns

[1] no germination data for R rate.

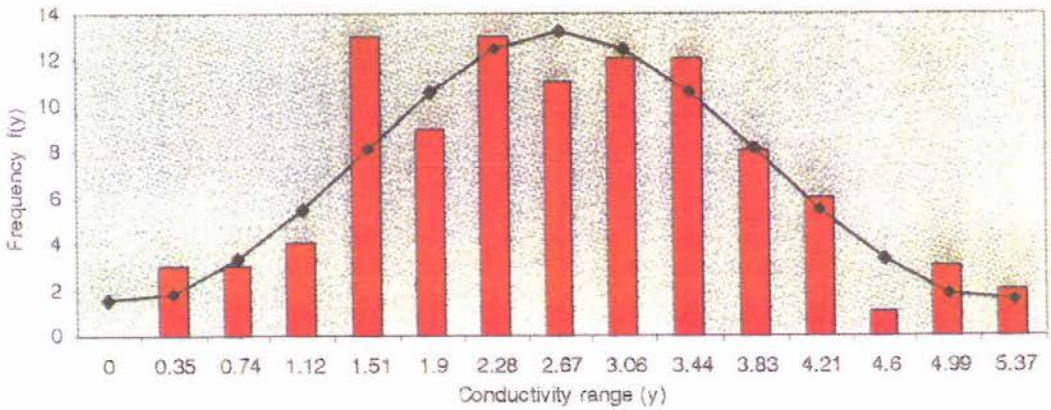
[2] differ significantly from the control at P< 0.05.

4.3 Determining tolerances for conductivity testing

One hundred different conductivity tests using four replicates each time from the same seed lot, resulted in a frequency table of one hundred ranges among the four replicates (Appendix 2-1).

Analysis using the computer program VAREST concluded that the observed range frequency was normally distributed according to the Chi^2 (χ^2) test (Figure 4.1; Appendix 2-2; Steel & Torrie 1980).

Figure 4.1: Observed (bar) and expected (line) frequency distribution of conductivity value range among 100 tests of four replicates of pea cv. Bolero



The sample mean and standard deviation, which are the population μ and σ, were 2.86 and 1.16, respectively (Appendix 2-2). According to the table of probability of a random value of $Z=(Y-\mu)/\sigma$ being greater than the values tabulated in the margins (Steel & Torrie 1980), Z is equal to 1.645 and 2.33 when the ranges fall within the 95 and 99% probability, respectively. Therefore the ranges (Y) of 4.77 and 5.56 are the marginal values at the 5% and 1% significance level.

CHAPTER 5: EFFECTS OF SEED TREATMENTS ON AA TESTING

5.1 Large seeded legumes

After AA, the germination of pea seed lots treated with *orthocide* at both rates either did not differ from, or was greater than the control (Table 5.1 and 5.2). For the systemic fungicide treatments, there was no clear trend. At the recommended application rate, the germination of *Apron TZ* and *Aliett Super* treated seeds did not differ from the control (no *thiram*) after AA, but the result for *Apron 35 SD* was seed lot dependent (Table 5.1 and 5.2). Germination after AA was significantly reduced by the double application rate for both seed lots and all three systemic fungicides.

All three systemic fungicides reduced the post AA germination of soybean at both application rates (Table 5.3). The recommended application rate result for *orthocide* must have been an anomaly as post AA germination was not reduced at double this rate (Table 5.3).

Adding *thiram* to the AA control seed lot before germination testing was again seed lot dependent. There was no effect on germination in the first Pania seed lot (Table 5.1), but there was a significant increase for the second Pania seed lot (Table 5.2).

During AA tests, storage fungi developed on seeds of some of the systemic chemically treated or untreated controls; for example *Apron 35 SD*, *Apron TZ* treated and control for seed lot 2 of pea cv. Pania (Table 5.2). *Penicillium* spp. and *Mucor* spp. (Hampton pers. comm.) developed on control 1 of soybean (Table 5.3).

The SMCs after AA in the garden pea experiments were from 33.3 to 37.0%. In four separate experiments, the ranges were 34.8-37.0%, 35.0-36.9%, 33.3-36.4%, and 33.4-36.8%, respectively. However, for the control-untreated seeds, the SMC ranged from 34.8- 36.8% (Table 5.1 and 5.2).

Table 5.1: Effects of seed treatment with systemic and protectant fungicides at the recommended application rate (R) and double that rate (D) on germination, vigour and SMC after ageing for seed lot 1 of pea cv. Pania

Treatment (R)	Germination (%)		SMC (%) after AA	Treatment (D)	Germination (%)		SMC (%) after AA
	Before AA	After AA			Before AA	After AA	
Apron 35 SD	94 *[1]	64 *	36.0	Apron 35 SD	92	60*	35.4
Apron TZ	98	77	36.5	Apron TZ	80*	47*	35.8
Aliett Super	98	75	36.0	Aliett Super	96	53*	36.5
Orthocide	98	85	36.2	Orthocide	100	71	36.5
Control 1[2]	99	77	36.3	Control 1	99	80	35.7
Control 2[3]		74	35.1	Control 2		80	35.5
LSD (P< 0.05)	4	10		LSD (P< 0.05)	7	10	

[1] differ significantly from the control 1 at P<0.05.
[2] germination without thiram.
[3] germination with thiram.

Table 5.2: Effects of seed treatment with systemic and protectant fungicides at the recommended application rate (R) and double that rate (D) on germination, vigour and SMC after ageing for seed lot 2 of pea cv. Pania

Treatment (R)	Germination (%)		SMC (%) after AA	Treatment (D)	Germination (%)		SMC (%) after AA
	Before AA	After AA			Before AA	After AA	
Apron 35 SD	96	73*[1]	34.3	Apron 35 SD	95	75	33.9
Apron TZ	96	71	35.0	Apron TZ	91*	65*	35.6
Aliett Super	97	69	35.3	Aliett Super	94	64*	36.0
Orthocide	98	76*	35.9	Orthocide	98	82*	35.8
Control 1[2]	97	63	34.7	Control 1	98	75	35.8
Control 2[3]		79*	34.4	Control 2		81*	36.4
LSD (P< 0.05)	ns	8		LSD (P< 0.05)	5	5	

[1] differ significantly from the control 1 at P<0.05.
[2] germination without thiram.
[3] germination with thiram.

Table 5.3: Effect of fungicide seed treatment of soybean at the recommended application rate (R) and double that rate (D) on germination before and after accelerated ageing (AA)

Treatment (R)	Germination (%)		Treatment (D)	Germination (%)	
	Before AA	After AA		Before AA	After AA
Apron 35 SD	94	54*	Apron 35 SD	84*	35*
Apron TZ	89*[1]	39*	Apron TZ	88	32*
Aliett Super	88*	57*	Aliett Super	92	54
Orthocide	91	65*	Orthocide	91	72*
Control 1[2]	95	75	Control 1	91	59
Control 2[3]		75	Control 2		77*
LSD (P<0.05)	6	8	LSD (P<0.05)	6	12

[1] differ significantly from the control 1 at P<0.05.
[2] germination without thiram.
[3] germination with thiram.

5.2 Cereal

At the recommended rate, maize seeds treated with chemicals had the same germination before or after AA testing (Table 5.4). At double the recommended rate, *benlate* plus *captan* reduced the post AA germination considerably (Table 5.4).

Table 5.4: Effects of seed treatment of maize cv. CF96005 with systemic and protectant chemicals at the recommended application rate (R) and double that rate (D) on germination and vigour

Treatment	Germination (%)		Treatment	Germination (%)	
(R)	Before AA	After AA	(D)	Before AA	After AA
Vitaflo 200	99	98	Vitaflo 200	97	98
Benlate+ captan thiram	98	96	Benlate+ captan thiram	99	72*[1]
	99	98		99	98
control 1[2]	98	97	control 1	98	96
control 2[3]		97	control 2		92
LSD (P<0.05)	ns	ns	LSD (P< 0.05)	2	5

[1] differ significantly from the control 1 at P<0.05.
[2] germination without thiram.
[3] germination with thiram.

CHAPTER 6: RESULTS OF CD TESTING WITH A COMPARISON OF TWO METHODS OF RAISING SMC, AND SWEDE DORMANCY

6.1 Small seeded species-onion and swede

Initial SMCs of the seed lots of onion and swede used in the experiments are listed in Table 6.1.

Table 6.1: SMC of onion and swede seed lots before the CD test

	SMC (%) of seed lots				
Species	A	B	C	D	E
Onion	6.6	6.1	6.3	6.2	
Swede	8.2	8.0	8.5	7.1	6.8

The water added method resulted in a lower SMC than that of desired SMC of onion and swede seed lots (19 and 20%, respectively) in all three runs (Table 6.2 and 6.3), with the exception of an anomaly for onion seed lot A in run 1 whereas the filter paper method resulted in SMCs very close to the desired ones. SMC obtained by the water added method had a slightly higher variance than the filter paper method with the exception, once again, for onion seed lot A in run 1 (Table 6.2 and 6.3).

Table 6.2: SMC achieved in CD testing of onion seed lots using two methods of raising SMC

		SMC (%)		
Method	Seed lots	Run 1	Run 2	Run 3
Water Added	A	21.8 ± 1.0[1]	18.7 ± 0.2	17.6 ± 1.0
	B	17.9 ± 0.3	18.4 ± 1.0	17.5 ± 0.8
	C	18.3 ± 0.4	18.3 ± 0.3	18.3 ± 0.2
	D	18.0 ± 0.5	18.1 ± 0.1	17.8 ± 0.3
	Mean	19.0 ± 1.8	18.4 ± 0.5	17.8 ± 0.7
Filter Paper	A	18.4 ± 0.7	19.1 ± 0.1	18.9 ± 0.3
	B	19.1 ± 0.2	19.2 ± 0.2	19.1 ± 0.2
	C	18.5 ± 0.8	18.9 ± 0.2	18.9 ± 0.3
	D	19.0 ± 0.1	19.2 ± 0.3	19.0 ± 0.2
	Mean	18.7 ± 0.6	19.1 ± 0.2	19.0 ± 0.2

[1] mean (four replicates) ± standard deviation.

Table 6.3: SMC achieved in CD testing of swede seed lots using two methods of raising SMC

Method	Seed lot	SMC (%)		
		Run 1	Run 2	Run 3
Water Added	A	19.4± 0.2[1]	18.9 ± 0.2	17.9 ± 0.5
	B	19.0± 1.0	18.6 ± 0.5	17.3 ± 1.0
	C	19.9 ± 0.2	19.9 ± 0.6	18.2 ± 0.6
	D	18.3 ± 1.5	19.4 ± 0.3	17.3 ± 0.9
	E	19.5 ± 0.2	19.6 ± 0.4	18.1 ± 0.4
	Mean	19.2 ± 0.9	19.3 ± 0.6	17.7 ± 0.7
Filter Paper	A	21.0 ± 1.0	20.1 ± 0.2	19.9 ± 0.2
	B	20.4 ± 0.5	19.8 ± 0.7	19.8 ± 0.2
	C	19.7 ± 0.3	20.0 ± 0.2	19.7 ± 0.5
	D	19.5 ± 0.2	19.4 ± 1.0	19.9 ± 0.2
	E	19.9 ± 0.3	19.7 ± 0.4	19.5 ± 0.9
	Mean	20.1 ± 0.7	19.8 ± 0.6	19.7 ± 0.4

[1] mean (four replicates) ± standard deviation.

Both methods of raising SMC produced CD test results which consistently ranked lot C and B as most and least vigourous seed lots respectively, in all three runs, though the post CD germination percentage in run 3, where the between paper germination method was used, was significantly lower than that in runs 1 and 2, for which the top of paper germination method was employed (Table 6.4-6.6).

The water added method resulted in a slightly but significantly lower germination percentage than that of the filter paper method (Table 6.4-6.6).

Table 6.4: Post-CD germination percentage of onion seed lots treated at 20^{0}C using the top of paper germination method after ageing at 45^{0}C for 24 h, after two methods of raising SMC (run 1)

Seed lot[1]	Water added method (%)	Filter paper method (%)	Mean
A	69[2]c[3]	70 c	69 c
B	56 d	72 c	64 c
C	85 a	88 a	86 a
D	75 bc	83 ab	79 b
	LSD (P<0.05) = 9		
Mean	71 b	78 a	LSD (P<0.05) = 7
	LSD (P<0.05) = 5		

[1] the pre-CD germinations of seed lot A, B, C and D were 87, 85, 95 and 92% respectively.

[2] results were the mean of four observations.

[3] means with the same letter are not significantly different at P< 0.05.

Table 6.5: Post-CD germination percentage of onion seed lots treated at 20°C using the top of paper germination method after ageing at 45°C for 24 h, after two methods of raising SMC (run 2)

Seed lot[1]	Water added method (%)	Filter paper method (%)	Mean
A	68[2] c[3]	70 c	69 b
B	49 d	55 d	52 c
C	78 b	87 a	83 a
D	67 c	71 bc	69 b
	LSD (P<0.05) = 8		
Mean	66 b	71 a	LSD (P<0.05) = 6
	LSD (P<0.05) = 4		

[1] the pre-CD germinations of seed lot A, B, C and D were 86, 77, 95 and 88% respectively.
[2] results were the mean of four observations.
[3] means with the same letter are not significantly different at P< 0.05.

Table 6.6: Post-CD germination percentage of onion seed lots treated at 20°C using the between paper germination method after ageing at 45°C for 24 h after two methods of raising SMC (run 3)

Seed lot[1]	Water added method (%)	Filter paper method (%)	Mean
A	49[2] de[3]	60 bc	54 b
B	47 de	54 cd	50 b
C	66 b	76 a	71 a
D	43 e	65 b	54 b
	LSD (P<0.05) = 7		
Mean	51 b	64 a	LSD (P<0.05) = 5
	LSD (P<0.05) = 4		

[1] the pre-CD germinations of seed lot A, B, C and D were 84, 76, 88 and 78% respectively.
[2] results were the mean of four observations.
[3] means with the same letter are not significantly different at P< 0.05.

The standard germination tests of the five swede lots did not indicate dormancy (Appendix 3-1 and 3-2). However, a high but varying number of dormant seeds was induced in all five seed lots as demonstrated by the post CD germination conducted at 20°C (Table 6.7). However, this was much less pronounced when 20-30°C was used as the germination temperature (Table 6.8; Appendix 3-1 and 3-2).

There was no interaction for the number of dormant seeds between the two methods of raising SMC and seed lots in all three runs. However the methods and seed lots had

significant effects on the dormancy rate with the water added method, and seed lot A had a higher dormancy (Table 6.7).

Table 6.7: Percentage of swede seed for which dormancy was induced after ageing at 45°C for 24 h after two methods of raising SMC (20°C germination temperature)

Seed lot	Water added method (%)	Filter paper method (%)	Mean
A	42[1] a[2]	25 b	34 a
B	14 cd	15 cd	14 bc
C	9 cd	8 d	9 c
D	19 bc	15 cd	17 b
E	18 bc	13 cd	16 b
	LSD (P<0.05) = 10		
Mean	20 a	15 b	LSD (P<0.05) = 7
	LSD (P<0.05) = 4		

[1] results were the mean of four observations.
[2] means with the same letter are not significantly different at P< 0.05.

Table 6.8: Percentage of swede seed lots for which dormancy was induced after ageing at 45°C for 24 h after two methods of raising SMC (20-30°C germination temperature)

Seed lot	Water added method (%)	Filter paper method (%)	Mean
A	11[1] a[2]	8 ab	9 a
B	5 bc	3 c	4 b
C	1 c	1 c	1 bc
D	1 c	0 c	0 c
E	1 c	1 c	1 c
	LSD (P<0.05) = 5		
Mean	3	2	LSD (P<0.05) = 3
	ns		

[1] results were the mean of four observations.
[2] means with the same letter are not significantly different at P< 0.05.

Pre-chilling after CD, but prior to germination at 20-30°C, had no significant effect on dormancy (Appendix 3-3). No data were obtained at 20°C.

Because induced dormancy could be a variable in ageing tests, further experiments using four seed working samples with different SMC from the same seed lot of swede cv. Doon Major were conducted.

There was less than 6% dormancy in the control (Appendix 4-1). But after artificial deterioration, the dormancy level in the four seed lots increased significantly as demonstrated by the post CD germination at 20°C (Table 6.9). Seed lot 1, which had the lowest SMC i.e., 7.0 % had the highest dormancy.

Table 6.9: Comparison of induced dormancy percentage of swede cv. Doon Major seed lots 1-4 after CD ageing at 45°C for 24h at four different moisture content and control germinated at 20°C (the filter paper method was used to raise SMC)

Seed lot	SMC (%)	CD (%)	Control (%)	Mean
1	7.0	35[1] a[2]	3 c	19 a
2	8.5	26 b	2 c	14 b
3	9.9	20 b	6 c	13 b
4	11.5	24 b	2 c	13 b
		LSD (P<0.05) = 7		LSD (P<0.05)= 5
Mean		26 a	3 b	
		LSD (P<0.05) = 4		

[1] results were the mean of eight observations.
[2] means with the same letter are not significantly different at P< 0.05.

However, when germinated at 20-30°C, seeds aged at both 45°C, 24h and 40°C, 48h showed no significant difference in dormancy compared to the control (Appendix 4-1).

Also, there was no significant difference in induced dormancy of seed lots between the two deterioration conditions, i.e. 45°C 24h and 40°C, 48h as revealed when germinated at both 20 and 20-30°C (Appendix 4-1).

The germination percentage of swede cv. Doon Major was normally 96-100% including normal seedlings produced from dormant seeds after pre-chilling treatment at 5°C. The germination after CD ageing ranged between 93-99% (Appendix 4-2). Because a high rate of dormancy was induced, the comparison of vigour status using CD germination is impaired and therefore suspect.

A few fresh seeds only germinated after the second cycle of chilling treatment.

6.2 Large seed species-pea and maize

When the filter paper method was used, the large seeded species achieved the desired SMC i.e., 20%, after 2-3 h with the lid on (garden pea cv. Bolero) and 6-8 h without the lid covering the plastic box (garden pea cv. Pania and maize cv. 96005 and 96003) under laboratory conditions (23^{0}C).

The water added method yielded almost the same SMC as the filter paper method did (Table 6.10, 6.12 and 6.13). There was no interaction between the two methods and the three seed lots of garden pea cv. Bolero for CD germination, and the two methods resulted in the same order of seed lots (Table 6.11), though the water added method produced a small but significantly lower CD germination (Table 6.11 - 6.13).

Table 6.10: SMC achieved following CD testing of seed lots 1, 2 and 3 (stored at 5, 20 and 25^{0}C for six months, respectively) of garden pea cv. Bolero after two methods of raising SMC

	SMC (%)	
Seed lot	Water added method	Filter paper method
1	20.0 ± 0.62[1]	20.7 ± 0.40
2	20.2 ± 0.35	20.8 ± 0.17
3	20.1 ± 0.24	20.6 ± 0.94
mean	20.1 ± 0.40	20.7 ± 0.55

[1] mean (four replicates) ± standard deviation.

Table 6.11: Comparison of the effect of two methods of raising SMC on CD germination for seed lots 1, 2 and 3 (stored at 5, 20 and 25^{0}C for six months, respectively) of garden pea cv. Bolero

Seed lot[1]	Water added method (%)	Filter paper method (%)	Mean
1	96[2] a[3]	99 a	97 a
2	95 a	98 a	96 a
3	77 b	82 b	79 b
	LSD (P<0.05) = 4		
Mean	89 b	93 a	LSD (P<0.05) = 3
	LSD (P<0.05) = 3		

[1] the control germinations of seed lot 1, 2 and 3 after storage were 98, 99 and 95% respectively.

[2] results were the mean of four observations.

[3] means with the same letter are not significantly different at P< 0.05.

Table 6.12: Comparison of the effect of two methods of raising SMC and their effect on post-CD germination for seed lot 1 of pea cv. Pania

Method	SMC (%)	Germination (%)
Control		99 a[2]
Water added method	20.0 ± 0.8[1]	94 b
Filter paper method	19.7 ± 0.3	98 a
		LSD (P< 0.05)= 3

[1] mean (four replicates) ± standard deviation.
[2] means with the same letter are not significantly different at P< 0.05.

Table 6.13: Comparison of the effect of two methods of raising SMC of maize cv. CF96003 and CF96005 and their effect on post-CD germination

Cultivar	Method	SMC (%)	Germination (%)
CF96003	Water added method	19.7 ± 0.1[1]	95 b[2]
	Filter paper method	20.0 ± 0.2	98 a
			LSD (P< 0.05) = 3
CF96005	Water added method	19.8 ± 0.2	95 b
	Filter paper method	19.7 ± 0.3	98 a
	Control		100 a
			LSD (P< 0.05)= 2

[1] mean (four replicates) ± standard deviation.
[2] means with the same letter are not significantly different at P< 0.05.

CHAPTER 7: EFFECTS OF HARDSEEDEDNESS BREAKING BY H_2SO_4 AND SANDPAPER ON VIGOUR TEST RESULTS

Sandpaper scarification treatment for 30 to 40 minutes for lotus cv. Maku and 50 to 90 minutes for white clover cv. Sustain had no effect on hard seed, germination, conductivity value and AA germination compared to the control (Table 7.1 and 7.2).

H_2SO_4 treatment for five minutes had little effect whereas 15 minutes effectively broke hard seeds in both species, especially in lotus cv. Maku. However, the conductivity value was increased and the AA germination reduced greatly by both H_2SO_4 treatments (Table 7.1 and 7.2).

SMC after AA ranged from 49.5 to 55.6% for white clover cv. Sustain and from 47.1 to 53.6% for lotus cv. Maku (Table 7.1 and 7.2).

After the AA test, the percentages of hard seeds of white clover cv. Sustain and lotus cv. Maku were significantly reduced from 14 to 10% and 6 to 2%, respectively compared to before the AA test (Table 7.1 and 7.2). Fungi, particularly *Penicillium* spp., developed on the seeds of all H_2SO_4 treatments during AA, with the most severe being for the 15 minutes treatment.

H_2SO_4 treatment, especially the five minutes treatment, increased germination of lotus cv. Maku seeds (Table 7.2). Most abnormal seedlings in the control had no roots (data not presented).

Treatment	Germination (%)	Hard seed content (%)	Conductivity (µs/cm/g)	AA germination (%)	Hard seed content After AA (%)	SMC after AA (%)
H_2SO_4 5min.	63 bc[1]	13 bc	67.9 b	28 d	9 bc	51.4
H_2SO_4 10min.	60 c	10 cd	70.9 ab	36 c	5 cd	54.2
H_2SO_4 15min.	66 abc	6 d	72.3 a	14 e	2 d	54.2
Control	69 ab	15 ab	46.0 dc	44 ab	11 b	50.4
sandpaper 50min.	72 a	17 a	48.8 c	40 bc	20 a	49.6
sandpaper 70min.	68 abc	17 a	46.9 dc	48 a	12 b	50.1
sandpaper 90min.	65 abc	20 a	43.3 d	43 abc	10 bc	54.2
LSD (P<0.05)	8	5.0	4.0	7	6.0	

[1] means with the same letter in each column are not significantly different at P< 0.05.

Table 7.2: Effects of H_2SO_4 and sandpaper treatment on lotus cv. Maku hard seed content, germination and vigour test results

Treatment	Germination (%)	Hard seed content (%)	Conductivity (µs/cm/g)	AA germination (%)	Hard seed content after AA (%)	SMC after AA (%)
H_2SO_4 5min.	84 a[1]	6 a	41.4 c	37 b	3	49.3
H_2SO_4 10min.	79 a	6 a	52.7 b	17 c	1	47.6
H_2SO_4 15min.	80 a	1 b	61.1 a	7 c	1	48.3
Control	67 b	9 a	33.3 d	52 a	4	49.4
sandpaper 30min.	66 b	7 a	35.2 d	56 a	2	50.8
sandpaper 35min.	59 b	8 a	35.4 d	52 a	3	50.5
sandpaper 40min.	63 b	6 a	33.6 d	48 ab	4	53.3
LSD (P<0.05)	11	3.4	2.9	14	ns	

[1] means with the same letter in each column are not significantly different at P< 0.05.

CHAPTER 8: DISCUSSION

Most of the seed lots used in the present study had a high germination percentage which was appropriate for vigour testing, except for sweet corn cv. Illini Gold and lotus cv. Maku (Table 4.1).

8.1 Seed treatment effect on conductivity

8.1.1 Large seeded legumes

The conductivity test is widely used for large seeded legumes as a vigour test to predict seed field emergence and storage ability (Matthews 1981; Perry 1984b). Any chemical seed treatment applied should have no detrimental effect on conductivity, even at double the rate (Anonymous 1996). In the present study of fungicide seed treatment, the results for soybean, Broad bean, French bean and one of the garden pea seed lots suggested that the fungicides when applied at the recommended rate had no effect on conductivity (Table 4.2 and 4.3), and thus no negative effect on seed vigour. This agreed with previous work which showed that *thiram*, *captan* and *carboxin* had no effect on soybean conductivity (McDonald & Wilson 1979; Loeffler *et al.* 1988; Eua-umpon 1991). However, there was an exception for seed lot 2 of garden pea (Table 4.2), as there was a small but significant increase in conductivity. Although the two seed lots of garden pea had similar quality (Table 4.1 and 5.1-5.2), the physical condition of an individual seed lot can affect its response to seed treatment (Hurd 1921; Christensen & Moore 1942). Many seeds have invisible seed coat fissures (Shull, cited from Hurd 1921). Although there were no visible cracks of the seed coat of the pea seed lot 2, there might have been microscopic cracks not visible to the naked eye, which would result in more fungicide accumulated inside the seed and embryo, thus being more toxic to the seed. This is supported by the report that the toxic effect (reduced germination) of copper sulphate was greater for wheat seeds with injured coats, and was greatest for seeds when coat was injured over the embryo (Hurd 1921). Thus, injury of the seed coat, particularly over the embryo region, increases the

chances of phytotoxicity (Hewett & Rennie 1986). It is possible that this occurred for pea seed lot 2, but this was not determined.

The effect of seed treatments on conductivity of soybean seed at the recommended rate after two months of storage was different compared to that when seed was first treated. Conductivity was increased, especially by *Apron TZ*, but conversely germination also increased (Table 4.5). When applying slurry to seed, a small amount of fungicide may penetrate the seed coat. During the storage period, the chemicals maybe had a negative effect on the seed, although seed metabolism was not very active because of low SMC. However, the chemical can move into seed even at low SMC. The more concentrated the chemical inside the seed, the more damage it could do. But, the negative effect of chemicals did not influence germination, which was less sensitive than vigour. The reason why germination increased was presumably that fungi had a greater deterioration effect (Halloin 1986). The positive effect of fungicides (i.e. preventing fungal growth), outweighed the negative effect of their phytotoxicity. The chemical effect may interact with storage duration and fungal infection. If fungal infection is profound after long storage, and if the chemical can prevent fungal growth, treated seeds can avoid accelerated deterioration by fungi, therefore resulting in higher germination if not vigour. The beneficial effect of soybean seed treatment at the recommended rate during storage was obvious after 24 months (Van Toai *et al.* 1986). *Captan + thiram* and *malathion* treated seeds had lower conductivity and higher germination after storage (Saraswthi *et al.* 1995). In the present study, the storage time was only two months after seed treatment which was not long enough for fungi to deteriorate seeds significantly. Therefore, the toxic effect of chemicals, especially the systemic ones, was the major cause for the conductivity increase. One of the few studies on systemic fungicide seed treatment on conductivity reported conflicting results in two pea cultivars after six months of storage after treatment with *benomyl* (Nascimento & Cicero 1991). Conductivity was unaffected for pea cv. Pomak and the value changed for cv. Mikado. Mikado had a higher viability (92-98%) than that of Pomak (70-81%). Higher vigour seeds are less influenced by stress (Matthews 1981) and the chemical effect may interact with the vigour status of seed lots, because of self-repair and detoxification of seeds involved (Coolbear 1995). The erratic results of

Nascimento & Cicero (1991), once again, could be attributed to the invisible cracks of seed coat that allowed an increase in the toxic effect of the seed treatment.

At double the recommended rate, in most cases, systemic fungicides increased conductivity (Table 4.2 and 4.3). There are no reports about the effect of chemical seed treatment on seed physiological change. However, a few studies have investigated the effect of the systemic fungicide *benomyl* on permeability and membrane structure of the leaves of bean and wheat seedlings (see Spotts *et al.* 1975). The *benomyl* absorbed by bean seedling roots resulted in significant changes in membrane function of leaf cells by modifying the lipoprotein complex and the polarised structure of the membrane water disrupted by ozone (Spotts *et al.* 1975). It is not however possible to directly compare seed and leaf cells as the leaf cell may have distinct different metabolism because of different moisture contents and other factors.

One possible reason for the conductivity increase may be that the systemic chemicals penetrated the seed and were translocated inside the seed at a higher rate during imbibition. When the concentration was higher, they had a greater effect on seed metabolism, membrane structure and thus more leakage. The more concentrated the chemicals in distilled water, the higher the conductivity (Table 4.4), although the chemical effect was reduced to near zero as there was little effect on conductivity after application to seeds at the recommended rate. However, at double the rate, some chemicals may have been washed out into water, and thus increased conductivity. This might be another reason to explain the increase in conductivity.

Fungicides can prevent or delay fungal invasion of seeds and seedlings during germination and field emergence, thus enhancing seed performance. AOSA (1983) and Copeland & McDonald (1995) believed that the conductivity test failed to evaluate improved performance following chemical seed treatment. But, more accurately, the conductivity test can not detect the fungal effect (e.g. Loeffler *et al.* 1988). This is true when newly treated seeds are conductivity tested, as in the present study. However, after long storage, the conductivity test can detect the enhanced effect of seed

treatment, e.g. Van Toai *et al.* (1986) and Saraswthi *et al.* (1995), because of the decrease in fungal deterioration of the seed.

Mostly, there was little influence of systemic and protectant fungicides seed treatment at the recommended rate, or even at double the rate on conductivity, provided storage fungi were not involved. The commercial seed treatment rate normally is within the range used in the experiments. The present study adds more knowledge and confidence that removal of seed treatment prior to the conductivity test (AOSA 1983) is not necessary for most protectants and systemic fungicides and insecticides, if chemicals are used at around the recommended rate. Loeffler *et al.* (1988) suggested that caution should be taken when testing newly released or other chemicals that have different formulations. In the present study, the fungicides had a wide range of conductivity in distilled water (Table 4.4), but when binding to seed, their effect was reduced greatly. If the theory, especially based on chemistry knowledge, is the same, then, the conclusion that the chemically seed treated can be directly conductivity tested without removing the chemical is valid.

There is also an interpretation issue. Statistically significant differences of less than 3 μS/cm/g in conductivity value do not necessarily mean there is a difference in vigour.

Now, it seems that more co-operation is needed, between seed technologists, physiologists and pathologists to understand more of the relative importance of the fungal effect (Halloin 1986); also with seed technologists and chemists to understand the effects of chemical treatment and achieve more understanding of the assumptions and variables involved in the conductivity test.

8.1.2 Cereal

At the recommended rate, the chemicals had no phytotoxic effects, as demonstrated by no conductivity increases or germination reduction. At double the rate, the same pattern was obtained except that *Vitaflo 200* significantly increased conductivity of maize cv. 96005 and *Benlate* reduced germination of sweet corn cv. Jubilee (Table 4.7-

4.9). It seems that fungi had a more important function in seed ageing and loss of viability during germination (Table 4.8) and storage (Table 4.10) than did phytotoxicity of chemicals.

No toxic effect was detected as conductivity did not change after two months of storage, but the germination of the control decreased significantly for sweet corn cv. Jubilee (Table 4.10). The main source of leakage for cereal is from the embryo, which is a small portion of the seed. Thus the presence of the deteriorated embryo, caused by phytotoxicity of chemicals in the present study, may only have little effect on the conductivity compared to the control (Bruggink *et al.* 1991). The increased germination of treated seed lots (Table 4.10) in the present study was mainly due to the fungi-control effect during germination rather than storage, because there was only two months of storage (see Section 8.1.1 for more discussion).

The conductivity results (Table 4.7 and 4.8) agreed with the conclusion of Parera *et al.* (1996) that conductivity of sweet corn seeds is higher than that of maize seeds. The reduced vigour of sweet corn can be attributed to the physiological and biochemical effects of the elevated sucrose levels present during seed formation and faster imbibition because of sh2 mutant endosperm.

Normally, maize, sweet corn, and wheat had lower conductivity than that of large-seeded legumes (see Section 4.2.1 and 4.2.2).

8.1.3 Small seeded legumes

Compared to the fungicides used to treat large-seeded legumes and cereals, the insecticides had more effect on conductivity of white clover (Table 4.13). This might be attributed to a larger ratio of surface: weight which resulted in more chemical on the seed. However, germination was not affected at both seed treatment rates (Table 4.13). This might be, as discussed in Section 8.1.1, because no fungi were involved and the chemicals affected vigour but not viability.

The three cultivars had similar a germination plus hard seed content (Table 4.1). Pitau and Huia cultivars had higher germination than that of Kopu. However, their conductivity values were not lower than that of Kopu (Table 4.13).

It has been suggested that the conductivity test is not reliable for small cotyledon seed (Matthews 1981; Perry 1984b). In the present study, the conductivity differences among the white clover cv. Kopu control during three runs was larger than that between chemically treated lots and the control (data not presented). It was unclear whether this was due to the unreliability of the conductivity test for small seeded legumes, or uniformity of the seed lot, because it is more difficult to obtain a uniform small seed sample than obtaining it from a larger seed sample. However, the inability of the conductivity test for lotus (Rowarth & Sanders 1996) and red clover (Wang & Hampton 1989) to predict seed potential performance has been reported. Repeatability of the test is important. More research is needed.

Irrespective of the reliability of the conductivity test for small seeded species, 50 seeds as recommended in the Vigour Testing Handbook (Hampton & TeKrony 1995) was not appropriate (Table 4.12). A small difference in the conductivity reading, which may be caused by the seeds or conductivity meter, can result in a very big difference in conductivity, because the very small weight of 50 seeds was employed to calculate the conductivity (Table 4.12). Therefore, a specific amount of seeds should be used, such as 0.3g for white clover in the present experiment (Table 4.13), and 0.5g for red clover (Wang 1989).

8.2 Tolerances

According to the calculation, 4.77 and 5.56μS/cm/g were the tolerances at the 5 and 1% significance level respectively; therefore, the present tolerance of 5μS/cm/g between the highest and lowest replicate value for a seed lot is suitable for this pea seed lot. However the seed quality of the lot used was excellent, as revealed by the high post CD germination of seed lot 1 (Table 6.11). Because random sampling variation is bigger when seed lot quality is lower (Bould 1986), if a seed lot with similar germination but lower vigour was tested, the tolerance would be expected to be

a little larger. However, 5μS/cm/g, according to the calculation, is the tolerance at about the 3% significance level. Thus the present tolerance as recommended in the handbook, i.e. 5μS/cm/g (Hampton & TeKrony 1995) is reasonably appropriate for pea. It is also reasonably suitable for other large seeded legumes because they have similar conductivity compared to peas (Table 4.2 and 4.3). When determining further tolerances, it will be better to use a few seed lots with different seed vigour, but all with high germination.

For cereal species, there should be a lower tolerance, because of their lower conductivity values than for large seeded legumes (see Section 4.2.1 and 4.2.2). The low value is due to the smaller embryo that is the main source of leakage (Bruggink *et al.* 1991). For small-seeded legumes, because of higher conductivity of e.g. white clover (Table 4.13), red clover (Wang 1989) and lotus (Hampton *et al.* 1994), a higher tolerance, i.e. more than 5μS/cm/g, might be expected. However, the tolerances for cereal and white clover will only need further research if it is concluded that the conductivity test is reliable for these species.

8.3 Seed treatment effect on AA testing

Systemic fungicides at the recommended rate had a phytotoxic effect on soybean as revealed by lower AA germination of treated seeds than that of control 1 (Table 5.3), but this was not the case for two seed lots of garden pea cv. Pania (Table 5.1-5.2), and maize cv. 96005 (Table 5.4). However, at double the recommended rates, all systemic fungicides reduced post AA germination in legumes with one exception in seed lot 2 of garden pea and soybean. Seeds with a high SMC are easily penetrated by chemicals so that phytotoxicity to seeds may result (Davies 1965). Therefore, in the AA conditions of high temperature (40-45^{0}C) and RH (nearly 100%) for three days, the fungicides could enter into the seeds more readily and in greater quantity than normal. But the phytotoxic effect to the seeds should be an interaction among the dose of chemical, cultivar and vigour status of the seed lot because of difference in metabolism (Sheridan & Grbvac 1983) and detoxification ability (Coolbear 1995). In the present study, soybean seeds were physiologically old, as demonstrated by relative lower, though

acceptable, germination (Table 4.1) than garden pea and maize. Thus the soybean seeds might have lower self-repair and detoxification ability and be more susceptible to chemical phytotoxicity even at the recommended application rate (Table 5.3). Although garden pea and maize had a higher vigour, at double the application rate the toxic effect resulted in lower post AA germination in many cases (Table 5.1-5.2 and 5.4). The present study showed that different chemicals, such as systemic and protectants and even different systemic chemicals, had different phytotoxic effects in AA testing. But the mechanism of phytotoxicity could not be detected in the present study.

Detrimental effects of fungi were also involved, because of the general lower germination of control 1 than of control 2 (which was *thiram* dusted after ageing but prior to germination (Table 5.2-5.3)). In a few cases, fungi also grew in seed lots treated with systemic fungicides (Table 5.2) because they did not have as broad a spectrum to kill pathogens as the protectants did. Storage fungi such as *Aspergillus niger* and *A. glaucus* may greatly affect test results (Gupta *et al.* 1993). An AA test of soybean seeds dusted with *thiram* before AA gave a better indication of seed performance under a range of different seedbed moisture and temperature conditions than did the results from seeds not dusted with *thiram* before AA, because of the control of saprophytic fungi (Boersma *et al.* 1996).

Previous studies suggested that all seed lots AA tested should either be treated or, preferably, untreated (Baskin 1981) and the results from treated and untreated seed lots should not be compared because the seed treatment could alter results (Baskin 1981; AOSA 1983). In the AA test, the results indicate the complicated combined effects of vigour status of the cultivar, phytotoxicity, and fungal development on the seed. The question of whether the chemical seed treatment and fungi play the same role in the AA test as in field emergence and storage is unknown. If there is a big difference, the AA test method must consider controlling fungi and removing the chemical treatment. A general conclusion cannot be obtained in the present study. However, the AA test can be utilised to compare vigour of seed lots treated with the same chemical at the similar application rate.

Seed treatment did not influence post AA SMC (Table 5.1 and 5.2). Therefore, the AA SMC range recommended by Hampton & TeKrony (1995) is applicable for chemically treated seed lots. However, Schneider & Renault (1997) reported that in saturated air, treatment enhanced seed moisture uptake at low application rate, and slowed it at a higher rate, implying that chemical seed treatment may be a new variable in the AA test. However how much effect it has is unknown.

8.4 CD testing- a comparison of two methods of raising SMC

The present study indicated that the time needed to achieve the desired SMC using the filter paper method was dependent on seed size, initial SMC, blotter moisture and RH of the environment. Whether a lid was used on the box was the most important factor, because this altered the RH and blotter moisture. Covering the box with a lid reduced imbibition time for garden pea from 6 to 2-3 h. To achieve the desired SMC using the filter paper method requires frequent and repeated weighing. However, this is expensive in time and labour. Few workers have studied CD testing compared to AA testing. One possible reason is because of the workload of frequently weighing seeds. An easy but reasonably accurate method to raise SMC to the desired level is needed.

The water added method resulted in a lower SMC than that desired in onion and swede (Table 6.2-6.3). This might be because of errors in using the micro-pipette or evaporation of droplets of water on the side of foil bags during heat sealing (Powell 1995). In the present experiments, only 0.04-0.10ml distilled water per bag, mainly depending on seed thousand weight and initial SMC, was required to achieve the desired SMC. Thus a very small error in using the micro-pipette, or moisture loss could result in a significant reduction in final SMC. But, for large seeded-species, such as garden pea and maize, the SMC attained was very close to the desired SMC (Table 6.10, 6.12 and 6.13). For large seed species, around 4ml water was needed to achieve the desired SMC. Therefore, a small amount of water loss or micro-pipette operation error would not influence the desired SMC to the same degree. Powell (1995) reported greater variability in SMC among replicates of rape seeds from using the water added method than in the filter paper method, and attributed this to inexpert use of the micro-

pipette needed for the addition of small amounts of water and evaporation during heat sealing (Powell 1995). But, the present study showed that the variation could be reduced greatly by experience. The largest SMC and variation attained, i.e. seed lot A of onion in run 1 (Table 6.2), occurred because of placing the micro-pipette inside the foil bags, resulting in water outside the micro-pipette being deposited into the bags. The method was changed for the following experiments. The variation of SMC of onion and swede obtained by the water added method was a little higher than the filter paper method (Table 6.2 and 6.3). This may be because, once again, problems were faced with achieving the exact small amount of water required. Another reason may stem from the methodology of the SMC determination after CD. According to the ISTA Rules (ISTA 1996), 4-5 or 10g seeds, depending on the diameter of the container, is required to test the SMC. However, the SMC of the seeds after CD was determined using 50 seeds (Hampton & TeKrony 1995). It is appropriate for large seed, e.g. pea because 50 seeds weighs about 5g (Table 4.1), but great variation may result for small seed species using 50 seeds, which would weigh only 0.2-0.3g for onion and swede. It is well known that the smaller the seed sample size, the larger the variation (Thomson 1979; Bould 1986). However, the two methods achieved almost the same variation for garden pea and maize (Table 6.10, 6.12 and 6.13).

For the CD test of onion, although the water added method resulted in lower SMC (Table 6.2), a lower CD germination was also found when compared to the filter paper method (Table 6.4- 6.6), implying that some factor(s) were more important than SMC in affecting the germination test result. One of them might be because of imbibition damage, because of fast water uptake resulting in membrane disruption and seed deterioration (Pollock *et al.* 1969; Powell 1986), as low temperature (10^0C) is known to enhance imbibition damage (Powell & Metthews 1978) and the sealed bags were equilibrated at 10^0C. Whether the seed metabolism initiation process which occurred in the filter paper method, in which the seeds absorbed moisture for a few hours at a more suitable temperature, contributed to higher CD germination was unclear, and there has been no comparison of imbibition rate for the two methods. However, a slightly lower germination is probably not important, because the vigour index is a relative, not absolute value. Furthermore, the water added method resulted in the same seed lot

ranking as the filter paper method (Table 6.4-6.6 and 6.11). There was no interaction between the two methods for onion, garden pea and swede in determining vigour and dormancy. These all suggested that the water added method is an efficient method and could supersede the filter paper method used in CD testing, provided the use of the micro-pipette is accurate.

For onion, the between paper method resulted in much lower germination after CD (Table 6.6) than that of the top of paper method (Table 6.4 and 6.5) because of excess moisture of the germination paper and small requirement for moisture by small seed. Thus, between paper method was not ideal for onion germination. However, both the germination methods were in accordance with the ISTA Rules (1996). Therefore, the germination test itself, although recognised and said to be standardised, is not yet completely standardised in terms of definition because the amount of water used in the substrate is not specified, and because of the optional germination temperatures (Hampton 1995). Increased moisture content of the substrate towel reduced maize germination significantly (Phaneendranath 1980) because excessive moisture of the substrate could restrict aeration (Justice 1972) and the germination process of maize is inhibited by high substrate water potentials (Silva & Filho 1990).

8.5 Induced dormancy of swede in CD testing

Dormancy of the swede seed lots from different cultivars (Table 6.7), and from the same cultivar but with different SMC (Table 6.9), was induced during CD testing. However, dormancy of swede seed germinated at 20°C after CD has not previously been reported (e.g. Powell 1981; Powell & Matthews 1985). Hilhorst & Toorop (1997) also reported that AA conditions of high temperature and SMC induced dormancy of tomato seeds. Furthermore, only a few dormant seeds of cv. Doon Major germinated only after the second pre-chilling treatment, implying that the induced dormancy had different intensities. This agrees with the viewpoint of Vleeshouwers *et al.* (1995) that dormancy of a seed is not an all-or-nothing property.

Different vigour status might affect the degree of induced dormancy. Seed lot A of swede, which had the highest induced dormancy (Table 6.7 and 6.8), had the lowest CD germination (Appendix 3-2). A cultivar effect might also be involved, because the seed lots were from different but unknown cultivars.

The water added method produced a higher dormancy in seed lot A than the filter paper method did, but not in other seed lots (Table 6.7). The seed lot of cv. Doon Major with the lowest SMC (7.0 %) had the highest dormancy (Table 6.9). The two methods of raising SMC, that is, the filter paper method and the water added method affected the seeds differently. The former method involved the process of a few hours imbibition of the seeds on a moist blotter at laboratory temperature (23^0C), which might trigger the germination metabolism. The later method may have caused more imbibition damage. However, what physiological mechanisms led to the results is unclear.

Pre-chilling did not induce dormancy. Of course pre-chilling treatment is a method used to break dormancy (ISTA 1996). Raising SMC on a blotter at 23^0C in the laboratory or at 10^0C for 24h for SMC equilibrating was also unlikely as the reason to induce dormancy. The only factor responsible for inducing dormant seed was the CD conditions, i.e. high temperature at certain SMC.

Some seed species are temperature sensitive, and will not germinate when subjected to high temperature at imbibition, such as lettuce (*Lactuca sativa* L.), especially cultivar 'Grand Rapids' (Maguire 1980). The induction of secondary dormancy is often stimulated by elevated temperature. This may be one of the reasons why chilling is effective in dormancy breaking (Hilhorst & Toorop 1997). Bewley & Black (1994) demonstrated that dormancy of *Ambrosia trifida*, *Avena sativa* and *Taraxacum megalorhizon* were induced at temperatures above the maximum for germination. The high temperature might result in the loss of key receptors for nitrate or in the plasma membrane, which was related to dormancy (Bewley & Black 1994).

However, the results showed that pre-chilling seeds after deteriorating, but prior to germination at 20-30^{0}C, had no significant effect on dormancy breaking compared to no pre-chilling (Appendix 3-3). This was attributed to the obscure effects of the 20-30^{0}C germination regime. The 20-30°C condition can overcome dormancy (Table 6.7 and 6.8; Appendix 4-1). Vleeshouwers *et al.* (1995) showed that alternating temperatures can effectively break dormancy.

Therefore, temperature has a complicated dual role in regulating dormancy and germination of seed, but had different patterns. The relief of induced secondary dormancy is physiologically different from the breaking of primary dormancy (Vleeshouwers *et al.* 1995).

Results from artificial ageing as a vigour test for swede species showed that the test should be used with caution. Caution also should be given to those species that have similar characters to swede when the ageing (AA and CD) tests are carried out, such as *Brassica napus*, and other *Brassica* spp. They may have similar metabolism and all need pre-chilling treatment before germination (ISTA 1996).

Dormancy is an important variable affecting vigour test results. Is there a different seed reaction to artificial ageing or soaking when seed is in a dormant compared to a non-dormant state? It is certain that at any degree of dormancy, seeds continuously react to their environment by adjusting their level of dormancy to the changing environment, implying the seeds are not inactive in the dormant state (Vleeshouwers *et al.* 1995).

Because dormancy was induced at the 45°C 24h or 40°C 48h ageing conditions, it may be not reliable to compare vigour of the seed lots by CD germination. This is because the seeds might tolerate the stress conditions in the dormant state.

However perhaps one way to overcome the imposition of dormancy after CD ageing of swede would be to run the germination test at 20-30°C and not 20°C. Another method would be to require prechilling before the post-CD germination test.

8.6 Effects of methods of hard seed breaking on vigour testing

H_2SO_4 treatment for 15 minutes effectively broke hard seeds, but seed vigour was greatly reduced. H_2SO_4 treatment for five minutes did not significantly break hard seed. However, the AA germination or conductivity value was still reduced or increased greatly (Table 7.1 and 7.2). The main factor is that H_2SO_4 erodes seed tissues (Brant *et al.* 1971). When hard seeds with thick coats were softened, the thinner coated seeds, including hard seeds and normal seeds, could be over-treated and damaged (Yi *et al.* 1994). It is well documented that seed coat integrity is one factor that influences the amount and rate of leakage of electrolytes from seed, such as in pea (Powell & Matthews 1979; Larson 1968), onion and leek (*Allium porrum* L.) (Beresniewicz *et al.* 1995). Seed with an injured or removed coat results in faster water uptake, thus more imbibition damage and enhanced leakage, and also fungi invasion, thus a reduced AA germination.

Therefore, the method of H_2SO_4 treatment is better used in seed lots of very high percentage of hard seeds and uniform coat thickness (seed lots with more uniform maturity).

Hare & Rolston (1985) investigated the effect of H_2SO_4 on germination of lotus cv. Grasslands Maku seed samples containing only hard seed. While this is the best method to study the methods of hard seed breaking and germination, it can not be used in detecting the effect of hard seed breaking methods on seed vigour, just because the present vigour tests can not detect the vigour status of hard seed.

The sandpaper method had no effect on breaking hard seed and seed vigour of white clover cv. Sustain and lotus cv. Maku (Table 7.1 and 7.2). This is inconsistent with the result of Viado (1989) who found that sandpaper reduced hard seed content of 16 lucerne seed lots from 38.4 to 21.4% using the same grade paper and machine. The primary reason was that the un-adjustable rotation speed of the scarifier was too low (9.8 rpm). The operating speed of scarification equipment is crucial but dependent on the kind of scarifiers used and treatment time. The optimum speed for lotus cv. Maku

seed is 900 rpm (Hare & Rolston 1985), 200 rpm for lucerne (Viado 1989) and 120 rpm for penngift crownvetch (Brant *et al.* 1971).

Therefore, appropriate treatment, e.g. chemical concentration, speed of the scarifier, duration of treatment is likely different for different seed lots, because of different species, seed history and hard seed content.

H_2SO_4 treatment, especially the five minutes treatment, increased the germination of lotus cv. Maku seeds (Table 7.2). This was because of the low germination and vigour of the seed lot. When H_2SO_4 eroded part of the seed coat, but did not damage the seed, the root could more easily penetrate the coat and produce a normal seedling. Selection of seed lots with high germination for vigour testing is important and a prerequisite.

The hard seed content of white clover cv. Sustain and lotus cv. Maku was reduced after AA testing (Table 7.1 and 7.2), indicating that the ageing conditions i.e. high temperature or/and high humidity had the effects of softening hard seed; a result also reported for lucerne by Dehghan-Shoar (Hampton, pers. comm.).

CHAPTER 9: CONCLUSIONS

1. Seeds chemically treated at the recommended rate can be directly conductivity tested in large seeded legumes and cereals without removal of the chemicals. The reliability of the conductivity test for small seeded legumes needs further research. The conductivity of the seeds treated at double the recommended rate significantly increased, but not necessarily for all species, seed lots or chemicals.

2. Different chemicals, especially systemics, have different phytotoxic effects, even at the recommended rates, on post AA germination. The phytotoxicity is the interaction of the application dose of the chemicals, cultivars and vigour status of the seed lots. When comparing vigour of seed lots treated with the same chemicals, the seed lots can be directly AA tested. Caution is needed when seed lots treated with different systemic chemicals are vigour tested using the AA test, because different chemicals have different phytotoxicity which may not affect later seed performance.

3. The present tolerance for the conductivity test is suitable for garden pea and other large seeded legumes.

4. Both the water added method and the filter paper method provided a SMC for large seeded species very near the desired SMC for the CD test. Variance was small, and seed lot vigour ranking did not differ between the two methods of raising SMC. The water added method provided a reasonably acceptable SMC in terms of mean and variance for small seeded species, but is very dependent on the accurate operation of the micro-pipette and improvement of SMC determination methodology after the CD test. Therefore the water added method, after further refinement, will be able to be used for the CD test, superseding the filter paper method.

5. The CD test conditions, i.e., high temperature of 40 or 45^{0}C at near 20% SMC induced high seed dormancy in swede seed lots, but the extent varied with cultivars and SMC. Pre-chilling and 20-30^{0}C germination temperature broke the dormancy.

Therefore, caution should be given when swede and its close species are artificially aged.

6. H_2SO_4 treatment had little negative effect on germination, but significantly increased conductivity and reduced AA germination because of seed coat degradation and fungal invasion.

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APPENDICES

Appendix 1: Quality data of nine commercially treated seed lots of garden pea

		Germination (%)		SMC (%)		TSW (g)	
Cultivar	Treatment	Untreated seed	Treated seeds	Untreated seed	Treated seeds	Untreated seed	Treated seeds
Talbot	Apron 35 SD	99	94	12.8	13.2	205.3	206.6
Quantum	Apron 35 SD	95	97	11.8	12.4	218.8	219.9
	Apron 35 SD	91	95	12.1	12.8	199.8	213.4
Spring	Apron TZ + Peridiam	96	94	10.8	11.2	249.3	253.3
	Aliette Super + Peridiam	98	94	13.3	13.4	240.8	247.1
Resal	Apron 35 SD	91	87	11.8	12.3	219.7	208.7
Hailey	Apron 35 SD	94	90	11.8	12.0	185.2	190.2
Ravel	Apron TZ + Molybdate	97	97	11.3	11.8	216.8	222.4
Cabree	Apron TZ + Molybdate	97	96	11.9	12.6	177.5	191.0

Appendix 2-1: Frequency of conductivity ranges for 100 tests using four replicates of garden pea cv. Bolero

Conductivity range (µS/cm/g)	0.5	0.6	0.9	1.1	1.3	1.4	1.5	1.6	1.7	1.8	1.9	2.0	2.1	2.2	2.3	2.4	2.5	2.6	2.7	2.8	2.9	3.1
Frequency	1	2	1	2	1	1	2	4	5	4	1	3	3	2	4	2	5	2	5	5	1	3
Conductivity range (µS/cm/g)	3.2	3.3	3.4	3.5	3.6	3.7	3.8	3.9	4.0	4.1	4.2	4.3	4.4	4.5	4.6	4.8	5.0	5.1	5.3	5.4	6.4	
Frequency	5	1	3	5	4	1	2	3	1	2	2	2	1	2	1	1	1	1	1	1	1	

Appendix 2-2: Distribution of the conductivity ranges from 100 tests using four replicates of garden pea cv. Bolero

Program VAREST by I.L.Gordon

Analysis 1 Field 1

mean = 2.86200 (0.115896) n = 100

σ^2 = 1.343187 (0.19091231)

sd = 1.158960 CV = 0.4049

Non-Normality Chi^2 = 9.176 NS (0.6887) Distribution is NORMAL

Obs: - [Σ]

0 3 3 [6] 4 13 9 [26] 13 11 12 [36] 12 8 6 [26] 1 3 2 [6]

Exp:-

1.5 1.8 3.3 5.5 8.1 10.6 12.5 13.2 12.5 10.6 8.1 5.5 3.3 1.8 1.5

Class boundaries:-

0.351 0.737 1.124 1.510 1.896 2.283 2.669 3.055 3.441 3.828 4.214 4.600 4.987 5.373

Appendix 3-1: Control germination and post CD germination of swede seed lots excluding dormant seeds

Treatment	Germination temperature (^{0}C)	Germination of seed lots (%) A	B	C	D	E
Control	20	91	93	99	99	96
	20-30	95	97	98	100	97
Post CD	20	52[1]	81	91	81	84
	20-30	80[2]	91	97	97	97

[1] data were the means of the four replicates.

[2] data were the means of 16 observations of eight each from the filter paper and the water added method.

Appendix 3-2: Accumulated final germination and post CD germination of swede seed lots including normal seedlings produced before and after a chilling treatment at 5^{0}C for three days

Treatment	Germination temperature (^{0}C)	Germination of seed lots (%) A	B	C	D	E
Control	20	95	97	99	100	97
	20-30	96	98	98	100	98
Post CD	20	80[1]	91	96	95	96
	20-30	84[2]	93	98	98	98

[1] data were the means of the four replicates.

[2] data were the means of 16 observations of eight each from the filter paper and the water added method.

Appendix 3-3: Effect of pre-chilling at 5°C for three days after the CD ageing but prior to germination on dormancy of five swede seed lots germinated at 20-30°C

	Dormancy of the seed lots (%)				
Treatment	A	B	C	D	E
pre-chilling	4[1]	2	1	0	2
control	5	3	2	1	1

[1] data were the means of eight observations of four each from the filter paper and the water added method.

Appendix 4-1: Dormancy (%) of swede cv. Doon Major seed lots 1-4 (with SMC 7.0, 8.5, 9.9 and 11.5% respectively) as revealed by standard germination and post CD germination test

Treatment	Germination temperature (°C)	Ageing condition	Dormancy (%) 1	2	3	4
CD	20	45°C, 24h	37	27	15	24
		40°C, 48h	32	25	25	23
	20-30	45°C, 24h	3	1	1	3
		40°C, 48h	2	2	3	2
control	20		2	3	6	3
			5	1	6	1
	20-30		1	2	1	0
			1	0	0	0

Appendix 4-2: Accumulated final germination and post CD germination of swede cv. Doon Major seed lots 1-4 (with SMC 7.0, 8.5, 9.9 and 11.5% respectively) including normal seedlings produced before and after a chilling treatment at 5°C for three days

Treatment	Germination temperature (°C)	Ageing condition	Germination (%) 1	2	3	4
CD	20	45°C, 24h	93	94	94	96
		40°C, 48h	95	95	96	97
	20-30	45°C, 24h	96	94	99	98
		40°C, 48h	95	97	98	97
control	20		99	99	96	97
			99	100	100	98
	20-30		99	98	99	99
			99	99	99	99