A STUDY OF SEED VIGOUR TEST METHODOLOGY VARIABLES

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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.).