THE RELATIONSHIP BETWEEN SOYBEAN (*GLYCINE MAX* (L.) MERRILL) SEED QUALITY AND THE RESPONSE TO MOLYBDENUM SEED TREATMENT

by

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DECLARATION

I the under-signed declare that the work in this thesis contains my own original work that has never before been submitted as a whole or in part at any other University for the purpose of acquiring a degree.

Abstract

Experiments in KwaZulu-Natal showed that seed treatment with molybdenum (Mo) could double the yield and increase the protein content by 1.9% of soybeans grown on acidic soils. However, it was also found that soybean yield at five of the localities was reduced on average by 8% after Mo seed treatment. It was surmised that the yield reductions observed after Mo seed treatment were connected to the quality of the seed used for planting. The aim of this project was to assess the relationship between seed quality and Mo seed treatment and find a fast, easy quality assessment test that could be used to adapt Mo treatments according to seed quality.

The first investigation entailed assessing the quality of the seed obtained, from various seed lots, for planting. A number of different seed quality testing techniques were performed and they included the accelerated ageing test, tetrazolium test, conductivity test, standard germination test and an emergence test planted at different depths with incubation at different temperatures. All the test results were compared with the accelerated ageing test results, to find the test most closely correlated to the accelerated ageing test, which is regarded as the most accurate indicator of soybean seed vigour. It was found that the emergence test where the seeds where planted at 10 cm presented a close correlation with the accelerated ageing test. An assessment of seed quality revealed that the four different seed lots provided seed of three significantly different levels of quality, which could be used for further investigations.

The second investigation was concerned with the reaction of the seed of different quality levels to Mo seed treatment. Firstly, seeds from four different seed lots were treated with five different concentrations of Mo and planted under acidic conditions. The establishment was monitored up until six weeks, at which point the experiment was terminated. In the second planting, seeds from the four different seed lots were treated with six different concentrations of Mo and

planted under optimum pH conditions. Emergence was monitored and after thinning out the remaining plants were left to mature and produce seed. The emergence percentage results from both of these two plantings did not reveal the alleged positive effect Mo seed treatment has produced in the field and no definite relationship between seed quality and Mo seed treatment was observed.

The third investigation was concerned with the effect that osmoconditioning had on the reaction of seed to Mo seed treatment. Seeds from two seed lots, one of very poor quality and the other of good quality, were pre-treated with four different levels of poly-ethylene glycol (PEG 6000) and then treated with four concentrations of Mo. They were planted under optimum pH conditions and establishment was monitored. After being thinned out the remaining plants were allowed to mature and produce seed. The emergence percentage results revealed that the PEG pre-treatments greatly improved emergence percentages, especially in the poor quality seed. There were some positive effects of Mo seed treatment observed where the lower concentrations of Mo were used, but again, no definite relationship between seed quality and Mo seed treatment was found. PEG pre-treatment appeared to help make the seeds more "resistant" to the harmful effects of Mo.

Uittreksel

Navorsing in KwaZulu-Natal het aangetoon dat saadbehandeling met molibdeen (Mo) die opbrengs van sojabone op suurgrond kan verdubbel en die proteieninhoud met tot 1.9% kan verhoog. Daar is egter ook gevind dat sojaboonopbrengs op vyf lokaliteite met gemiddeld 8% gedaal het na saadbehandeling met Mo. 'n Vermoede bestaan dat die opbrengsverlagings wat voorgekom het na saadbehandeling met Mo verband hou met die kwaliteit van saad wat vir die aanplantings gebruik is. Die doel van die projek was om vas te stel wat die verband tussen saadkwaliteit en Mo saadbehandeling is en om 'n vinnige en maklike toets te vind om saadkwaliteit te bepaal om sodoende Mo behandelings aan te pas volgens saadkwaliteit.

In die eerste eksperiment is die kwaliteit van verskillende saadlotte wat verkry is, getoets. 'n Aantal verskillende tegnieke om saadkwaliteit te bepaal is uitgevoer. Die tegnieke was die versnelde verouderingstoets, tetrazoliumtoets, konduktiwiteitstoets, standaard ontkiemingstoets en 'n vestigingstoets waar saad op verskillende dieptes geplant en by verskillende temperature geïnkubeer is. Die resultate van die verskillende toetse is gekorreleer met die resultate van die versnelde verouderingstoets, wat beskou word as die toets wat die beste aanduiding gee van saadgroeikragtigheid. Daar is gevind dat sade wat 10 cm diep in sand geplant is, se opkoms die beste korrelasie met die versnelde verouderingstoets toon en dus as 'n goeie aanduiding van die kwaliteit van saad beskou kan word. Daar is gevind dat daar drie saadlotte is met duidelike kwaliteitsverskille wat gebruik kon word in verdere eksperimente.

Die tweede eksperiment het die reaksie van saad van verskillende kwaliteitsvlakke teenoor molibdeen saadbehandeling ondersoek. Eerstens is saad van vier verskillende saadlotte behandel met vyf verskillende konsentrasies Mo en onder baie suur toestande geplant. Die vestiging is gemonitor vir ses weke waarna die eksperiment gestaak is. Daarna is saad van dieselfde vier

saadlotte behandel met ses verskillende Mo konsentrasies en onder optimum pH toestande geplant. Vestiging is gemonitor en na ses weke is die plante uitgedun en twee plante per pot is gelaat om saad te produseer. Die vestigingspersentasie van beide die eerste en tweede plantings het nie die verwagte positiewe effek teenoor Mo saadbehandeling getoon nie en geen betekenisvolle verwantskap tussen saadkwaliteit en Mo saadbehandeling kon waargeneem word nie.

Die derde eksperiment het die invloed van osmokondisionering op die reaksie van saad op Mo saadbehandeling ondersoek. Saad van twee saadlotte, een van goeie kwaliteit en een van swak kwaliteit, is voorafbehandel met poli-etileen glikol (PEG 6000) en daarna met vier konsentrasies van Mo behandel. Die sade is onder optimum pH toestande geplant en die vestiging is gemonitor. Nadat dit uitgedun is, is die oorblywende twee plante gelaat om saad te produseer. Die vestigingspersentasies het getoon dat PEG voorafbehandelings vestiging betekenisvol verbeter, veral in die geval van lae kwaliteit saad. Daar was 'n positiewe effek van Mo saadbehandeling waar relatief lae konsentrasies molibdeen toegedien is, maar daar kon weereens nie 'n duidelike verwantskap tussen saadkwaliteit en Mo saadbehandeling waargeneem word nie. Dit blyk dat PEG voorafbehandeling die sade meer bestand teen die skadelike invloed van Mo gemaak het.

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Chapter 1: Literature Review

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1.1 Introduction

This project was initiated in reaction to a project performed by Farina, Thibaud and Channon (1997) in KwaZulu-Natal. The aim of their project was to investigate factors affecting the response of soybean to molybdenum (Mo) application. They planted a number of soybean plots with half plots planted to seed treated with Mo and the other half with untreated seed. In most cases they found that the treated seed produced significant positive yield responses, but in some cases the Mo treatment had negative effects on yield (Farina *et al.*, 1997). The reasons for these negative responses were unclear, but it was thought to possibly be associated with seed quality.

The cultivar that produced most of the poor results in the investigations in KwaZulu-Natal was Dumela. Seed of this cultivar and another commonly used cultivar (Prima) was obtained to use for the project. Literature was studied on soybean seed quality, what affects seed quality, what causes deterioration and how seed quality can be evaluated in soybeans. Thus, the aim of the first part of the project was to accurately assess the quality of the seed to be used for the project. To do this a number of different testing techniques were performed and the results evaluated and compared with each other. External seed characteristics were also evaluated and their contribution to seed quality was assessed. Along with this a seed nutrient content test was performed to assess seed nutrient status relative to seed quality. It was hoped that an easy, non-expensive test could be found that could be carried out on the farm and used as an accurate indication of quality so that Mo seed application concentrations could be adjusted according to the quality of the seed.

Secondly, once soybean seed quality had been established, the various quality seed lots were treated with various levels of Mo to see if a relationship could be found. Literature on the subject of Mo treatment on soybean seeds is sparse, thus the role of Mo was studied as a general subject.

Seed enhancement techniques were investigated as a means of improving seed quality, should there prove to be a relationship between poor seed quality and molybdenum application. Seed that had received osmoconditioning pre-treatments with polyethylene glycol (PEG 6000) were planted after receiving various concentrations of Mo seed treatment.

1.2 Seed quality

Seed quality can be divided into physical attributes and physiological attributes. Physical seed attributes that affect performance are characteristics such as etching, cracking, colour, size and shrivelling (Illipronti, Langerak & Lommen, 1997). Etching occurs when the growth of the enlarging embryo is so rapid that it expands beyond the elastic capacity of the seed coat, tearing it and producing seed coat etching (Illipronti *et al.*, 1997). Cracking of a seed is caused by mechanical damage inflicted upon the seed. The colour of seed can be either yellow or green, with green seeds being of a lower quality. There are contrasting opinions on whether size affects quality, but it definitely has an effect on some of the quality testing techniques. Shrivelling of the seed coat generally occurs when the seeds are exposed to adverse field conditions such as high temperatures and drought conditions (Franca Neto *et al.*, 1993).
Physiological seed attributes that affect seed performance are characteristics such as poor tissue and cells and poor membrane function. A seed should not be viewed as a complete entity because all its tissues/organs and organelles differ in form, chemistry, function and susceptibility to deterioration (McDonald, 1998).

Seed quality has a number of components that should be taken into consideration, namely genetic and mechanical purity of seeds, seed germination and vigour and seed health. However, this review will be focusing on seed germination and vigour tests.

1.2.1 Seed quality deterioration

To be able to evaluate seed quality one needs to have an understanding of what causes seed deterioration.

Soybean seeds are known for their rapid deterioration after they have reached physiological maturity. Physiological maturity is when the seed has reached it's maximum dry weight and it is at this point that it possesses it's greatest potential for germination and seed vigour (Copeland & McDonald, 1995). During seed deterioration, the first seed quality component lost is vigour, which is followed by a loss of germination capacity and finally death (loss of viability) (Trawatha, Tekrony & Hildebrand, 1995).

Seed quality deterioration is contributed to by factors such as seeds harvested immature or over-mature, physical abuse during harvesting and transport, improper storage and poor planting and handling (McDonald, 1998).

Field conditions during the developing stages of soybean seeds are also responsible for poor seed quality. The intensity and rapidity of deterioration of seed quality is affected by a number of factors such as temperature, relative humidity, moisture content of the seed, micro organisms, mechanical injuries and initial seed quality (Custodio & Marcos-Filho, 1997).

There are many reports of cases where hot temperatures and low moisture conditions occurred during seed fill, and as a result seeds of smaller size were produced which were often shrivelled. These reports state that shrivelled seeds show no difference in standard germination but have reduced vigour (as shown by accelerated ageing and conductivity tests) (Franca Neto *et al.*, 1993).

Where high temperatures and humidity are found both during seed fill and after physiological maturity, there is a higher number of abnormal seeds, usually shrivelled and smaller in size. These seeds have reduced germination and vigour, with vigour decreasing before germination capacity does. However, normal seeds are also produced under these conditions but it was found that these seeds also had reduced germination and vigour even though they appeared normal (Spears, Tekrony & Egli, 1997).

Drought stress without high temperatures will often produce smaller seeds in smaller numbers but with little effect on germination and vigour, except where the seed is shrivelled (Vieira, Tekrony & Egli, 1992).

Another reported cause of quality reduction is low levels of calcium in the seeds. Calcium uptake in the plant is closely related to water uptake, thus in drought conditions there is less transpirational movement and less Ca is taken up by the plant and distributed to the seeds (Smiciklas *et al.*, 1989). The primary effect of Ca deficiency in cells is loss of membrane integrity. Because soybean seed germination requires membrane reorganisation, the lack of Ca could make membrane reorganisation less efficient (Smiciklas *et al.*, 1989). Thus Ca deficient seeds have a lower germination rate, reduced vigour and higher conductivity levels due to an increase in the permeability of plasma membranes (Smiciklas *et al.*, 1989).

Low Ca in the root medium during seed fill is reported to produce seeds with a lower Ca concentration, a higher number of shrivelled pods and undeveloped seeds and a higher number of abnormal seedlings germinating from normal seeds (Keiser & Mullen, 1993).

Seed quality deteriorates when the cation balance is changed, especially when the B/Ca ratio is increased. Seedling development is positively related to seed Ca concentration and negatively related to seed B concentration (Keiser & Mullen, 1993).

Cation imbalances (low Ca levels) often occur when high relative humidity conditions result in reduced transpiration rates and increased concentrations of P, Mn, Fe, Zn and B in the seed (Keiser & Mullen, 1993). Ca is the only seed nutrient that is positively related to germination and thus reduced germination potential results if low amounts are deposited in the seed (Smiciklas *et al.*, 1989).

The reason for this, as mentioned above, is that Ca is closely related to membrane integrity and reorganisation is thus less efficient during germination and electrolyte leakage occurs.

Deterioration of seed quality during storage might occur due to the peroxidation of unsaturated fatty acids. Polyunsaturated fatty acids found in seeds are highly susceptible to peroxidation and high peroxidation levels reduce seed viability (Trawatha *et al.*, 1995). Another possible cause of deterioration is the accumulation of free radicals, which cross link with macromolecules and disrupt membrane functioning and enzyme activity (Trawatha *et al.*, 1995).

Large seeds are more easily damaged and so tend to be cracked more often, but otherwise shape, size and weight have little affect on the viability and vigour of seeds (Illipronti *et al.*, 1997). Some reports however, do state that low vigour is correlated with low seed weight (Gibson & Mullen, 1996).

Yellow coloured seeds are generally heavier than the green or green/yellow seeds and they have a lower electrolyte leakage than green seeds (Illipronti *et al.*, 1997).

Etched seeds are more susceptible to mechanical damage and so it is possibly not the direct effect of etching that reduces the quality of the seed (Gibson & Mullen, 1996).

1.2.2 Seed evaluation techniques

When assessing seed quality it is advisable not to use germination tests because these only give an indication of how the seeds fare under ideal conditions, which are far from conditions found in the field (Copeland & McDonald, 1995). For this reason vigour tests are better because they give an indication of field emergence.

For evaluating seed quality, direct and indirect techniques of testing are available. An example of a direct test is the cold test, which is the most commonly used direct test. This is when the seeds are subjected to adverse conditions by placing them in cold, wet soil. The seeds are under direct stress from temperature, moisture (imbibition) and micro organisms. They are then allowed to germinate under normal soil conditions (Copeland & McDonald, 1995).

Most of the tests used to measure vigour are indirect tests. Indirect tests measure specific physiological components of seeds (Copeland & McDonald, 1995). All of the tests described below are indirect tests.

1.2.2.1 Accelerated ageing test

The accelerated ageing test is one of the most commonly used tests because of its simplicity and ease of standardisation. It is where unimbibed seeds are subjected to high temperatures and high relative humidity and then placed under ideal conditions to germinate (Copeland & McDonald, 1995).

Low quality seeds deteriorate more rapidly than high quality seeds under these conditions (McDonald, 1998). The advantages of this test are that the temperature and duration of ageing are easy to manipulate, to create the best conditions for a specific crop (McDonald, 1998). The test can be used for individual seed evaluation and requires no additional training for correct evaluation (Copeland & McDonald, 1995).

1.2.2.2 Electrical conductivity test

The conductivity test is based on the principle that low quality seeds often leak more exudates than high quality seeds during the first few hours of imbibition (McDonald, 1998). This is due to the loss of membrane integrity, which is considered one of the primary physiological events that occurs during seed deterioration. Loss of membrane integrity occurs as a result of storage deterioration and mechanical damage (Copeland & McDonald, 1995). The test quantifies the amount of electrolytes that are released from imbibing seeds (Custodio & Marcos-Filho, 1997).

The amount of exudates leaked, is influenced by the stage of seed maturation, degree of seed ageing, incidence of damage and size of seed (Illipronti *et al.*, 1997; McDonald, 1998). Cracking results in increased leakage but etching has been shown to have no effect in one situation and to increase leakage in others (Illipronti *et al.*, 1997). Seed size is reported to influence conductivity readings and for this reason conductivity should be expressed on an area basis ($\mu A\ mm^{-2}$) to reduce variation due to weight. Readings should not be expressed on a weight basis because conductivity decreases as weight increases and thus one tends to overestimate seed quality of large seeds (Illipronti *et al.*, 1997). It has also been reported that soybean seeds at high moisture content establish a resistance to leakage (McDonald, 1998).

1.2.2.3 Potassium leachate test

A further development in leakage tests is to identify the specific compounds that are leaked. The main inorganic ions that are leaked are potassium, sodium and calcium. Reports show that potassium is a more efficient indicator than taking all the ions together (Custodio & Marcos-Filho, 1997). Thus the potassium leachate test is used to determine seed vigour. It requires an imbibition time of only 90 min and then a flame photometer is required to read the K leachate levels (Custodio & Marcos-Filho, 1997). Like the conductivity test it is a valuable test because it identifies different performance levels, but the K leachate test is a much quicker test. (The ordinary conductivity test should imbibe for 24 hours.) The K leachate test is also more sensitive to differences in seed performance and physiological seed quality (Custodio & Marcos-Filho, 1997).

In both the conductivity and potassium leachate tests, seeds are imbibed in groups of 25 and so the end result is an average of the 25 seeds. Thus, one is assuming that all of the 25 seeds are equally deteriorated (Copeland & McDonald, 1995). If the electrolyte leakage of individual seeds could be monitored it would be a better representation.

1.2.2.4 Tetrazolium test (TZ)

Another quick, inexpensive vigour test is the tetrazolium test. In contrast to the conductivity test, which only detects cracked and wrinkled seeds, the TZ test detects etched, cracked, non-yellow and wrinkled seeds (Illipronti *et al.*, 1997). This test is based on the principle that the TZ molecule reacts with hydrogen atoms released due to dehydrogenase activity in living tissue. This produces a red pigment and an evaluation of the staining pattern on the seed and the colour intensity is made (Copeland & McDonald, 1995). Thus, it is a subjective placement of seeds into vigour categories ranging from strong to weak and the test is subject to certain standardisation difficulties (Copeland & McDonald, 1995).

1.2.3 Seed enhancement

It was proposed that after having assessed the quality of the seed one could also consider seed enhancement that could possibly improve the seeds' quality and in turn improve their response to Mo application. Osmoconditioning, also known as seed priming is a possible technique of seed enhancement that could be used.

Priming is a hydration/dehydration process that is meant to enhance seed quality after it has been in storage for a period of time. It is accomplished by using osmotica such as poly-ethylene glycol (PEG) or various salts, or direct application of water. This is then followed by a drying process such as matriconditioning and drum priming (McDonald, 1998). The hydration is meant to initiate the earliest stages of germination and perhaps also initiate physiological repair of membranes and organelles damaged during storage (Copeland & McDonald, 1995). It is meant to result in more rapid and uniform seedling emergence (McDonald, 1998).

Although osmoconditioning affects membrane re-establishment it would seem that the intact membranes are very sensitive to the subsequent drying process (Armstrong & McDonald, 1992). Defects in the growing radicle tip can also result from the drying process (McDonald, 1998). However, very positive results have been obtained by both Park *et al.* (1999) and Braccini *et al.* (1999) with osmoconditioning or priming performed on soybeans. In both cases germination ability and establishment of aged seeds were improved.

1.2.4 Conclusion

The problem with seed quality evaluation is that seeds that come from the same seed lot can differ in quality and they can also have different reasons for their individual deterioration. Thus it is sometimes valuable to know what the most sensitive part of the seed is. For example, in soybeans the growing points of the embryonic axis are most prone to ageing (McDonald, 1998). This however, also shows that different parts of the seed age at different rates, thus only reinforcing the complexity of seed quality evaluation.

1.3 Role of molybdenum in plants

Apart from being an essential constituent of several important enzymes, Molybdenum has a range of non-specific roles in plant metabolism (Srivastava, 1997).

1.3.1 Role in nitrogen metabolism

Molybdenum is well known for the role it plays in nitrogen metabolism in crop plants. It is required for both nitrate reduction and biological nitrogen fixation (Srivastava, 1997).

In nitrate reduction Mo is a constituent of nitrate reductase, the enzyme that catalyses the reduction of nitrate into nitrite. High concentrations of nitrate in a plant have been reported to increase Mo requirements. Enzyme activity is closely related to Mo availability and Mo deficiency is diagnosed by monitoring the activity of nitrate reductase in plant foliage (Srivastava, 1997).

Mo is also required for biological nitrogen fixation, which is carried out by the root nodule bacteria (*Rhizobium*) in leguminous crops. Mo is a constituent of the enzyme nitrogenase, which facilitates the reduction of atmospheric dinitrogen (Srivastava, 1997).

Thus, being an essential constituent of N-fixing activity, an absence of Mo results in poor growth and activity of root nodules in leguminous plants. A study on soybeans showed that Mo application did not increase the number of root nodules, but did ensure better growth of each individual nodule (Srivastava, 1997).

As a result of Mo promoting the utilisation of absorbed nitrates and N fixation, it thus influences the protein content in plants.

1.3.2 Other roles of molybdenum

Mo is also a constituent of sulfite oxidase, an enzyme that mediates the oxidation of sulphite to sulphate in sulphur metabolism (Srivastava, 1997).

Mo influences carbon assimilation and the utilisation of carbohydrates and is indirectly involved in the synthesis of plant pigments. Thus where Mo is deficient, low sugar concentrations in plants is related to the failure of photosynthesis and poor growth is related to non-utilisation of carbohydrates (Srivastava, 1997).

Because of molybdenum's role in nitrogen and indirect role in carbohydrate metabolism, the reproductive phase of plants is more sensitive to a Mo deficiency than the vegetative phase and seed and seedling quality is greatly affected (Srivastava, 1997).

Reports also show Mo to help plants to be more drought resistant and disease resistant. Whether these are direct effects of Mo, or indirect effects of plant metabolism, is still uncertain (Srivastava, 1997).

1.3.3 Molybdenum in soybeans

In soybeans Mo deficiency reduces plant growth, the number of pods per plant, the number of seeds per pod, seed size, nodulation and total nitrogen and protein content of seeds (Gupta, 1997). Harris, Parker & Johnson, (1965) described Mo deficiency symptoms as "leaves twisted on stem and necrotic areas adjacent to midrib, between the veins and along the margins." Thus, the symptoms of this deficiency are typically those of nitrogen deficiency - a general yellowing of the foliage (Harris *et al.*, 1965).

Mo toxicity is detrimental to *Rhizobia* and can cause seedling injury in soybeans (Gupta, 1997).

Harris *et al.*, (1965) investigated whether the amount of seed-borne Mo would influence the response of the progeny to applied Mo. It was found that progeny of all the seeds that contained less than 2.6 ppm Mo, responded to sodium molybdate applied as a seed treatment. The progeny of seed containing 22.4 ppm Mo did not respond to the applied Mo.

This shows that not only the quality of the seed but also the Mo content of the seed must also be taken into account when assessing the plants response to Mo application.

1.4 Conclusion

Thus far very little research has been performed using Mo as a testing element on soybean production, especially as a seed treatment. This project was thus embarked upon with the knowledge that problems were going to be encountered due to lack of experimental experience and knowledge in this particular research field.

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Chapter 2: The investigation of external seed characteristics of soybeans in relation to seed quality and the comparison of various seed quality tests in soybeans.

Introduction

It is well known that soybean (*Glycine max* (L.) Merrill) seed quality deteriorates very rapidly after being harvested and during storage. Thus, when it comes to using the seed for replanting, the quality of the seed is often below optimal. Poor quality seed does not only result in decreased germination but also reduced seed vigour, i.e. the plant's ability to establish itself and develop after germination (Copeland & McDonald, 1995). Therefore, if farmers could determine seed quality before sowing, they can adjust sowing densities accordingly and regulate concentrations of molybdenum (Mo) pre-plant seed treatments in accordance with seed quality (see Chapter 3).

Germination percentage can be tested with a simple germination test, but this does not give any indication as to the vigour of the seed (Copeland & McDonald, 1995). There are a number of techniques for testing seed vigour. One of the most accurate and widely used tests for evaluating soybean seed quality in laboratories is the accelerated ageing test (McDonald,1998). This test is however not very user friendly for the average farmer, who does not have the required equipment to perform the test, nor the proper training to evaluate test results accurately. So in search for a simple vigour test other tests were performed, using the accelerated ageing test results as a control with which these tests could be compared. The other testing techniques performed were the conductivity test, the tetrazolium (TZ) test, the standard germination test and a basic emergence test where seeds were planted at a number of different depths, at different temperatures. These testing techniques can all be performed with minimal expense and training.

The literature on the effect external characteristics have on seed quality is often contradictory. Illipronti, Langerak and Lommen (1997) found that there was no difference between the conductivity of etched and non etched seeds, whereas Oliveira, Mathews and Powell (1984) found that seeds with split coats not only had higher conductivity readings, but also resulted in poorer emergence. Wrinkling of seed coats is said to reduce the quality of soybean seeds (Franca Neto *et al.*, 1993). It was decided to perform the conductivity test and tetrazolium test on different seed lots whose seeds were categorised according to prominent external characteristics to see if differences in quality would be highlighted. The seeds were categorised as etched seed, wrinkled seed and normal seed.

Internal seed nutrient content was also analysed to see if there was any correlation between nutrient content of the seed, external characteristics and seed quality.

The main objective of this study therefore, was to try and find a simple seed quality test that is closely correlated to accelerated ageing test results and thus can be used by a farmer as a good indicator of soybean seed quality. The second objective was to ascertain whether there is any correlation between seed quality and internal seed nutrient content or seed quality and external seed characteristics.

Materials and methods

Soybean seed from four different lots was obtained. Three successive harvests of seed of the cultivar Prima were obtained i.e. fresh seed that had been harvested in that season (Fresh); seed that had been in storage for one year (1

YR) and seed that had been in storage for two years (2 YR). The final lot of seed was of the cultivar Dumela, which had been in storage for one year (Dumela). Each of the four different seed lots were then divided up into four categories related to external characteristics. The categories were "etched", "wrinkled", "normal" and a "mixed" group which was a random sample taken out of each seed lot. Both the conductivity test and the tetrazolium (TZ) tests were performed on the four different seed lots having been divided into these four different categories.

The Conductivity Test

Four samples of 25 seeds were each weighed before being placed in 75 ml of distilled water for 24 hours. They were kept at room temperature (25°C) during this time (Copeland & McDonald, 1995). The readings were taken with a conductivity meter (Metrohm, 644 conductometer).

Tetrazolium (TZ) Test

Four samples of 25 seeds were placed between moist paper towels for 16 hours, at room temperature (25°C). This allowed slow imbibition to take place. The seeds were then covered by a 0.1% solution of 2,3,5-triphenyl tetrazolium chloride and left for 4 hours. The seeds were then rinsed and stored in tap water until analysed (Copeland & McDonald, 1995). Upon analysis the seed coat was removed and cut along the embryonic axis to show staining patterns. The light, only slightly pink seeds were of high quality or vigour and the darkly stained seeds, with white patches of dead tissue were of reduced vigour (Copeland & McDonald, 1995). The seeds were either classified as vigorous or having reduced vigour. Although no intermediate evaluation categories were included in this evaluation, they can easily be created by looking at the extent of the staining pattern on the seed. The percentage of vigorous seeds was calculated.

Seed Analysis

Seed of the four seed lots divided into the various categories were analysed for nutrient contents (Table 3) by the soil science section at the Infruitec institution of the Agricultural Research Council in Stellenbosch, using standard techniques.

For the following vigour tests only a random sample of seeds from the four different seed lots (Prima – Fresh; Prima – 1YR; Prima – 2YR and Dumela – 1YR) was evaluated.

The Accelerated Ageing Test

A minimum of 200 seeds (± 42 g) of each seed lot (Fresh; 1YR; 2YR & Dumela) were placed in Hoffman Accelerated Ageing trays used in conjunction with transparent polystyrene containers. The wire mesh used was standard 18 mesh. The seeds were placed in the trays in an unimbibed state and subjected to a constant temperature of 41 °C and a relative humidity of around 100% for 3 days in an accelerated ageing chamber (Hampton & TeKrony, 1995). The seeds were then removed from the stress conditions and placed under optimum germination conditions (standard germination test, see next section). A sample of seeds was taken from each tray to test the seed moisture content of the seeds immediately after being taken out of the chamber. The seeds should have a seed moisture content of 27 – 30% when they are removed from the chamber (Hampton & TeKrony, 1995).

Standard Germination Test

Three sheets of germination towel were placed on top of each other and rolled up into a scroll. The scrolls were soaked in distilled water and then squeezed out to ensure that each scroll weighed approximately 250 g. The top piece of paper was lifted and 50 seeds per scroll were placed in four rows. The paper was then replaced and the scroll was rolled up again and placed into a plastic bag. The packages were then placed in containers so that they were standing upright and placed into a germination cabinet at 30 °C. Seedling counts were conducted at four and seven days after seeding. The seedlings were evaluated for normal development, and the results were expressed as percentage normal seedlings for each repetition (AOSA, 1993).

After the accelerated ageing test six repetitions (six scrolls) per seed lot underwent the standard germination test. A further four repetitions (four scrolls) of each seed lot were germinated under these standard conditions, having received no pre-treatment.

Emergence Test at Different Depths

Five litre plastic ice-cream containers had holes drilled in the bottom for drainage and were then filled with sand obtained from the Berg River at Paarl, 45 km NE of Cape Town. The sand is classified as a grey sand with 1% clay, 9% silt, 27% rough/coarse sand, 31% medium sand and 32% fine sand. The pH (KCl) is 7.1, resistance (ohms) 1550, P content is 54 ppm, K content is 64 ppm, Ca content (%) is 4.05 and Mg content (%) is 0.09. The sand was moistened to field capacity with water. Seeds from the four seed lots were planted at three different depths, namely 2 cm; 5 cm & 10 cm. Ten seeds were planted per container and there were three replications. The containers were placed at three different constant temperatures, namely 15°C; 20°C and 24°C in growth rooms. The containers were checked for emergence on a daily basis for the first seven days and then only every second day after that. The seeds under temperature conditions of 15°C were allowed to incubate up until 34 days after planting, those under 20°C conditions up until 25 days after planting and those under 24°C up until 21 days after planting. Therefore the seeds were subjected to the different temperatures for roughly the same number of degree-days (ca 500). The emergence rates and percentages were recorded. The seeds were considered to be fully emerged once the cotyledons had been pulled clear of the soil.

All data were analysed by using the "means" command of the SAS statistical package to test for significant differences between means. The emergence percentage data was logit transformed before analysis. Where a significant interaction was found the "pdiff" command of "proc glm" was used to evaluate the individual interactions of the various treatment combinations. To compare different testing methods with varying units, a correlation analysis was done using Pearson's Correlation Coefficient. The "proc plot" command was used to illustrate the strength of the linear relationship between each of the various tests and the accelerated ageing test. The "proc corr" command was used to provide correlation coefficients as well as a p-value for each correlation (SAS, 1985).

Results and discussion

Table 1 shows the results of the two tests performed on the seeds that were grouped according to prominent external characteristics.

Table 1 Comparison of the conductivity and tetrazolium test results of the soybean seeds categorised according to external characteristics

Seed Description	Fresh		1 YR		2 YR		Dumela		Means	
	[1]C.T	[2] T.T	C.T	T.T	C.T	T.T	C.T	T.T	C.T	T.T
Etched	77	91	90	73	103	43	108	51	95 a	65 b
Mixed	56	83	87	83	99	66	114	42	89 ab	69 a
Wrinkled	59	93	97	90	86	86	89	48	83 bc	79 a
Normal	61	97	74	94	80	86	95	69	78 c	87 a
Means	63.3 a	91 a	87 b	85 b	92 b	70 b	101.5 c	53 c		

*Different letters indicate statistical significant differences, within each test method, at the 5% level.

[1]**C.T** = Conductivity Test (mS/cm/g)

[2]**T.T** = Tetrazolium Test (% healthy seed)

Table 2 The interactions found between the soybean seed lots and the seed categories in the conductivity test and the tetrazolium test

Conductivity Test			Tetrazolium Test		
Seed Lot	Seed Category	Interaction	Seed Lot	Seed Category	Interaction
FRESH	mixed	a	FRESH	mixed	a
FRESH	wrinkled	ab	FRESH	wrinkled	abc
FRESH	normal	ab	FRESH	etched	abcd
FRESH	etched	c	FRESH	normal	cdef
1 YR	normal	bc	1 YR	normal	ab
1 YR	mixed	cdef	1 YR	wrinkled	bcd
1 YR	etched	cdef	1 YR	mixed	defg
1 YR	wrinkled	efg	1 YR	etched	defgh
DUMELA	wrinkled	cdef	DUMELA	mixed	efgh
DUMELA	normal	defg	DUMELA	wrinkled	fgh
DUMELA	etched	gh	DUMELA	etched	fgh
DUMELA	mixed	h	DUMELA	normal	h
2 YR	normal	cd	2 YR	normal	abc
2 YR	wrinkled	cde	2 YR	wrinkled	cde
2 YR	mixed	efgh	2 YR	mixed	efgh
2 YR	etched	fgh	2 YR	etched	gh

The conductivity test interactions showed that the "etched" category of the Fresh seed was significantly different from the other categories of that seed lot. However, "etched", fresh seed was not significantly different from the normal and wrinkled seed of all the other seed lots except Dumela "normal" seed. This shows that the lowest quality seed of the Fresh lot was of similar quality to the highest quality seed of the other seed lots.

In all the seed lots, except 1 Year, "etched" seed was significantly different from the values of the "wrinkled" and "normal" looking seed, indicating a lower quality. It would seem that "normal" and "wrinkled" seed are not significantly different in quality.

The interactions between the results of the tetrazolium test do not provide such a clear picture. In most cases there was no significant difference between the different categories within a seed lot. This vagueness could be attributed to the subjective nature of the test.

Table 3 shows the nutrient content analysis of the seeds also divided according to external characteristics.

Table 3 Analysis of nutrient content of soybean seeds grouped according to external characteristics

Seed Type	N (%)	P	K	Ca	Mg	S	Cl	Na (mg/kg)	Mn	Fe	Cu	Zn	B	Mo
F-Normal	6.09	0.62	1.95	0.22	0.23	0.35	0.02	54	36	99	13	44	26	3.91
F-Etched	6.10	0.62	1.94	0.19	0.24	0.33	0.01	53	30	96	13	44	24	2.72
F-Wrinkled	6.13	0.60	1.90	0.21	0.23	0.32	0.01	65	33	94	13	67	24	3.53
F-Mixed	6.04	0.62	1.94	0.21	0.23	0.32	0.04	57	33	97	13	44	25	4.01
2yr-Normal	5.88	0.63	1.92	0.21	0.24	0.33	0.01	59	36	88	13	88	26	3.43
2yr-Etched	6.03	0.64	1.89	0.18	0.26	0.33	0.02	40	33	89	15	46	26	3.62
2yr-Wrinkled	5.74	0.62	1.92	0.20	0.24	0.30	0.01	47	34	84	13	54	24	2.87
2yr-Mixed	5.82	0.64	1.96	0.20	0.25	0.33	0.02	27	35	89	14	46	26	3.34
1yr-Normal	6.18	0.65	1.82	0.20	0.22	0.33	0.03	15	33	90	14	44	28	3.98
1yr-Etched	6.28	0.67	1.81	0.19	0.23	0.34	0.01	20	31	91	14	45	29	5.26
1yr-Wrinkled	6.08	0.65	1.83	0.20	0.22	0.35	0.02	11	32	87	14	44	28	4.06
1yr-Mixed	6.20	0.65	1.84	0.20	0.22	0.33	0.01	21	32	93	14	44	28	4.85
D-Normal	6.45	0.58	1.93	0.21	0.22	0.35	0.02	6	35	93	13	42	32	3.23
D-Etched	6.42	0.59	1.93	0.19	0.23	0.34	0.03	25	33	86	14	39	30	2.50
D-Wrinkled	6.40	0.57	1.90	0.20	0.22	0.33	0.01	26	35	88	13	40	29	2.35
D-Mixed	6.44	0.58	1.92	0.19	0.22	0.35	0.02	22	35	87	13	40	29	2.96

Little relationship could be observed between seed nutrient content and seed quality or the various external seed characteristics. A reference can be made to previous literature compiled by Keiser & Mullen (1993) which showed that low Calcium (Ca) concentrations in the seed could lead to a larger percentage of abnormal seedlings – an indication of lower seed quality. They established that the Ca concentration needed to be about 2.0 g/kg to realise a germination potential of 90%. All the seed samples had satisfactory Ca levels, except the "etched" seeds that were all below 2 g/kg (0.2%). This may be a contributing factor to the "etched" seed's poorer performance.

The ANOVA analysis of the results from the germination test planted at different depths showed that there was no significant difference between the results obtained under the different temperatures (p=0.3975).

Table 4 The statistical means of the emergence percentages at the different temperatures

Temperatures	15°C	20°C	24°C
Means (%)	56	62	57
T grouping*	A	A	A

*Letters representing statistical significant difference at the 5% level.

Temperature was thus excluded as one of the factors of this experiment. Further analyses were performed on the emergence results according to the different depths, which were significantly different (p=0.0001).

Table 5 The results from the four different tests that can indicate seed quality of the four different soybean seed lots

Seed Lot	Quality parameters						
	Vigor Test (Germ. % after acceler. ageing test)	Viability Test (Germ. %)	Conductivity Test (mS/cm/g)	Tetrazolium Test (% healthy seed)	Diff. Depth Germination Test (Germination %)		
					2cm	5 cm	10 cm
Fresh Prima	80 a*	89 a	63 a	91 a	100 a	83 a	70 a
1 year Prima	37 b	82 b	87 b	85 b	99 a	80 ab	46 ab
2 year Prima	1 c	50 c	92 b	70 b	81 b	60 bc	20 b
1 year Dumela	8 c	50 c	101 c	53 c	80 b	55 c	30 b

*Different letters in the same column indicate statistical significant differences at the 5% level.

The vigour test shown in the first column of Table 5 is the accelerated ageing test results which were obtained and used as the most accurate representation of the quality of the different seed lots. It is with these results that the rest of the tests were compared to show whether the individual tests are able to indicate seed quality accurately. Using SAS and performing an ANOVA analysis on the results of the vigour test the "Fresh" seed was shown to be significantly different from the "1 Year" seed lot, which in turn was significantly different from both the "2 Year" and "Dumela" seed lots. The latter two were not significantly different from each other (Table 5).

The results in the table are not directly comparable because they are not of the same units and so a correlation analysis was performed using SAS to compare all of the tests together. The Pearson correlation coefficients (R) are listed with the probability (P) below them (|R| under Ho: Rho=0 / N = 4) in Table 6.

Table 6 The relationship between the accelerated ageing test (vigour test) and the various other tests performed as indicators of seed quality

		Tetrazolium	Conductivity	Viability	Depth=2cm	Depth=5cm	Depth=10cm
Vigour	(R)	0.82076	-0.94125	0.92782	0.88262	0.88710	0.99341
Test	(P)	0.1792	0.0588	0.0722	0.1174	0.1129	0.0066

These results indicate that the germination test where seeds are planted at a depth of 10 cm is the test most closely correlated to the vigour test (accelerated ageing test), having the highest value of |R|. The conductivity test also has a high value for |R|, but the correlation coefficient for the 10 cm depth germination test was also significant with a probability less than 0.05. The conductivity test's probability falls just outside of this range. Both tests are good indicators of seed quality and could be used as simple tests. However, apart from the fact that the 10 cm depth germination test seems to be more accurate, it is also a more practical test and more representative of what could happen in the field. Farmers can probably relate better to an emergence percentage than to a conductivity reading measured in mS/cm/g. Because it is so easy to do at home, a farmer could use it as an indication of quality and then adjust sowing density to minimise effects of poor quality seeds. Soybean plant density in South Africa should be 30 – 40 plants /m² irrespective of row width (Protein Research Trust, 1999). Because soybean seed size can vary from 13 g/100 seed to 23 g/100 seed, the amount of seed needed varies from 40 kg/ha to 75 kg/ha (Protein Research Trust, 1999). Additional seed may be needed to compensate for loss in viability and vigour. Thus by collecting emergence percentage results from the 10 cm depth germination test an indication of quality can be obtained and a compensation can be incorporated. This would also be a valuable tool if a pre-treatment had a harmful effect on seed of poor quality. As shown in Chapter 4 there may be some relationship between seed quality and molybdenum application. Should further research show this to be true, applying molybdenum to poor quality seed would decrease emergence percentages more than no application. A rapid quality test would prove very valuable in a case like this.

Conclusion

From the results presented in this chapter, it can be concluded that out of the external characteristics of soybeans observed, only the etching of seed coats seemed to reduce seed quality. This can be deduced from the conductivity test results, being a relatively good indication of quality. These results are contradictory to those in the literature, where wrinkling of seed coats was shown to reduce seed quality (Franca Neto *et al.*, 1993).

The conductivity and 10 cm depth emergence test were shown to be simple, inexpensive tests that could be performed on the farm and which give a relatively good indication of seed quality. The 10 cm depth germination test proved to be the more accurate and more practical of the two. Future work should try to "calibrate" the 10 cm depth germination test in terms of establishment in the field, in order to enable farmers to adjust sowing densities according to the test results.

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Chapter 3: The relationship between soybean seed quality and level of molybdenum application

Introduction

Molybdenum (Mo) is well known for the role it plays in nitrogen metabolism in crop plants. It is required for both nitrate reduction and biological nitrogen fixation. In nitrate reduction Mo is a constituent of nitrate reductase, the enzyme that catalyses the reduction of nitrate into nitrite (Srivastava, 1997).
Biological nitrogen fixation is carried out by the root nodule bacteria (*Rhizobium*) in leguminous crops. Mo is a constituent of the enzyme nitrogenase, which facilitates the reduction of atmospheric N_2. An absence of Mo results in poor growth and activity of root nodules in leguminous plants (Srivastava, 1997).
Apart from being an essential constituent of several important enzymes, Mo has a range of non-specific roles in plant metabolism. Mo is a constituent of sulfite oxidase, an enzyme that mediates the oxidation of sulphite to sulphate in sulphur metabolism. Mo also influences carbon assimilation and the utilisation of carbohydrates and is indirectly involved in the synthesis of plant pigments (Srivastava, 1997).

Because of molybdenum's role in nitrogen and indirect role in carbohydrate metabolism, the reproductive phase of plants is more sensitive to a Mo deficiency than the vegetative phase and seed and seedling quality is greatly affected (Srivastava, 1997).

In soybeans Mo deficiency reduces plant growth, the number of pods per plant, the number of seeds per pod, seed size, nodulation and total nitrogen and protein content of seeds (Gupta, 1997). Harris, Parker and Johnson, (1965) described Mo deficiency symptoms as "leaves twisted on stem and necrotic areas adjacent to midrib, between the veins and along the margins". Thus, the symptoms of this deficiency are typically those of nitrogen deficiency - a general yellowing of the foliage (Harris *et al.*, 1965). Mo toxicity is detrimental to *Rhizobia* and can cause seedling injury in soybeans (Gupta, 1997).

Harris *et al.*, (1965) investigated whether the amount of seed-borne Mo would influence the response of the progeny to applied Mo. It was found that progeny of all the seeds that contained less than 2.6 ppm Mo, responded to sodium molybdate applied as a seed treatment. The progeny of seed containing 22.4 ppm Mo did not respond to the applied Mo. This shows that not only the quality of the seed but also the Mo content of the seed must also be taken into account when assessing the plant's response to Mo application.

Research done in KwaZulu-Natal by Farina, Thibaud and Channon (1997) on soybeans grown at various localities, showed that soybean yield increased with molybdenum seed treatment in areas with molybdenum deficient soils and where the soil pH is low. However, at some localities and with some soybean cultivars molybdenum seed treatment caused a decrease in yield. It was postulated that seed quality could play a role in this phenomenon.

The objective of this study is to investigate the relationship between seed quality and molybdenum seed treatment under acidic and optimum conditions.

Methods and materials

The cultivar shown to produce lower yields in Natal is the cultivar Dumela (Farina, *et al.*, 1997). Thus, Dumela seed was specifically obtained to use in this project. Seed from three consecutive years of the cultivar Prima was also obtained, with the freshest seed having been harvested a month before the project commenced. The quality of the four different seed lots (Prima – Fresh (Fresh); Prima – 1 year storage (1 Yr); Prima – 2 year storage (2 Yr) and Dumela

– 1 year storage (Dumela)) was established using standard procedures (Accelerated ageing test procedure shown in Chapter 2.) The results showed that the Fresh seed was of high quality, the 1Yr was of medium to low quality and the 2 Yr and Dumela were both poor quality seed. The seed lots were analysed for Mo content in the seeds and the results showed that all the seeds contained levels of Mo higher than 2.66 ppm. (Fresh=3.54 mg/kg; 1 Yr=4.54 mg/kg; 2 Yr= 3.32 and Dumela=2.76). A full nutrient content analysis is shown in Table 3 of Chapter 2.

The four different quality seed lots were then treated with varying levels of sodium molybdate as shown in Table 1 below. The seeds were counted out and weighed and then levels of Mo were applied per kg of seed. Distilled water at the rate of 10 ml per 1 g of sodium molybdate was used to dissolve the Mo, which was then applied directly to the dry seeds (Wouters, *et al.*, 1997). In the field 1 g Mo per kg of seed is the normal application rate (Farina *et al.*, 1997).

Ten seeds per pot were then planted 2 cm deep in plastic plant pots (20 cm diameter) containing *ca.* 3 kg of acid-washed sand (Wouters, *et al.*, 1997). The sand is classified as a grey sand with 1% clay, 9% silt, 27% rough/coarse sand, 31% medium sand and 32% fine sand. The pH (KCl) is 7.1, resistance (ohms) 1550, P content is 54 ppm, K content is 64 ppm, Ca content (%) is 4.05 and Mg content (%) is 0.09. *Rhizobium* bacteria were sprinkled onto the surface of the pots and washed in after the seeds were planted. The plants were fertigated with a balanced nutrient mixture with a low (15% of normal) N content. The nutrient mixture contained 312 mg kg^{-1} K, 160 mg kg^{-1} Ca, 48 mg kg^{-1} Mg, 28 mg kg^{-1} N, 30 mg kg^{-1} P and 272 mg kg^{-1} S. Micro-nutrients were added as prescribed by Wouters *et al.* (1997) except for Mo, which was omitted. De-ionised water was used for the nutrient mixture. The plants were grown in glass houses with an average temperature that ranged from 10°C at night to 35°C during the day.

Under normal conditions, using normal tap water, a standard, balanced feeding solution usually has a pH of about 5.8. However, because deionised water was used for this investigation it resulted in the feeding solution having a pH of 3.5. This was only discovered once the seeds had already been planted. It was decided to continue with the acidic conditions to see what the reaction to Mo under these conditions would be. The seeds were allowed to germinate and establish themselves. Seedling emergence was monitored daily for 12 days and thereafter every second day for a further 12 days after which no further seedlings established. The plants were then harvested after six weeks.

The experiment was then repeated, but this time the pH of the feeding solution was adjusted to 5.9 (Protein Research Trust, 1999) by adding potassium carbonate. Again seedling emergence was monitored daily for 12 days and thereafter every second day for a further 12 days after which no further seedlings established. At this point all plants were removed from the pots except two. These two plants were allowed to grow into mature plants and produce seed.

The percentage establishment per pot was calculated for both experiments. The seed yield of each mature plant was recorded for the plants grown under normal pH conditions.

The "means" command of the SAS statistical package was used to test for significant differences between means. The establishment percentage data was logit transformed before analysis. Where a significant interaction was found the "pdiff" command of "proc glm" was used to evaluate the individual interactions of the various treatment combinations. A regression analysis was also performed on the emergence percentage data. This was done using the regression format of SAS to find values for x and y intercepts, which were then used to formulate quadratic equations that described the lines of each seed type (SAS, 1985).

Table 1: The layout of the two experiments

1. Acid Conditions	2. Optimum Conditions
•Four seed types of varying quality were used namely: ***Fresh-Prima; 1YR-Prima; 1YR-Dumela & 2YR-Prima***. (order is from good to poor quality)	•Four seed types of varying quality were used namely: ***Fresh-Prima; 1YR-Prima; 1YR-Dumela & 2YR-Prima***. (order is from good to poor quality)
•Molybdenum levels used: ***0 g ; 0.4 g ; 1 g ; 4 g & 8 g*** Mo per 1Kg seed.	•Molybdenum levels used: ***0 g ; 0.4 g ; 1 g ; 4 g ; 8 g & 12 g*** Mo per 1 Kg seed.
•**Five** repetitions of each were planted with 10 seeds in a pot.	•**Four** repetitions of each were planted with 10 seeds in a pot.
•The feeding solution given to the plants had a pH of about **3.5**.	•The feeding solution given to the plants had a pH of about **5.9**.
•The experimental layout was a **4X5** factorial with five replications.	• The experimental layout was a **4X6** factorial with four replications.

Results and discussion

There was a significant interaction (p=0.0025) between seed lot and Mo levels for the first investigation performed under acidic conditions.

Figure 1 shows a representation of the emergence percentage means of the different seed lots under acidic conditions. Seedling emergence from both the Fresh and 2 Yr seeds tended to decrease as Mo rate increased. The same trend was observed in the case of the 1 Yr and Dumela treatments, except that emergence increased as the Mo treatment was raised to 8 g kg^{-1}. For this reason it was decided to include another Mo level, namely 12 g Mo, for the second investigation where the plants were grown under optimum pH conditions.

The most noticeable factor about these trends is that in all cases the best emergence was obtained where no Mo was applied. Thus in all cases Mo treatment had no significant effect or a negative effect on emergence.

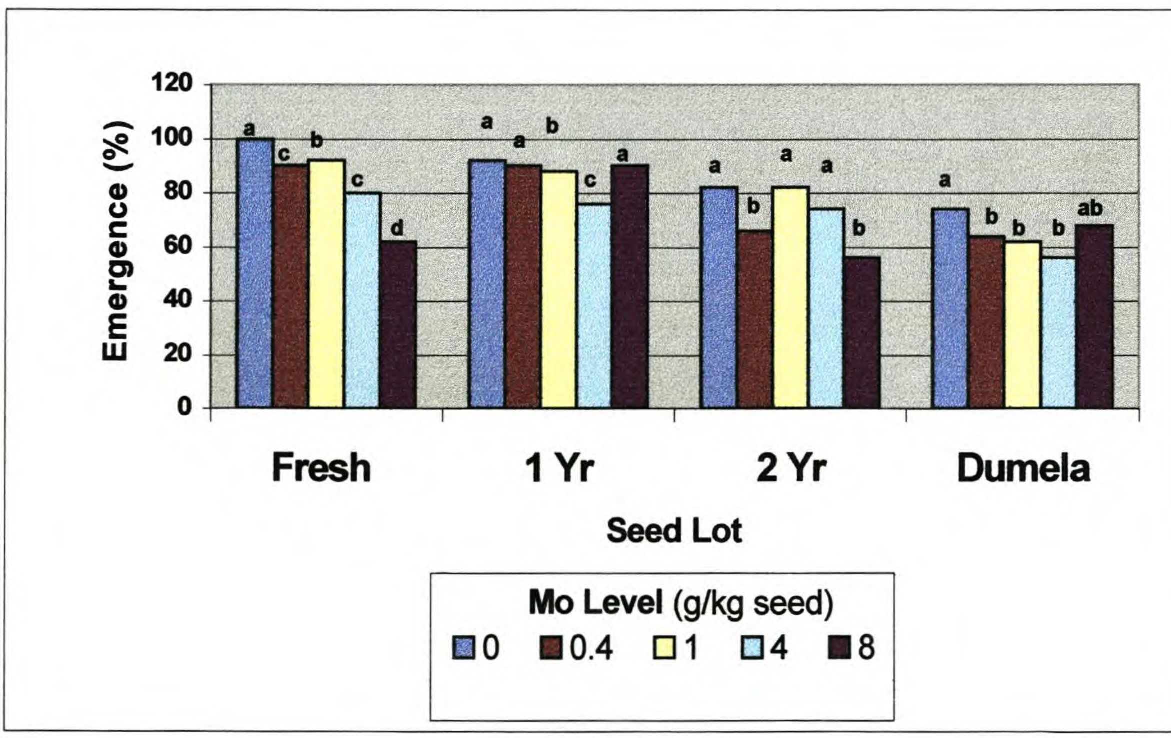

Figure 1 The effect of various levels of sodium molybdate on emergence percentage means of soybean seeds from different seed lots grown under acidic conditions.

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

There was a significant interaction (p=0.0003) between seed lot and Mo level in the second investigation performed under optimum pH conditions. Figure 2 shows the emergence percentage means of the plants grown under optimum pH conditions. The graphs indicate maintenance of a performance level for the lower concentrations of Mo (0 g; 0.4 g and 1 g). Above these levels there are sharp declines in emergence and so it is safe to assume that these higher concentrations (4 g; 8 g and 12 g) of Mo become increasingly toxic to the seeds.

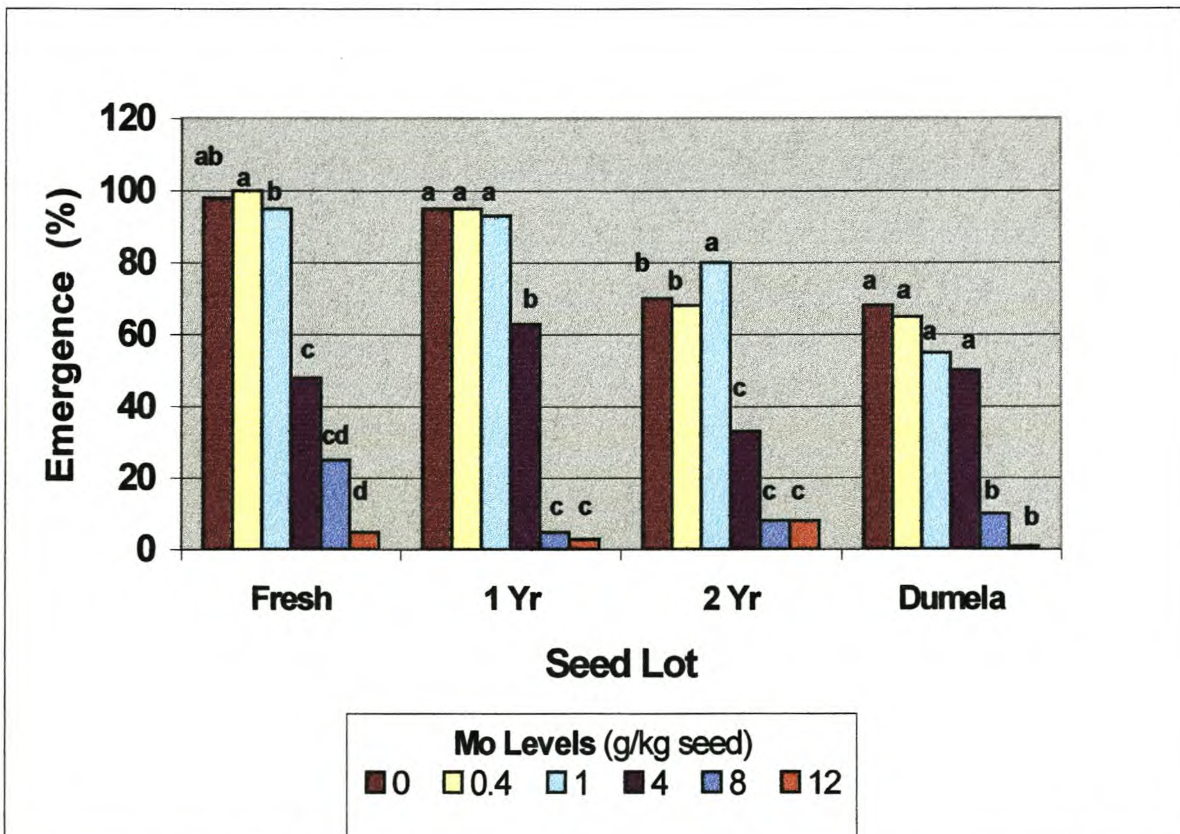

Figure 2 The effect of various levels of sodium molybdate on emergence percentage means of soybean seeds from different seed lots grown under normal pH conditions.

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

The Fresh and 1 Yr seed lots treated with low concentrations (0 g, 0.1 g and 1 g) of Mo maintained seed emergence levels. If the 2 Yr – 1 g Mo results are accurate and not due to experimental error, a positive effect of Mo is shown in this instance. However, there are no similar effects shown by the Dumela seed lot, which is also a poor quality seed lot. Dumela was the seed lot with the lowest Mo content in the seeds (2.76 mg kg^{-1}) and so according to Harris *et al.* (1965), this seed lot should have had the largest reaction to Mo application.

Figures 3 and 4 are regression graphs drawn from the emergence results of the two plantings. These graphs show clear differences in reaction trends. Under the acidic conditions the Mo application is shown as having little effect on the 1 Yr and Dumela lots because of their positive reaction to the 8g Mo application. Thus they are represented by an almost straight line where there is no significant difference between the x intercept and the final point on the line.
The regression lines under acidic conditions did not decrease as fast or to as low levels as the regression lines representing the seed lots under optimum pH conditions. The seeds planted under optimum pH conditions reacted far more to the higher concentrations of Mo. This could be explained by the fact that under acidic conditions Mo is fixed in the soil in forms unavailable to plants or soil micro-organisms (The United States Dept. of Agri, 1957). Therefore Mo may have had a smaller effect on establishment under the acidic conditions, as it did under the optimum pH conditions.

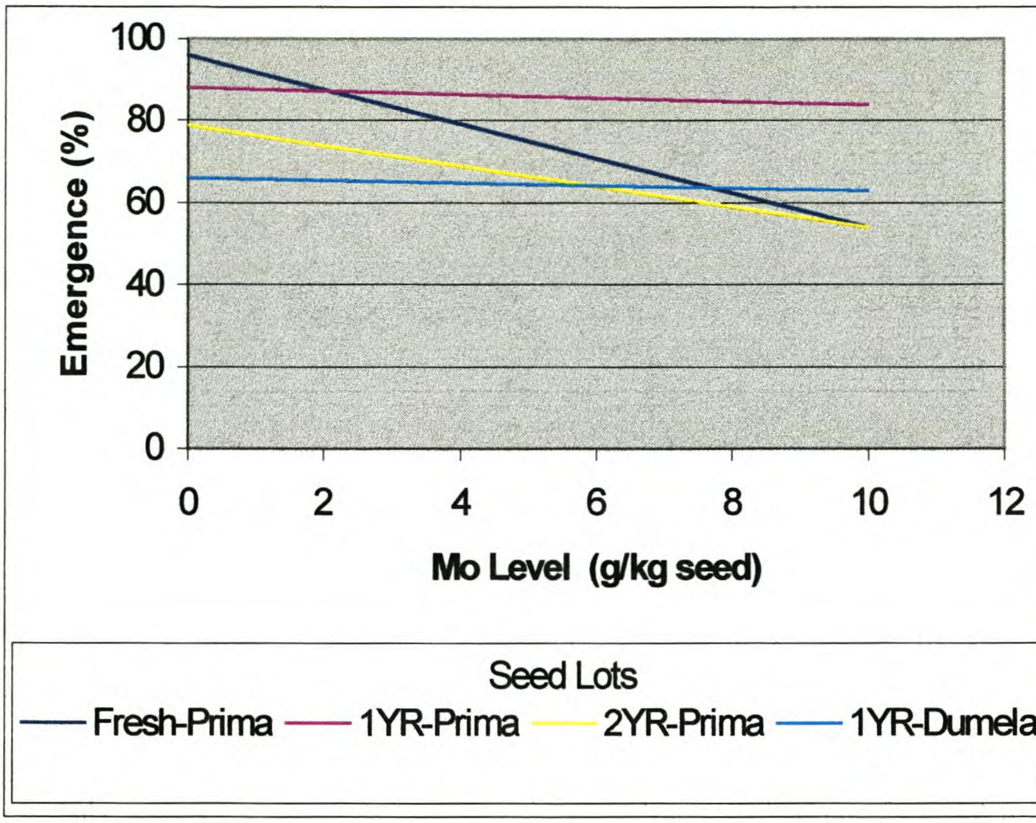

Figure 3 The regression graph of the emergence of the different lots of soybean seeds grown under acidic conditions after treatment with varying levels of sodium molybdate.

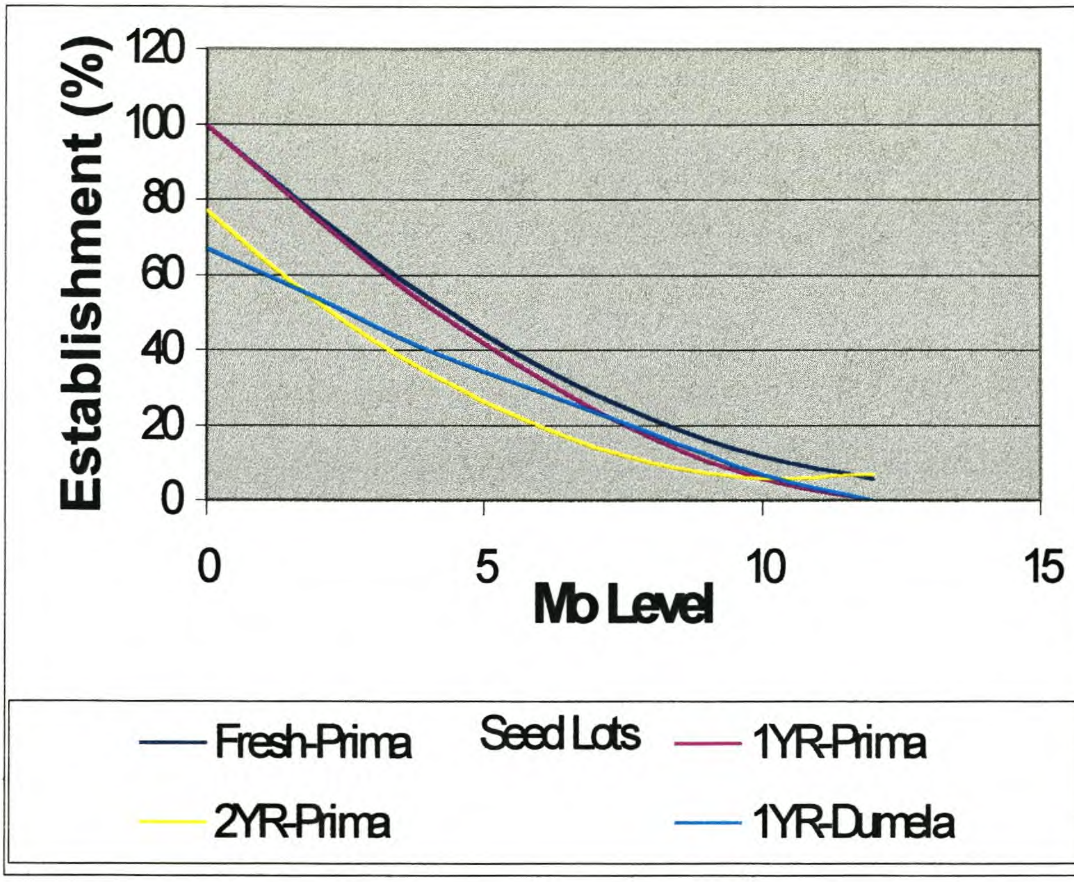

Figure 4 The regression graph of the emergence of the different lots of soybean seeds grown under normal pH conditions after treatment with varying levels of sodium molybdate.

Once the plants grown under optimum pH conditions had reached maturity and the seeds were harvested, the seeds of each plant were counted, the number of pods were counted and then the seeds were removed from the pod and the total weight of the seeds from each plant was obtained. Upon completing an ANOVA analysis on all three parameters, it was evident that all three gave similar results. So for future reference, results can be obtained by just counting the number of pods produced by each plant.

Figure 5 shows the total weight of seed obtained from the plants. There was a significant difference only between the various Mo concentrations (p=0.0001). Again a decreasing trend in total seed weight with increasing levels of Mo revealed itself, with exception of the Fresh and 2 Yr seeds where an initial increase in total seed weight occurred before a decrease with the higher, more toxic, levels of Mo. It can be stated that the higher levels of 8 g and 12 g of Mo have toxic effects on the seeds and that the positive effect of 8 g Mo that was observed in the first planting, could have been due to the acidic conditions.

The increases in total seed weight observed in the Fresh and 2 Yr seed lots with a low (0.4) Mo level, may be due to the positive effect of Mo becoming evident in the mature plants. However, then the Mo effect has little to do with seed quality because the Fresh and 2 Yr seed lots are on either end of the quality scale (See Chapter 2). Again these deviations could be accounted for by experimental error. The experimental conditions were not ideal because the seeds were planted in sandy soil and irrigation was applied overhead and so a type of leaching effect may have occurred. This may have resulted in uneven amounts of Mo leaving the pots and possibly given rise to irregular results. The fact that the negative effect of Mo manifested itself in the form of poor establishment, may also have been an influencing factor. When the pots were thinned out, only two seedlings were left to develop in each pot, regardless of whether the pot had originally had ten or only two established seedlings in it. To a large degree the two strongest seedlings would also have been left in each pot. Thus, due to both of these factors a big source of variation was removed. Therefore the yield decreases obtained here were due to poorer growth and would have been amplified by establishment decreases under field conditions.

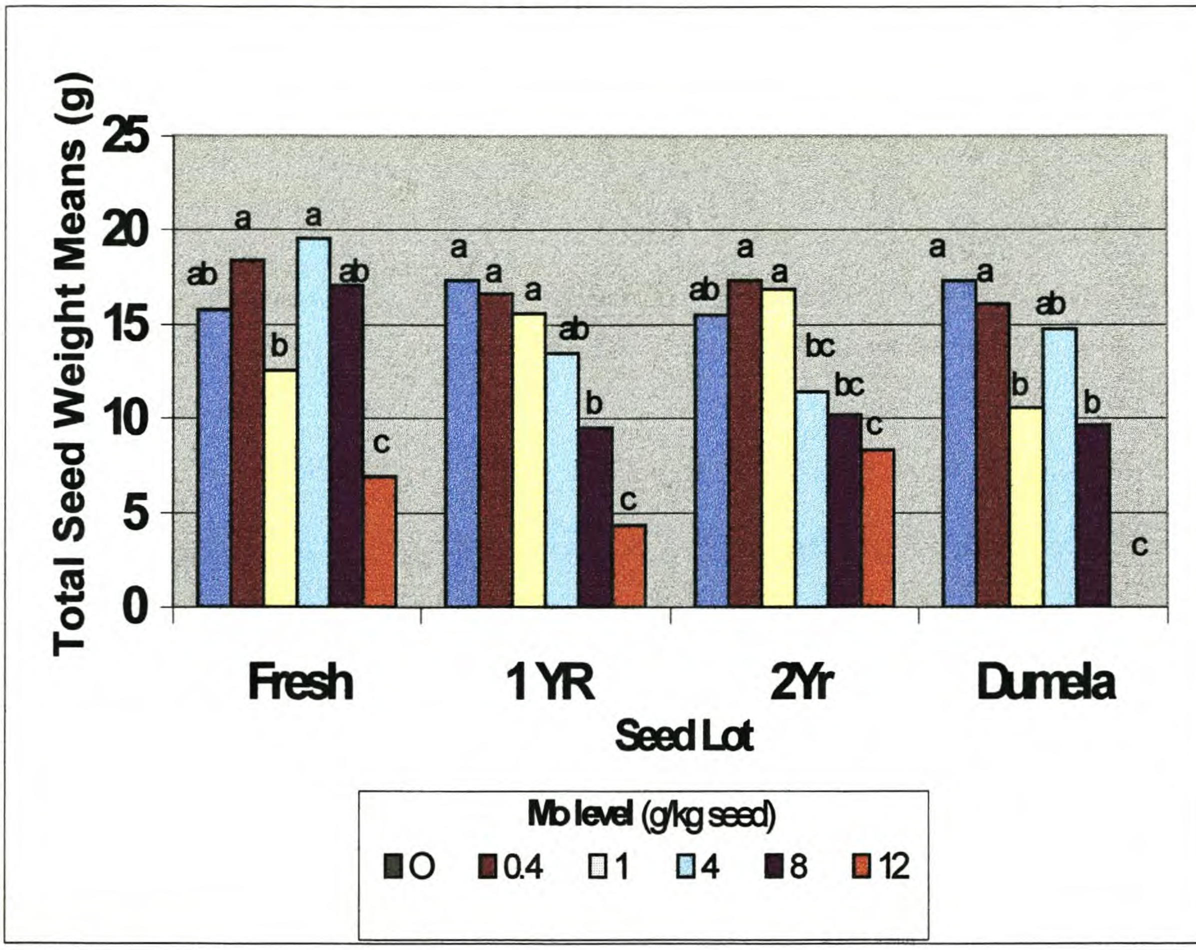

Figure 5 Total seed weight means from the yield of the soybean plants grown from different seed lots under normal pH conditions, after treatment with various levels of sodium molybdate.

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

It would be advisable if future investigations are carried out under conditions closer to field conditions. This could be done by planting the seeds in soil instead of sand and by applying water from below to the pots to obtain a more realistic water regime. The other possibility is that the poor reaction to Mo obtained in KwaZulu-Natal may be linked to soil type and conditions. So it would be advisable if further investigations could be carried out in the soil type from the area. Only 1-2 g of Mo should be used for future trials as it is clear that the higher levels of Mo are toxic to the seeds. Other methods of application should also be investigated. Perhaps a ground application can be tried after the seeds have been planted, or a foliage application on the newly emerged seedlings. It is not known exactly when the seedling starts utilising Mo. In the beginning stages of development the seedling relies very much on stored nutrients in the seed. Perhaps Mo should only be applied once a basic root system has been established to get maximum benefits. A final point that can be made is that the seeds that did not receive any Mo treatment at all should have shown Mo deficiency symptoms because the experimental environment was meant to be Mo free. Either the seed had enough Mo stored in it to provide all the Mo it required, or there was Mo still getting into the experimental system, because these were the plants that performed the best.

Conclusion

Despite the varying trends of both plantings, a constant finding was that under most situations Mo had a negative effect or little or no effect on establishment and not the positive effect that was expected. These results are possibly due to incorrect experimental conditions. It was a very artificial environment in which the experiment was carried out. The positive effects of Mo application observed in the field are possibly due to interactions between soil and Mo and the soybean plant.

Thus, at this stage it would not seem that seed quality plays a direct role in the reaction to Mo application. However, should the conditions under which the experiment is carried out be more closely correlated to field conditions, different results may be obtained.

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Chapter 4: The effect of poly-ethylene glycol pre-treatment and molybdenum application on soybean seed performance

Introduction

Soybean (*Glycine max* (L.) Merrill) seed treatment with molybdenum generally increases soybean production in low pH soils in Kwa-Zulu Natal (Farina, Thibaud & Channon, 1997). However, in certain instances soybean production was decreased after seed treatment with molybdenum (Farina *et al.*, 1997). It was thought that low seed quality played a role. Low quality seed may result in lower yields due to a lower establishment percentage as well as a lower and more erratic rate of establishment (McDonald, 1998). A seed pre-treatment procedure, called osmoconditioning involves imbibition of low quality seed in a solution with a low osmotic potential (McDonald, 1998). This treatment appears to facilitate membrane repair in the seed (McDonald, 1998) and also slows the rate of imbibition (Saha, Mandal & Basu, 1990). This results in a higher percentage and a less erratic germination of seeds (McDonald, 1998). Excellent results with osmoconditioning have been achieved in species with small seeds, notably some vegetable species (McDonald, 1998). Recent studies carried out in Brazil show very positive results being obtained from soybeans that had undergone osmoconditioning (Braccini *et al.*, 1999). It was thought that if soybeans undergo an osmoconditioning pre-treatment and membrane repair takes place, then the membranes would be less permeable to Mo, resulting in less Mo damage in poor quality seed.

In this experiment, the effect of osmoconditioning and molybdenum treatment on the emergence percentage, emergence rate and seed yield of soybean of the cultivar Prima was investigated.

Material and methods

For this investigation two lots of soybean seed were used, both of the cultivar Prima. The one was a lot of recently harvested, good quality seed, named "Fresh" and the other a lot of poor quality seed that had been in storage for 2 years, named "2 Yr". The seeds were subjected to a pre-treatment of which the osmoconditioning agent was poly-ethylene glycol (PEG 6000). Four levels of PEG (0, 179, 224 and 262 g L^{-1}) were used. This corresponds to osmotic pressures of 0, -0.4, -0.6 and –0.8 bar (Armstrong & McDonald, 1992). Distilled water was used in all cases. Seeds (300) were imbibed in 200 ml of the various solutions at 25 °C for 24 hours. The PEG treatments were aerated for the duration of the pre-treatment. After 24 hours the seed was rinsed thoroughly in distilled water and subjected to a treatment of four sodium molybdate concentrations *viz*. 0, 1, 4 and 8 mg Mo kg^{-1} seed (Wouters *et al.*, 1997).

Ten seeds per pot were planted 2 cm deep in plastic plant pots (20 cm diameter) containing *ca*. 3 kg of acid-washed sand (Wouters *et al.*, 1997).). The sand is classified as a grey sand with 1% clay, 9% silt, 27% rough/coarse sand, 31% medium sand and 32% fine sand. The pH (KCl) is 7.1, resistance (ohms) 1550, P content is 54 ppm, K content is 64 ppm, Ca content (%) is 4.05 and Mg content (%) is 0.09. *Rhizobium* bacteria were sprinkled onto the surface of the pots and washed in. The plants were fertigated with a balanced nutrient mixture (Wouters *et al.*, 1997) with a low (15% of normal) N content. The nutrient mixture contained 312 mg kg^{-1} K, 160 mg kg^{-1} Ca, 48 mg kg^{-1} Mg, 28 mg kg^{-1} N, 30 mg kg^{-1} P and 272 mg kg^{-1} S. Micro-nutrients were added as prescribed by Wouters *et al.* (1997) except for Mo, which was omitted. De-ionised water was used for the nutrient mixture. The experimental layout was a 2X4X4 factorial with three replications. Seedling emergence was monitored daily for 12 days and thereafter every second day for a further 12 days after which no further seedlings established. At this point all plants were removed from the pots except two. These two plants were allowed to grow into mature plants and produce seed.

The percentage establishment was calculated. The number of days it took for 50% emergence to occur was calculated as an indication of rate of emergence and the seed yield of each plant was recorded. The "means" command of the SAS statistical package was used to test for significant differences between means. The establishment percentage data was logit transformed before analysis. Where a significant interaction was found the "pdiff" command was used to evaluate the individual interactions of the various treatment combinations (SAS, 1985).

Results and discussion

The interaction between PEG pre-treatment and the molybdenum concentration for seedling emergence was significant (p=0.0149) and the individual interactions showed that:

PEG 1 Mo 0

PEG 1 Mo 1

PEG 2 Mo 0

PEG 2 Mo 1

PEG 3 Mo 0

for the combined results of the seed lots were all not significantly different from each other. The interaction between PEG pre-treatment, molybdenum concentration and seed lot was not significant.

Even though the "Fresh" seed was already of high quality, a positive effect is still seen from the PEG pre-treatment (Figure 1). In all instances the 1 g of Mo applied improved, or at least maintained the same emergence percentage as the untreated seed. So it would seem that seed of higher quality reacts well to a light application of Mo. It is clear that Mo treatment of 4 g and 8 g per kg of seed is toxic to the seeds and has detrimental effects on emergence. The PEG treated seed appears to be more "resistant" to the 4 g Mo application but not the 8 g.

The positive effect of PEG pre-treatment on soybean seeds of poor quality is clearly shown in Figure 2. Thus, it can be said that a pre-treatment with PEG can greatly improve the emergence ability of soybean seed of poor quality. These results correlate with results obtained by Braccini *et al.* (1999). They showed that osmoconditioning with PEG 6000 improved seed germination and vigour compared to untreated controls. They also subjected the osmoconditioned seed to accelerated ageing and found that the seed maintained its quality during accelerated ageing. Similar results were obtained by Park *et al.* (1999) where priming aged seeds improved germination and also resulted in good stand establishment in field trials.

It would seem that Mo application, even at an application rate of 1 g, has a detrimental effect on emergence of poor quality seed. Even though membrane repair may have taken place resulting in a better emergence ability, the seed still remain sensitive to Mo. Their sensitivity is lessened by the PEG pre-treatment, but not to the degree where they react more positively to Mo application. So if the osmoconditioning is meant to facilitate membrane repair then it is not only the state of the membranes that make a seed sensitive to Mo.

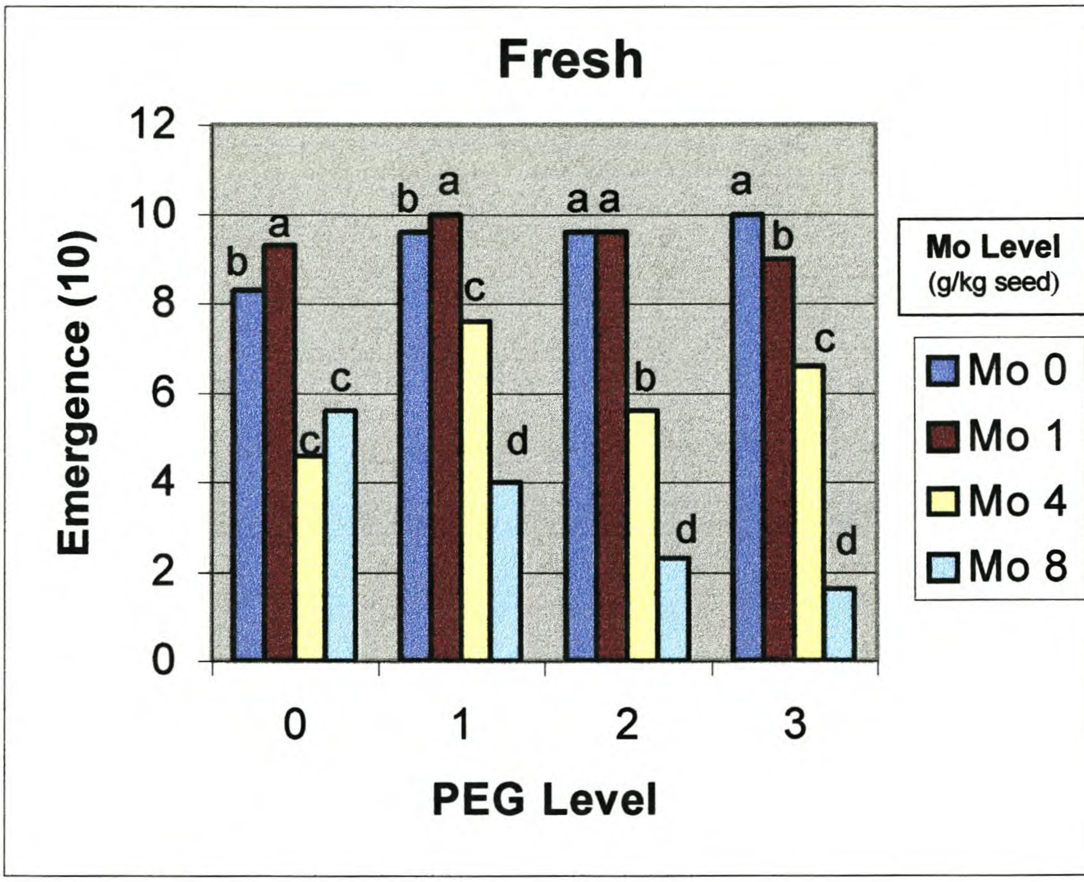

Figure 1 The emergence means of the "Fresh" soybean seed lot under the various PEG pre-treatments and Mo treatments (Mo 0 = 0 g $MoKg^{-1}$; Mo 1 = 1 g $MoKg^{-1}$; Mo 4 = 4 g $MoKg^{-1}$; Mo 8 = 8 g $MoKg^{-1}$).

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

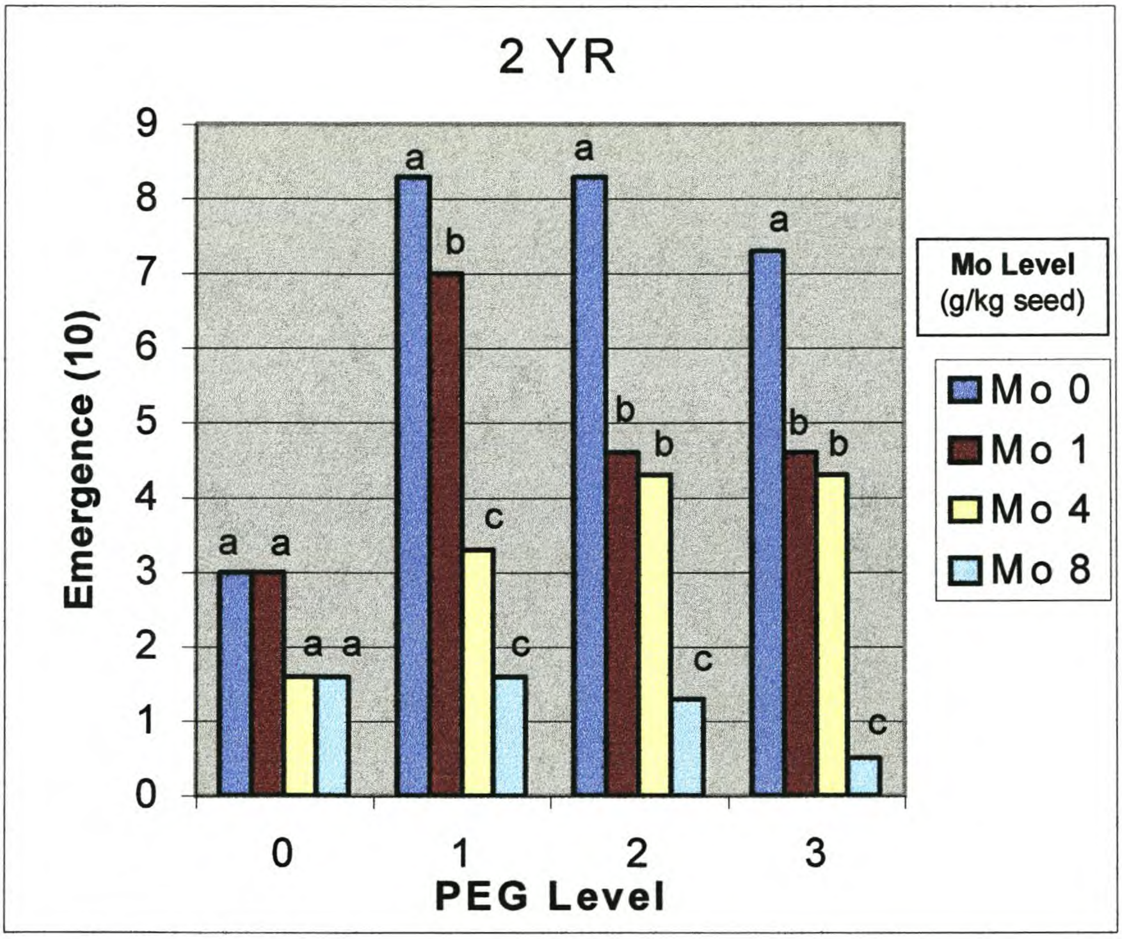

Figure 2 The emergence means of the "2 Yr" soybean seed lot under the various PEG pre-treatments and Mo treatments (Mo 0 = 0 g $MoKg^{-1}$; Mo 1 = 1 g $MoKg^{-1}$; Mo 4 = 4 g $MoKg^{-1}$; Mo 8 = 8 g $MoKg^{-1}$).

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

There seemed to be little effect on the germination rate of the "Fresh" seed from the PEG pre-treatment except that it slowed down the rate of germination of the seed that had been treated with 8 g of Mo (Figure 3). The molybdenum application showed the same results as have been seen with the germination percentage where 0 g and 1 g give similar results and 4 g and 8 g give progressively worse results.

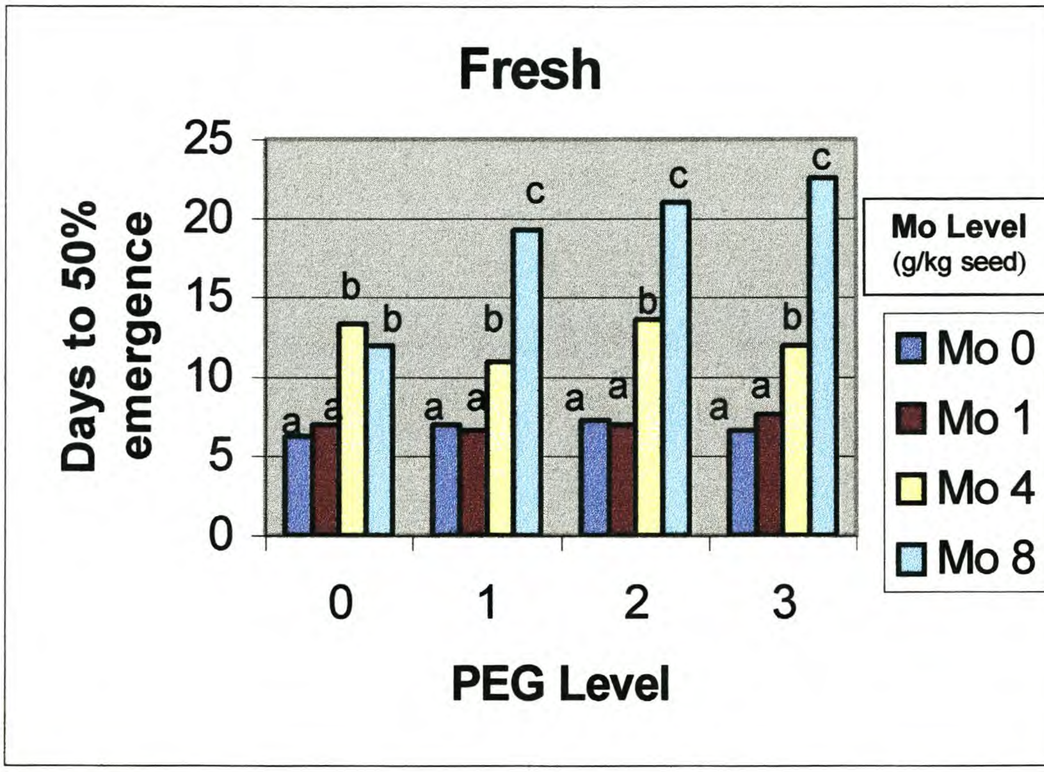

Figure 3 The rate of germination of the soybean seeds from the "Fresh" seed lot represented as the number of days it took for 50% of the seeds to germinate (Mo 0 = 0 g $MoKg^{-1}$; Mo 1 = 1 g $MoKg^{-1}$; Mo 4 = 4 g $MoKg^{-1}$; Mo 8 = 8 g $MoKg^{-1}$).

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

PEG pre-treatment had a large effect on increasing the germination rate of the poor quality seed (2 Yr) under the lower concentrations of molybdenum. Where there was no PEG pre-treatment the germination was slow at all levels of molybdenum (Figure 4).

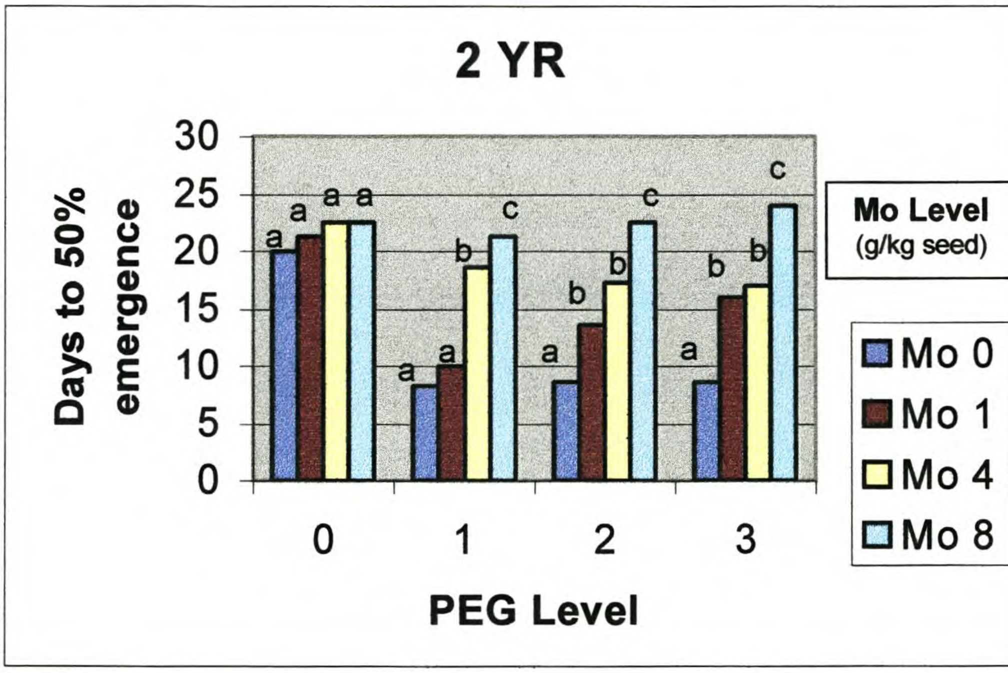

Figure 4 The rate of germination of the soybean seeds from the "2 Yr" seed lot represented as the number of days it took for 50% of the seeds to germinate (Mo 0 = 0 g MoKg-^{1}; Mo 1 = 1 g MoKg-^{1}; Mo 4 = 4 g MoKg-^{1}; Mo 8 = 8 g MoKg-^{1}).

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

Looking at the total weight of seed harvested from the plants under the different treatments it is clear that there was little effect from the individual treatments on the two seed lots (Figure 5 and 6). This could be due to the fact that the seeds were planted in sand and they were irrigated from an overhead system which may all have resulted in a type of leaching effect, removing excess Mo in the soil. Thus the individual effects of the different Mo applications may have been lost. The fact that the negative effect of Mo manifested itself in the form of poor establishment, may also have been an influencing factor. When the pots were thinned out only two seedlings were left to develop in each pot, regardless of whether the pot had originally had ten or only two established seedlings in it. To a large degree the two strongest seedlings would also have been left in each pot. Thus, due to both of these factors a large source of variation was removed.

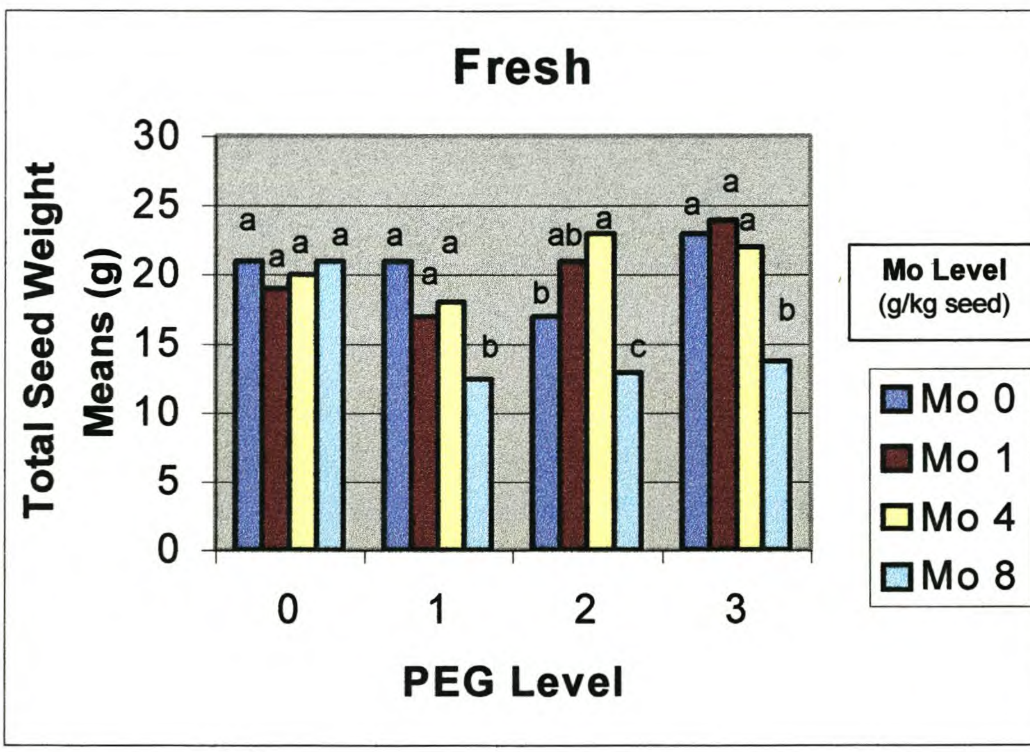

Figure 5 The total weight of soybean seed harvested from the "Fresh" seed lot plants.

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

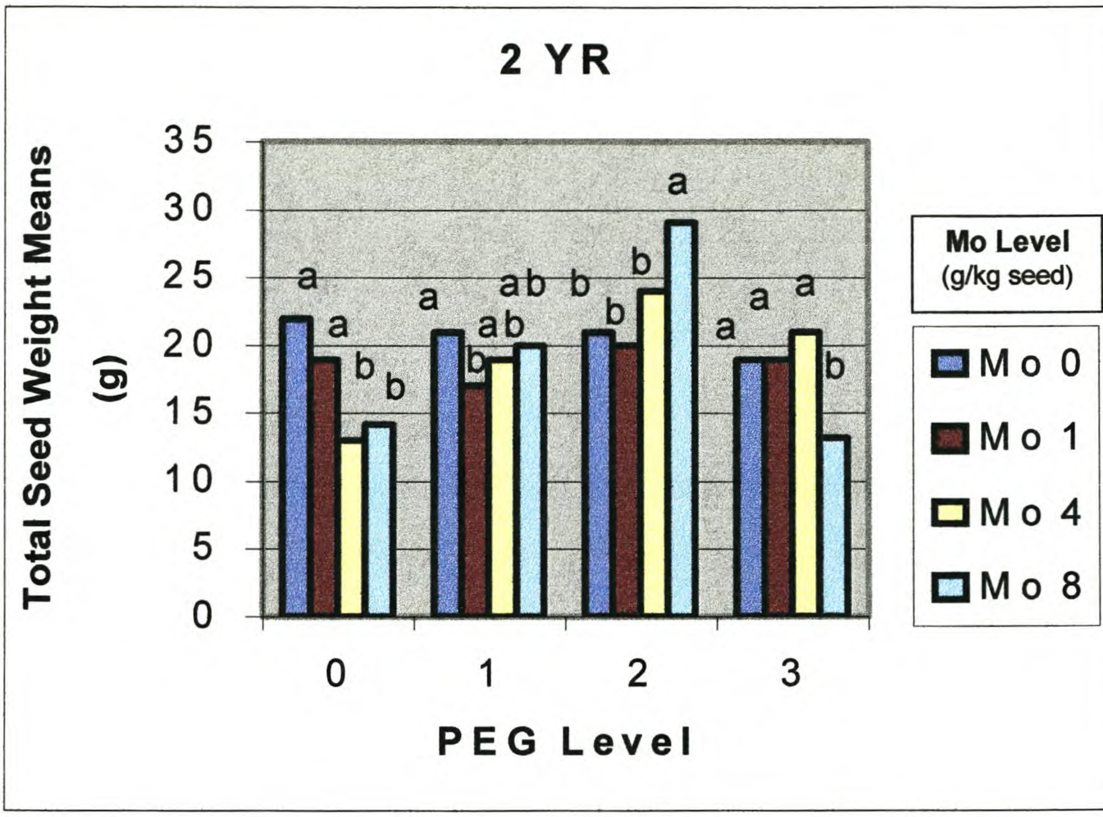

Figure 6 The total weight of the soybean seeds harvested from the "2 Yr" seed lot plants.

*Letters on the graph represent significant differences at the 5% level between the various levels of Mo within each seed lot.

Conclusion

There appears to be a positive effect of a low concentration of molybdenum being applied to healthy seed of high quality. This must be the effect experienced from molybdenum application in the field, but which was not found in Chapter 3. Where poor quality seed is involved the application of molybdenum has detrimental effects on emergence. The emergence of the seeds is greatly improved by the PEG pre-treatment. The prevention of further damage by slower imbibition and any membrane repair mechanisms that takes place during osmoconditioning helps improve emergence. But these mechanisms are not the only factors that allow a seed to utilise molybdenum effectively for the combination of their effects could not prevent the poor quality seed from remaining sensitive to the molybdenum. However, it did help improve the seeds resistance to it. It can therefor be said that there is a relationship between seed quality and damage caused by molybdenum but further research is required to clarify this.

The most exciting aspect of this research is the positive effect of PEG pre-treatments on the performance of soybean seed. These results warrants further research on the subject of osmosconditioning of soybean seeds.

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Chapter 5: Conclusions

Unfortunately, the results expected from the molybdenum investigation were not forthcoming. The most unexpected result is that the molybdenum applications did not have the positive effect on the soybeans, as was reported in the field studies. The reasons for this may be connected to ineffectual experimental conditions under which the project was carried out. However, there were indications that the better quality seed reacted more positively to molybdenum application and so the possibility that there is a relationship between seed quality and molybdenum application can not be entirely ruled out. For further investigations on this subject, the positive effect of molybdenum seed treatment must first be expressed before seed quality can be brought into the investigation as a parameter.

The PEG pre-treatment of soybeans shows promise for further investigations. The positive effects observed in this project correlates with findings in the literature.

The effects of external characteristics on seed quality remains an illusive subject as the results obtained in this project contradict, once again, results of previous literature.

The most positive finding of this investigation is the result that the 10 cm depth germination test may prove to be a reliable simple method of testing seed quality. Should further investigations reconfirm this, it may prove to be a very useful tool to use in practice. Further investigations should look at calibrating the results of the test to adjust sowing densities for low quality seed. Should further investigations show that reactions to molybdenum seed treatment is related to seed quality then a calibration system could be found where molybdenum must be regulated according to the quality of the seed.