THE ROLE OF SEED COATING IN THE ESTABLISHMENT AND GROWTH OF *MEDICAGO SATIVA L.* CULTIVARS

by

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DECLARATION

I, Leana Nel declare that the thesis/ dissertation, which I hereby submit for the degree MSc (Agric) Pasture Science at the University of Pretoria, is my own work and has not previously been submitted by me for a degree at this or any other tertiary institution.

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L. Nel

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ABSTRACT

The role of seed coating in the establishment and growth of Medicago sativa L. cultivars

by

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The use of coated seed to establish crops is not a novel practice. Seed coatings have been used on small seeded crops to improve the handling ability by making the seed unit larger and heavier. Producers can therefore calibrate their sowing equipment more efficiently and wind will not cause as much drift at sowing. Seed coating can have an added benefit for leguminous crops if the symbiotic inoculant (*Rhizobium*) is added to the coating. This saves the producer time and allows peace of mind that inoculation was done by trained professionals.

Other than the inoculation, there are some other constituents in the seed coating that can have benefits to the plant. It can, however, be theorized that added nutrients or pesticides will be beneficial to the plants only if these nutrients are deficient in the growth medium or when pests are present. This study evaluated the effect of seed coating on the life stages of germination, emergence and survival, seedling growth and ultimate yield of mature lucerne (*Medicago sativa* L.) plants, comparing the results with non-coated seed. Two cultivars were used in the evaluation, SA Standard and SuperCuf, to determine if the effects would be similar, or would genetic differences between the cultivar play a significant role. These life stages (germination, emergence and seedling growth) were chosen due to the importance of these stages to the success of establishment. Fast and uniform

germination will result in a uniform stand with strong competition against weed infestation. The effect of growth medium on the emergence of seedlings and the interaction between the seed coating and the growth medium was important to determine to identify limitations in the use of seed coating. Changes in the growth of seedlings in terms of some physio-morphological characteristics will assist in identifying parameters influenced by the coating. It was, however, essential to not only do these trials under ideal agricultural conditions, but to identify if similar results would be obtained from stressed conditions, such as salinity, which is a growing concern for crop production areas. The question of whether seed coating will influence the ultimate production of the crop could then be answered.

It was found that the method in which germination is tested can have a significant outcome for the results obtained. When the Jacobsen apparatus was compared with the use of petri dishes, using specification according to ISTA, it was found that the water movement in the Jacobsen apparatus overcomes concentrated nutrient conditions, especially for SuperCuf. Under saline conditions the coated SA Standard seed had higher germination than the non-coated seed, therefore overcoming inhibitions imposed by the salinity. It is clear that the coating influences germination of lucerne and the interaction with the seed environment is significant. It is also clear that the genetic differences between cultivars are significant and results should not be applied across all lucerne cultivars.

When the emergence percentage had been determined in different growth media, namely a commercial growth media, a sandy loam soil and silica medium, it was found that the emergence was influenced by the media. Even though the emergence of seedlings are mostly determined by the nutrients in the cotyledons, some growing conditions did cause lower emergence for non-coated SA Standard seeds and was overcome by the use of coated seed. When the growing conditions were manipulated with saline irrigation it was found that coated SuperCuf had a higher emergence % than the non-coated treatments when irrigated with the 750 $\mu S.cm^{-1}$ water. From the data collected from this trial, it can be concluded that, even though the use of seed coating does not always influence the emergence of lucerne seedlings, seed coating does have an influence on the emergence, but it is dependent on the growth medium quality in terms of nutrient composition and salinity. Similar to the germination trial, the genetic influence of the different cultivars was noticeable.

To determine the differences caused by seed coating to the physio-morphological characteristics (stem height, leaf area etc.) of lucerne, a pot trial was conducted using different irrigation treatments, municipal water (180 µS.cm^{-1}), 500 and 750 µS.cm^{-1} water, created with NaCl. It was found that the physio-morphological characteristics were highly correlated, i.e. the stem height, leaf area, number of leaves and dry matter production changed in relation to each other. There were, however, differences in this correlation coated and non-coated seed treatments, when irrigated with 500 µS.cm^{-1} water. For the seedlings grown from coated seed, the correlation between shoot dry matter yield and the other parameters were low, while the seedlings growth from non-coated seed, leaf area was not correlated with the other parameters. It was concluded that the tolerance mechanism for salinity for plants irrigated with 500 µS.cm^{-1} water, caused more differences than the other water treatments.

Taking into consideration that coating influences the germination and emergence of lucerne and that the two cultivars react differently to the seed coating, the yields obtained from field trials could then be interpreted if differences were observed. Three field trials were established, namely a trial established in autumn (established in 2009) and second trial established in spring (established in 2010) which were sown at 25 kg.ha^{-1}, while a third trial established spring (established in 2010) was sown at 5 different sowing densities, namely 80%, 90%, 100%, 110% and 120% of recommended sowing density (25 kg.ha^{-1}). It was found that the pasture stands established with SA Standard, did not show many differences between the coated and non-coated seed treatments and were mostly restricted to the second growing season, where the non-coated seed treatments had significantly higher dry matter yield than the coated seed treatments. Stands established with SuperCuf, displayed more variation between the seed treatments and the non-coated seed treatments had higher yields in the first season. It was, however, found that the stands established with coated SuperCuf seed had lower stem: leaf ratio’s, indicating that a better quality fodder can be produced from coated seed. The data from the sowing density trial showed very little difference between the yields of the stands established with coated and non-coated SA Standard seed. Differences were, however, observed between seed treatments at 90% and 120% of the recommended sowing density, where the stands established with non-coated seed had higher yields than the stands established with coated seed. It can be concluded that under these trial conditions, the use of coated seed had very little influence on the yield of the lucerne stands. The observed differences suggest that the lucerne growth under these few conditions, the stands established from non-coated seed had better yield, but the stands established from coated seed had better quality. It is, however, more likely that there will be no differences between the seed treatments. Data from the sowing density trial also led to the conclusion that stands established at 20% less seed will not result in lower yields if the stand establishment is successful. The similarity between the seed treatments and the sowing densities suggests that the number of plants per area were the same, caused by seedling mortality during the high growth rate in the early growing stage, or the morphological characteristics, such as number of stems per plant and number of leaves per stem, adapted to result in similar yields and quality.

Chapter 1

Literature Review

The role of seed coating in the establishment and production of *Medicago sativa* L.

L. Nel, W. F. Truter, A. K. J. Surridge-Talbot, N. J. Taylor

Background

Medicago sativa L. (lucerne also internationally known as alfalfa) is a deep-rooted perennial legume that has been under investigation for many years as a forage species. Its value and popularity stem from its suitability as a forage species for both monogastric and ruminant animals, due to the high crude protein content (18-20% CP), good amino acid profile, high digestibility and palatability (Meyer 1999, Van Oudshoorn et al. 2001). Depending on the establishment and management of the stand, the quality of the forage, in terms of crude protein (6.25 X N), fibre and digestibility, are variable (Hoy et al. 2002, Lattimore 2008). However, lucerne's value is not only in its application as an animal forage, it also has important value in soil conditioning and protection, as well as the capacity to lower production costs of other crops. These attributes are mostly due to the nature of growth and physiology of lucerne.

A fascinating description of the root system of lucerne is presented by Coburn (1912) in *The Book of Alfalfa*. The tap root system has been known to penetrate as deep as 39 meters. This extreme depth of the root system is an indication of the lengths the plant can go to, to obtain water, making it drought resistant and suitable to reduce dryland salinity (Coburn 1912, Humphries and Auricht 2001, Marshall et al. 2008, Pannell and Ewing 2006). There are also large quantities of fibrous roots that form while the older ones decay, increasing the amount of humus in the soil and creating openings for air, water and fertilizers to penetrate. The contribution of humus to the soil is not the only contribution to soil health. Nitrogen fixation is an important attribute, as it not only provides N to the plant, but also to the soil (Peoples et al. 2002, Tubb 1976, Vance et al. 1979).

As early as 1887, the concept of nitrogen fixation and the relationship between the bacteria, generally called Rhizobia, and legumes was identified (Catroux et al. 2001, Peoples et al. 2002). It also became apparent that some legume species need to be inoculated when established in areas where they have not been cultivated previously, as in the case of Australia where commercially available legumes were all introduced (Deaker 2004). The symbiotic relationship is species specific, although ‘cross inoculation groups’ exist. Lucerne has a specific relationship with *Sinorhizobium meliloti* (previously known as *Rhizobium meliloti*) but this micro-organism also inoculates other *Medicago* species, *Melilotus* species and *Trigonella foenum graecum* (Hartmann et al. 1998, Somasegaran and Hoben 1985). In the mid 1990’s, approximately 250 million hectares of legumes were grown worldwide. According to Kinzig, as cited by Xavier et al. (2004), this resulted in about 9 x 10^{10} t of dinitrogen (N_2) fixed per year. The full potential of this relationship has, however, not yet been met, as poor inoculation is still causing establishment failure in many parts of the world (Deaker 2004). Importantly, nitrogen fixation is not only valuable for legume production, as legumes are often planted with other crops, since nitrogen fixed by the legume is not used entirely by the legume itself and is released and made available for other plants to use (Ledgard and Steele 1992).

In cropping regions of Australia, lucerne is incorporated into systems to reduce groundwater recharge and improve the sustainability of grain production by preventing dryland salinity (Humphries and Auricht 2001). Australia is, however, not the only country afflicted by dryland salinity. According to Pannell and Ewing (2006) some states of the United States of America, and other regions of Canada, Thailand, Turkey, India, Argentina and South Africa have problems with dryland salinity. Dryland salinity is not the most common cause of salinity, as salinity is most commonly associated with irrigation (Brady and Weil 2002, Pannell and Ewing 2006, Rietz and Haynes 2003). Prevention or minimisation of dryland salinity is based upon creating the largest possible soil water deficit before the rainy season (Anon, 1993, cited by Crawford and Macfarlane (1995)). Since lucerne has a high evapotranspiration rate and has access to deep underlying soil water due to its deep root system, it is a more highly suitable species for creating a larger soil water deficit than most other crops (Scott & Sudmeyer, 1993, cited by Crawford and Macfarlane (1995)). Even though this is not the most common attribute of lucerne, it is a valuable application in the conservation of soils.

As with most plants, the environmental conditions most suited for lucerne production, stem from its origins. However, due to the long history of lucerne and the plant breeding advances that have been made, the genotype of the plant can be selected to suit the environment. The end result of this development is that there are only a few selected areas where lucerne production is not suitable. Characteristics considered when selecting an area for lucerne production are water availability, soil pH, soil depth and clay content (Van Oudshoorn et al. 2001). Ideal conditions described by Van Oudshoorn et al. (2001) are a soil pH range of 6.7 to 6.9, a soil depth of at least 1.2 m, clay content less than 35%, with enough water from either precipitation or other water resources suitable for irrigation purposes.

Table 1 depicts the contribution of some countries to global lucerne production. It is clear that lucerne production is one of the major production systems in countries with moderate climatic conditions, while countries like South Africa and Australia generally have a dry climatic condition and production is limited by water availability. According to Table 2, the production of lucerne varies somewhat under different water availabilities. The nutritional requirements, however, differ between the water availabilities, mostly due to the high yield potential and growth rate associated with the availability of water, but also due to nutrient leaching from the soil.

Table 1 Countries where lucerne production is extensively practiced, comparing the area of land cultivated with lucerne, the area of cultivated land in the country and the percentage of this cultivated land that is occupied by lucerne, listed in ranking order (Anonymous 2009b, Van Oudshoorn et al. 2001)

Countries	Area cultivated with lucerne per country (km^2)	Area of cultivated land per country (km^2)	% of cultivated land in the country planted with lucerne
Argentina	75000	284342	26.4
France	5660	22715	24.9
Italy	13000	104377	12.5
Bulgaria	3990	35199	11.3
Hungary	3375	47684	7.1
USA	105990	1669302	6.4
Canada	25443	474681	5.4
Greece	1980	37985	5.2
Romania	4000	95384	4.2
New Zealand	1012	33395	3.0
Russia	33750	1192300	2.8
Poland	2580	125590	2.1
South Africa	3000	157246	1.9
Spain	3326	184981	1.8
Germany	1900	117793	1.6
Mexico	2450	268072	0.9
China	9600	1504350	0.6
Australia	1155	471550	0.2

Table 2 The production potential of lucerne (*Medicago sativa L.*) under different water quantity scenarios and the requirements of phosphorus and potassium under these conditions (Anonymous 2009a)

Rainfall (mm annum^{-1})	**Production** (kg dry matter (DM) ha^{-1})	**Optimum P (Bray 1) requirements** (mg kg^{-1})	**Optimum K requirements** (mg kg^{-1})
600	6000	20	150
800	10000	20	150
Irrigation	15000-25000	30	150

In Fig. 1, the production areas of lucerne in South Africa are shown. The different colours show the different production intensities, i.e. the areas where lucerne is produced intensively, extensively and the areas where lucerne can potentially be cultivated extensively if adapted cultivars can be utilized. The intensive production areas are also associated with river systems, like the Orange River and Vaal River and dams, e.g. the Bloemhof Dam where irrigation schemes are possible. There is, however, high potential areas in KwaZulu-Natal where lucerne is not produced due to the low pH of the soil and the high incidence of leaf diseases (Van Oudshoorn et al. 2001). Temperature is also an important factor facilitating plant growth and is shown in Table 3. Higher temperatures are associated with higher metabolic rates and subsequent faster growth and higher yield potential.

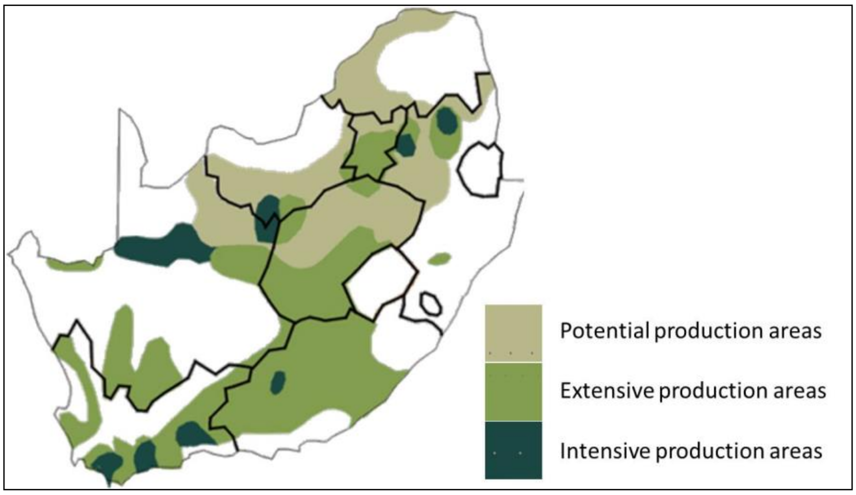

Fig. 1 The areas where lucerne is planted in South Africa and the intensity of lucerne production as represented by the different colours. (Van Oudshoorn et al. 2001).

Table 3 The differences in yield potential of lucerne in different areas in South Africa compared with the average maximum temperature in January (Anonymous 2009a)

Area	Average maximum January Temperature (ºC)	Yield potential (DM ha^{-1})
Bloemfontein	30	20
Wepener	29	17
Germiston	26	12
Kimberley	33	25

As in most commercial plant production systems, the profit margin is the main focus of the producer and research done on these plants is usually focused on lowering the cost of production or increasing the value, in terms of production or yield, of the product per area. The cost of producing lucerne pastures is ever increasing due to poor plant establishment and high fertilization cost. The success of establishment is influenced by the environment in which the plant is seeded, competition (Fawcett and Harvey 1978, Rice 1979), poor inoculation (Catroux et al. 2001, Evans et al. 1980) and poor management practices. These factors can be managed by ameliorating the soil and/ or using the seed as a vector for amelioration. Management practices, like fertilizer applications, irrigation and pest control, have improved the establishment and yield of lucerne (Van Oudshoorn et al. 2001). The yield increases must, however, justify the input costs. Principles to increase the profit margin of legume production also include inoculant technologies, growth regulators and bio-protectants. Technological development has allowed for even greater improvements and savings by adding the above mentioned components to the seed (Van Oudshoorn et al. 2001).

Seed coating technology is thus a new science involving the collaborative inputs from many pesticide, chemical and seed companies. Each company usually has their own seed coating technology. These technologies are comprised of various constituents, which may be introduced onto the seed or they may be applied individually as needed. Coatings can include polymer technology, microbial inoculation, growth regulators, systemic and contact pesticide treatment and micro and macro nutrient applications. Each constituent has an important function and can contribute to fewer establishment problems, or to overcome harsh environmental conditions causing poor establishment of crops. These coatings can be specific to production areas, which are associated with specific production problems. An example of this would be where the early planting of maize in the United States would allow a sufficiently dry seedbed, but would expose the young seedlings to late spring frost, various pests and diseases and would result in variable emergence in the stand (Vyn and Murua 2001). These researchers have investigated polymer coatings which regulate imbibition by seeds by changing the polymer’s structure from an impermeable crystalline phase to a permeable amorphous phase, when the temperature is adequate for emergence and survival. The sowing method, availability of water and other environmental factors will influence the success of the seed coating, but the coating may assist the genetic ability of the seed to overcome some environmental conditions.

Problem statement

The cost of producing lucerne pastures is high due to poor establishment, high fertilization costs and degraded soil properties due to poor agricultural practices. The success of establishment is influenced by the seed environment, competition, poor inoculation and poor management practices. Management practices have improved the establishment and yield of lucerne. If less money can be spent on fertilization to ameliorate the soil, while not compromising yield, production costs will be lower and profit margins will increase. The coating of seed with what is needed by the seed and seedling is the basis of this theory. Coated seed could be more expensive than normal seed, but a comparison between the costs of the two practices should reveal the significance of coated seed.

Introduction

The decision to produce lucerne in a specific area requires some information to illuminate the advantages and disadvantages of the chosen production system. With the requirements of the production system and the availability of new technologies, a well informed decision can be formulated to suite the producer. The availability of coated seed can influence the production of lucerne as the equipment used for establishment can easily be calibrated with the larger heavier particles. The constituents in the seed coating also have some theoretical advantages, such as pre-inoculation with rhizobia and the supplementation of nutrients. It is logical to expect healthier plants when there are deficiencies in the growing environment, but there is a question about whether there will be differences in plant development when deficiencies are induced. Saline irrigation water introduce significant amount of ions into the growth medium and can influence the uptake of nutrients in the medium (Bewley and Black 1994, Hadas 2004, Halmer 2004). Knowing the quality of the environment where the seed needs to germinate and develop into a productive plant, is essential in developing an appropriate and productive unit.

Seed coating treatments

The various constituents of seed coatings have been adapted from cultivation practises which have been applied over many years. Brockwell (1962) describes adding lime and other organic material to the seed to aid in inoculant survival and nodulation success, or the addition of agro-chemical applications or nutrient applications to aid in the survival of the seed and seedling. Seed treatments have the advantage that the components are at seed level, making the placement ideal for retrieval by roots. Combining nutrients, inoculants and pesticides on the seed, has the further advantage that due to the low quantity being applied, the environmental impact is less than conventional application, not to mention the advantage of solving many potential problems with one solution (Ashraf and Foolad 2005, Liu and Litster 1993). The addition of smaller quantities has further efficiency and cost advantages (Harman 1991, Scott and Blair 1988).

Constituents of seed coating used with lucerne seed are:

1. Inoculants: *Sinorhizobium meliloti*
2. Nutrients: Micronutrients, such as Mo, are very important for legume production. Macronutrients are placed as a nutrient source for the inoculants, but can also be used by the young seedling
3. Pesticide: Pesticides, such as fungicides and insecticides, that are suited for seed applications
4. Polymer: a carrier for the constituents and protection for the inoculant against the other constituents.

The function of inoculants as seed treatments

The use of microbes with seed has been documented extensively (Bashan et al. 2002, Bromfield 1984, Deaker et al. 2004, Fraser 1966, 1975, Harman 1991, Hayman and Tavares 1985, Pijnenborg et al. 1991). Different uses of microbes include inoculation, biological control, growth and nutritional responses. Each of these uses can improve the yield of leguminous crop stands, but is hardly ever applied together due to competition effects between microbial species (Harman 1991). For the purpose of lucerne production, only the inoculants will be discussed.

Inoculation of legume crops is the largest voluntary release of micro-organisms in agriculture (Catroux et al. 2001). It is a practice that has been performed for many years, as it increases nitrogen fixation and yield in legume crops (Catroux et al. 2001). The increase in inoculant use has drawn the interests of researchers, to increase efficiency and to make it an accessible practice for any farmer.

Inoculation of legumes is species specific, but individual strains of inoculants can also be more effective in specific environments and on different legume cultivars, as presented in Table 4 (Bromfield 1984, Xavier et al. 2004). A comparison of dry mass of the inoculated and uninoculated plants shows that the strains are all symbiotically effective. According to the dry mass differences, MB10 Spc was least effective, even though no differences in number of nodules were observed. Effective inoculation is dependent on the selection of the most effective strains, a suitable carrier and appropriate additives, which will all influence the survival rate of the rhizobia on the seed (Bashan et al. 2002, Xavier et al. 2004).

Table 4 The response of *Medicago sativa* L. cultivars Apollo, Saranac and Vernal, grown in jars, inoculated with individual Sinorhizobium meliloti strains and in mixtures of two strains (Bromfield 1984)

Inoculant Strains	**Shoot dry mass (mg.plant^{-1}) Mean results of 10 plants**				**Nodule no. per plant Mean results of 10 plants**			
	Apollo	**Saranac**	**Vernal**	**Mean**	**Apollo**	**Saranac**	**Vernal**	**Mean**
2011 Str	64.0	41.4	85.5	63.6	29.5	25.4	30.6	28.5
MB10 Spc	42.4	38.0	45.7	42.0	29.8	26.4	26.3	27.5
2001 Kan	76.0	55.3	58.8	63.4	28.2	26.3	23.1	25.8
2011 Str + MB10 Spc	50.8	43.2	51.0	48.4	22.1	28.5	40.3	30.3
2011 Str + 2001 Kan	64.3	48.8	45.8	53.0	30.0	27.1	22.7	26.6
MB10 Spc + 2001 Kan	41.0	31.9	40.5	37.8	24.0	31.8	23.9	26.5
Uninoculated	11.9	9.2	10.2	10.4				

Rhizobia will infect root hairs when sensing a lack of available nitrogen. Once the nodules have formed, nitrogen is fixed from the air and made available to the plant. Not all of this nitrogen is used by the host plant and thus the nitrogen level in the soil increases, making it available to other crops, such as grass sown between the legumes or the next crop to be sown there in a rotational cropping system. The amount of nitrogen added to soil is dependent on the efficiency of the inoculant, the type of legume, the period it is grown and the nutrient content of the soil (Evans et al. 1980, O'hara et al. 1988, Somasegaran and Hoben 1985).

There are many ways to inoculate legumes, like lime or peat pelleting, liquid spraying, sowing porous granules with the seed or buying pre-inoculated seed. Buying pre-inoculated seed or granules eliminates many preparation procedures for the farmers (Brockwell et al. 1975, Fraser 1966, 1975, Gault and Brockwell 1980, Herridge et al. 2002, Rice et al. 2001). Other considerations for selecting the inoculation method includes the equipment needed for the treatment, for instance if the granules are the same size and mass as the seed it can be sown together without separation.

All species of rhizobia used to inoculate seeds are sensitive to environmental conditions, with the three main reasons for rapid death being desiccation, soluble seed coat exudates and unfavourable temperatures during storage (Deaker et al. 2004). Researchers have tried to overcome the rapid demise of rhizobia by storing the pre-inoculated seeds at low temperatures and by using additives, like polymer coatings and nutrients, in the inoculant formulation (Deaker et al. 2004). Researchers like Elegba and Rennie (1984), had a simpler strategy, involving placing more rhizobia on the seed to start with, however, at some point this ceases to be a solution and thus other solutions must be sought to improve inoculation.

Desiccation causes poor survival of rhizobia on legume seeds. As the surface of the seeds dries, the bacteria die, especially once the individual bacterial cells are exposed to low relative humidity (Deaker et al. 2007). There have been several investigations that have revealed the importance of the medium in which the bacteria are suspended (suspending medium) in the recovery of cells after dehydration (Deaker et al. 2007). Media such as sugars, amino acids, polymers and clay minerals all show improvement in the recovery of bacterial cells in both freeze-dried and vacuum-dried formulas (Deaker et al. 2007). Bashan et al. (2002), Deaker et al. (2007) and Hartwig et al. (1990) all indicated the improvements that could be made in the seed coating field, focussing on the suspending medium and binding agents. These products increase the tolerance of rhizobia to desiccation by adding growth media and moisture (Deaker et al. 2007). This work also shows the importance of different suspending media. In Table 5, the loss of viable *Rhizobium leguminosarum* bv. *trifolii* is shown after the first four hours and up until the first 24 hours following application (Deaker et al. 2007). By numbers alone, GL05 (a polymer used to suspend bacteria) protects the bacteria the best. Even though moisture is critical to survival, there is no direct correlation between moisture content and the ability of a polymer to protect bacteria from desiccation.

The importance of inoculation is evident in the improvement of nodulation and yield (Deaker et al. 2004). The utilisation of nitrogen from fixation reduces production costs. This not only makes it important for developed agricultural systems, but also for developing countries, where nitrogen fertilisers are not affordable (Sparrow and Ham 1983). Improvement of inoculation techniques and technologies can increase survival rate of inoculants, making inoculation more efficient. Carriers and quality of inoculants have been and are the focus of many studies involving legumes. The aim of the majority of these studies was to make inoculation easier and more reliable for the legume producer (Deaker et al. 2004, 2007, Fraser 1966, Sparrow and Ham 1983, Xavier et al. 2004).

Table 5 The survival of a *Rhizobium leguminosarum* bv. *trifolii* strain after vacuum-drying in different polymers and the moisture properties of the polymers (Deaker et al. 2007)

Polymer	**Net loss of viable cells (log_{10})**		**Moisture properties at 98% relative humidity**	
	0-4h	0-24h	Moisture content (% dry weight)	Water activity
GL05	1.6[a]	1.7[a]	35.6	0.87
NL05	1.7[a]	3.6[b]	29.3	0.90
KL05	3.3[b]	5.7[c]	38.1	0.85
No Polymer	3.7[b]	6.7[d]	ND	ND
MC	2.9[a]	>6.9[d]	68.0	ND
PVP	3.1[b]	>6.9[d]	75.4	ND

*GL05 = polyvinyl alcohol with MW of 29.4
NL05 = polyvinyl alcohol with MW of 22
KL05 = polyvinyl alcohol with MW of 29
MC = Methylcellulose
PVP = polyvinyl pyrollidone
NC = not determined

Nutrients

Fertilisation of soils is one of the most common practices in agriculture. The science behind this practice has developed over time making it more precise and a more cost efficient practice. By adding only the nutrients required by the plant in the right amounts and ratios, the producer can decrease expenses, whilst still producing high yields (Hardy et al. 2009, Meyer et al. 2002, Raun et al. 1999, Van Oudshoorn et al. 2001, Yolcu and Turan 2008).

Since nutrients are removed from soil by the crops and other nutrients are lost by leaching, it is recommended that a fertilisation program be worked out from a soil analysis done at the site. Tissue analysis can determine the status of nutrients in the crop stand and can then be used to determine if trace elements should be included in a maintenance fertilisation program. Deficiencies and toxic levels can be determined through tissue analysis as a result of interactions and the transformation of minerals in the soil, which influence the availability of nutrients (Hardy et al. 2009, Helalia et al. 1996). Table 6 provides the amount of nutrients expected to be removed by lucerne in one growth season, which can be used to compare the results of a tissue analysis (Van Oudshoorn et al. 2001).

Table 6 The removal of nutrients (g nutrients per ton dry matter) by lucerne during one growth season (Anonymous 2009a)

	Removal per ton DM (g. ton^{-1})	
Nutrient	**USA data**	**ARC data**
P	3000	2700
K	24000	21000
Ca	15000	13000
Mg	3000	2700
S	3000	2700
B	40	No data available
Mn	60	No data available
Fe	170	No data available
Zn	30	No data available
Cu	10	No data available
Mo	1	No data available

*ARC = Agricultural Research Council

The application of nutrients to the seed coat only satisfies some of the requirements of the plant for a limited time. Soil applications for long-term production should not be ignored, especially macro nutrients, like phosphorus and potassium, which are applied in large quantities (20 mg kg^{-1} P and 150 mg kg^{-1} K for lucerne) (Van Oudshoorn et al. 2001).

Phosphorus is a very important nutrient for legumes. Its function in the plant is mainly for the transformation of energy (conversion of ADP to ATP) and carbohydrates (conversion of starch into sugars) (Miles et al. 2000). Adequate P at plant establishment will favour strong seedlings by stimulating root growth and tillering, making it a stronger competitor against weeds (Scott and Blair 1988, Van Oudshoorn et al. 2001).

Soil properties, such as soil pH and clay content, influence the availability of P (Table 7). Phosphorus requirements are also influenced by specific crop requirements. Legumes have higher P requirements than grasses do, due to a lower efficiency of utilisation in legumes and the higher yields associated with some legumes, for example lucerne. All of these requirements can be satisfied by a P fertilisation program. The advantage of applying some P to the seed can be justified due to the importance of P for the inoculant. By stimulating the inoculant to colonise the roots, efficient nodulation and crop production can be expected (Gourley et al. 1993, Miles et al. 2000, Scott and Blair 1988).

Table 7 The amount of phosphorus required to raise the soil test P level by a single unit in different soils containing different percentages clay (Miles et al. 2000)

Soil	Area	Clay %	pH (KCl)	P requirement (kg P ha^{-1})
Avalon	Dundee	11	4.5	3.4
Katspruit	Kokstad	30	4.5	6.3
Clovelly	Rietvlei	47	4.2	13.2
Hutton	Cedara	53	4.0	16.4
Griffin	Nottingham Road	61	4.0	15.8

Potassium is found in three forms in the soil. The first is the non-exchangeable form that is trapped in soil particles as minerals. The second form is the exchangeable form, which is held by electrostatic forces and is generally not available to plants. The third form is generally a minute fraction, but is in solution and is available to the plant. The general importance of K in plants is clear when it is deficient, causing a lower tolerance to drought, frost and fungal diseases (Berg et al. 2003, Miles et al. 2000).

Potassium can be immobile in the soil and K mobility decreases with liming, because liming increases the cation exchange capacity (CEC) of soil. During liming, other antagonists to K are introduced, namely calcium, magnesium and sodium, making K less available to legumes (Ashley et al. 2006, Berg et al. 2003, Miles et al. 2000).

Sulphur is assimilated into plants in an inorganic form even though in the soil it is found mainly in organic matter. Soils with low organic matter, sandy soils and soils far from cities are often deficient in S, therefore creating a need for its amelioration (Miles et al. 2000, Van Oudshoorn et al. 2001).

The anion sulphate (SO_4^{2-}) is important in legumes and other crops, because of its incorporation into organic molecules, for example: the amino acids cysteine and methionine. It is also important for growth and nitrogen fixation. Nitrogen occurs with S in a 14:18 ratio in lucerne, indicating the amount of S needed by the plant (Miles et al. 2000).

Micronutrients are required in very small quantities, but are essential in the development of plant components such as roots and play an important role in plant physiology. Micronutrients, due to the extremely low quantities required by crops and animals, can be added into a normal fertiliser programme, or they can be added directly to the seed. Quantity of micronutrients can cause problems indirectly, because toxic effects can easily become evident. A factor influencing this toxicity is the relative immobility of micronutrients in the plant, with the exception of Molybdenum (Gupta et al. 2001). Chelates (an organic metal complex) can be used to manipulate the availability of micronutrients by choosing a soluble or less soluble form, creating a resource pool that makes micronutrients available over time, avoiding a toxic effect even though toxic quantities are present (Brady and Weil 2002, Viets 1962). The justification of applying micronutrients to seed has been discussed in various articles (Forde and Lorenzo 2001, Hackett 1964, Van Oudshoorn et al. 2001). The importance of micronutrients is not comparable to the amount of the mineral needed, because most crops are relatively sensitive to deficiencies (Gupta et al. 2001). Table 8 shows the relative sensitivity of a few crops to different micronutrient deficiencies.

Table 8 Relative sensitivities of different crops to micronutrient deficiencies (Martens and Westermann 1991)

CROP	B	Cu	Fe	Mn	Mo	Zn
Lucerne	High	High	Medium	Medium	Medium	Low
Bean	Low	Low	High	High	Medium	High
Clover	Medium	Medium	-	Medium	Medium	Low
Grass	Low	Low	High	Medium	Low	Low
Pea	Low	Low	-	High	Medium	Low
Rye	Low	Low	-	Low	Low	Low
Sorghum	Low	Medium	High	High	Low	High
Soybean	Low	Low	High	High	Medium	Medium

Molybdenum is an essential micronutrient for protein synthesis, nitrogen fixation and forms part of some enzymes, for example nitrate reductase (Miles et al. 2000, Mulder et al. 1959). The most common cause of Mo deficiency in pastures is attributed to low soil pH and is usually observed as a nitrogen deficiency in legumes (Gupta et al. 2001, Van Oudshoorn et al. 2001). If the soil is adequately limed, Mo deficiencies are usually eliminated (Van Oudshoorn et al. 2001). Care should be taken not to over supply Mo to forage species as it is poisonous to animals. Molybdenum shows antagonistic relationships with Cu and S and synergistic effects with P in the soil. Toxicity is therefore reached even sooner when the Cu concentration in the soil is low (4 mg kg^{-1}), such as weathered sandy soils (Gupta et al. 2001). A plant tissue Cu: Mo ratio less than 2 will cause Mo toxicity (molybdenosis) in ruminant animals, while the plant reaction will differ depending on the species involved. Molybdenosis can also be caused by high soil pH levels, which increases the availability of Mo (Brady and Weil 2002, Van Oudshoorn et al. 2001). The Agricultural Research Council's (ARC) recommendation for Mo application for lucerne production, is 100 g ha^{-1} initially and biennial corrections with a topdressing. Molybdenum is also important for the uptake of other micronutrients like Iron. According to Monreal and Villalvilla (1982), Fe uptake is depressed when Mo concentrations are lower and higher than the required amount. Molybdenum has proven to be the most important micronutrient for lucerne production and should be managed with care, to prevent these deficiencies and toxicities (Miles et al. 2000).

Boron is an important micronutrient for high yielding legumes as it is necessary for protein synthesis (Miles et al. 2000, Van Oudshoorn et al. 2001). Boron availability is limited in both acidic and alkaline soils. Since there is a shortage in any soil other than a neutral one, B is known to be the only micronutrient essential in South African fertilisation programs for lucerne (Van Oudshoorn et al. 2001). Addition of B and lime has shown positive effects on lucerne produced on acidic soils (Pinkerton and Simpson 1986). To include B in seed coatings is, however, not recommended, since it can be toxic to the seed, thereby causing germination failure. Most annual crop producers use broadcast application of B, whereas foliar applications are often used for perennial fruit and nut trees (Gupta et al. 2001, Martens and Westermann 1991).

The micronutrients Zinc, Copper, Manganese and Fe are also classified as heavy metals found in a cationic form in soils. In South Africa, these micronutrients are rarely deficient. Availability may decrease in soils that have been limed, but these metals are sufficiently available to pastures, like lucerne in South Africa (Gupta et al. 2001, Miles et al. 2000). Iron deficiency is commonly known to be a “physiologically induced disease” and is rarely caused by a lack of Fe in the soil. Iron interacts with B, Cu, Zn, Mo and Mn in an antagonistic manner, causing deficiencies in the plant. Acidifying alkaline soil has shown to increase the availability of Fe and overcoming deficiency symptoms. Iron deficiencies can, however, be overcome by foliar sprays, using a chelated form of Fe in the soil (Brady and Weil 2002, Gupta et al. 2001).

According to Gupta et al. (2001), Zn additions resulted in significant benefits for legumes, but only when supplied together with Mo. Even though Zn might be sufficient for forage growth, additions can improve grazing livestock performance when Zn is low in the soil. The ARC recommends an addition of Zn when the soil concentration is below 1 mg Zn kg^{-1} soil (Van Oudshoorn et al. 2001).

Other nutrients are rarely added and recommendations are therefore only made by plant nutritionists. Care must be taken to not supply too many of these nutrients, else toxicities will prevail in plants and animals (Miles et al. 2000).

Pesticide treatments for seed

Pesticide treatment of crops is one of the most valuable developments in agriculture. Pesticide applications, which include fungicides, insecticides and herbicides, have many advantages and have become an essential component of crop production (Van Oudshoorn et al. 2001). Even though it is an essential component of crop production, there are some environmental impacts that are only realised when the boundary is overstepped. Seed application overcomes some environmental problems by a simple quantitative aspect, as by adding less pesticide to the environment, a lower incidence of environmental degradation is evident (Skinner et al. 1997).

Seed application of pesticides, is mostly restricted to fungicides and insecticides, but some development has allowed limited herbicide applications. The development of this aspect of seed coating technology is strongly linked to carriers of the chemicals and is a major field of development and research where seed coating technology is concerned (Green and Beestman 2007).

There are a limited number of chemicals that can be applied to the seed coat, where the active ingredient will address the pest problem. This will depend on the plant species involved and the pest that is predominant in the specific production system.

Table 9 gives the active ingredients of insecticides and fungicides that can be applied to seed. The action of the active ingredient is also important, but it should be considered that contact action only applies to seed predators, whilst systemic action includes the rest of the pests(Adkisson 1958, Allen et al. 1961, Anonymous 2007, Hacskaylo et al. 1964, Reynolds et al. 1957).

Table 9 Pesticides that can be used as seed treatments (Anonymous 2007)

Active ingredient	Agrochemical Use	Application *	Action
Benfuracarb	Insecticide	LS	Systemic
Carbaryl	Insecticide	WS	Contact
Carbosulfan	Insecticide	DS	Systemic
Gamma-BHC	Insecticide	DS	Contact
Hydramethylnon	Insecticide	FS	Contact
Imidacloprid	Insecticide	FS/ WS	Systemic
Thiamethoxam	Insecticide	FS/ WS	Systemic
Thiocarb	Insecticide	FS	Contact
Benomyl	Fungicide	DS/ WS	Systemic
Captab	Fungicide	FS	Contact
Carboxin/thiram	Fungicide	FS	Systemic/ Contact
Cypermethrin/ triadimenol	Fungicide	DS/ WS	Contact /Systemic
Difenoconazole	Fungicide	FS	Systemic
Fludioxonil	Fungicide	FS	Contact
Fludioxonil/mefenoxam	Fungicide	FS	Contact
Imazalil/ iprodione	Fungicide	FS	Systemic/ Contact
Mancozeb	Fungicide	DS/WS	Contact
Metalaxyl	Fungicide	WS	Systemic
Metalaxyl – M (Mefenoxam)	Fungicide	ES	Systemic
Prochloraz Manganese chloride	Fungicide	DS/WS	Contact
Silthiopham	Fungicide	FS	Contact
Tebuconazole	Fungicide	ES	Systemic
Thiram	Fungicide	DS/ WS/ FS	Contact
Tolclofos-methyl	Fungicide	WS	Contact
Triadimenol	Fungicide	DS/ FS	Systemic
Triticonazole	Fungicide	FS	Systemic

*DS = Powder for dry seed treatment; FS = Flowable concentrate for seed treatment; LS = Solution for seed treatment; WS = Water dispersible powder for slurry treatment; ES = Emulsion of seed treatment

When choosing a pesticide to use on seed, the seed coating and method of application should coincide, for instance, a dry seed treatment should not be used in a fluid system. In Table 9 these different application methods are described. The method of application is not linked to a method of action, but is rather linked to the carrier (Anonymous 2007). The carriers used can have just as big an effect on production as the chemicals in them.

Polymer Technology

As mentioned previously in the section on inoculants and pesticide treatments for seed, carriers are very important. These carriers are usually some form of polymer. The use of these polymers in seed coating vary between changing the shape and size of a seed, to complex characteristics like changing the point of water permeability to synchronise germination and emergence with environmental conditions (Vyn and Murua 2001).

When choosing a polymer to coat seed, objectives must be clear. In the case of lucerne, the polymer is a carrier for the inoculant, nutrients and pesticides, but when these components are combined, there may be an interaction and the inoculant may not survive. To circumvent this problem, more than one polymer can be used. Harman (1991) gave a description of different seed coatings or treatment methods including some advantages and disadvantages on the biological component, regarding the technique (Table 10). Integrated biological and chemical treatment methods are a developing technology that allows for more theoretical solutions to address production challenges. Each of the techniques used, may have a carrier that best suits the procedure and as new seed treatments develop, new polymers and additives develop that meet management objectives (Vyn and Murua 2001).

Table 10 Advantages and disadvantages of using different seed coating procedures (Harman 1991)

Seed Treatment Procedure	**Colonization of microbes before planting**	**Timing favouring microbes**	**Amendments**	
			pH Control	Pesticide selection requirements
Conventional slurry or planter box	NO	NO	NO	NO
Solid matrix priming (SMP)	YES	NO	YES	NO
Double coating	NO	YES	YES	NO
Integrated biological and chemical treatments	NO	?	NO	YES

Development and production of lucerne

Growth and development of plants begins with the germination of the seed and is followed by the growth of the seedling, until the plant starts to flower and produce seed again. There are definable stages of development for lucerne (Undersander et al. 1997, 2007). From the germination process until establishment, the seed and the seedling is vulnerable to the environment. While the seedlings are still dependent on the endosperm, any factor delaying, or making photosynthesis ineffective, can prevent establishment. Establishment is only reached when current photosynthesis can provide the photosynthates to support the plant (Meyer 1999). The success of crop establishment will be a factor of management during this period. After this period, the factors influencing lifetime yield become the focus of management practices. These factors include time of first harvest, type of harvesting, harvesting interval, fertilisation and nodulation (Van Oudshoorn et al. 2001).

Under optimal growing conditions, the germination process of lucerne takes about 5 days (Meyer 1999). The International Seed Testing Association (ISTA) makes provision for lags in seed germination, therefore giving 15 days when germination percentage can be determined (Anonymous 2006). Factors, other than the osmotic characteristics of water, such as soil temperature and pH, chemicals like herbicides, growth regulators, allelopathic and autotoxic chemicals, and characteristics peculiar to the seed, such as dormancy and hard seededness, influence germination (Fawcett and Harvey 1978, Meyer 1999, Ross and Hegarty 1980) .

The growth medium is an important factor to consider when investigating germination. Soil is the growth medium which provides moisture, nutrients and protection for seed. Three characteristics of soil which influence germination are soil temperature and soil pH and the soil water content. These influence the activity and availability of components in the soil. Higher soil temperatures increase the rate of germination of most species, but only influence the final germination percentage of lucerne when the temperature is below 2.8°C because imbibition only occurs above 2.8°C (Undersander et al. 1997). Temperature also influences the activity of enzymes in biochemical processes of cell division and differentiation, occurring during germination (Campbell and Farrell 2003). Soil pH plays an important role in terms of availability and mobility of ions and its cohesion to organic colloids. The negative charge on these molecules is extremely high in neutral alkaline soils, making it attractive to cations (Brady and Weil 2002). Optimal soil pH for lucerne cultivation ranges from 6.7 and 6.9 (Van Oudshoorn et al. 2001). Lucerne seeds and the roots are sensitive to pH as it influences the solute concentrations in the soil water component. The concentration and ratios of nutrients in the solution influences plant tissue by influencing normal water and nutrient uptake by tissue (El-Kherbawy et al. 1989).

When all these characteristics are taken into consideration within a management plan and seedbed preparation, a successful stand is likely. The higher the germination percentage, the more seedlings will emerge and the higher the competition will be, pushing out intruders like weeds and creating a uniform stand. The uniformity of the stand increases the quality of the product, being hay, silage or grazing. It also increases grazing safety of animals as it decreases the chance of the presence of other poisonous plants. Germination rate is also a factor that will influence the success of establishment. If all or most seed germinates within a short span of time, the uniformity of the stand will increase. Even if a uniform stand emerges, management of seedlings and successful harvesting methods are still necessary to successfully produce lucerne (Fick and Mueller 1989, Undersander et al. 1997).

A great deal is known about lucerne production, including timing of cultural practices and scheduling harvests to maintain the health and vigour of a stand. These practices are largely dependent on the developmental stage and the partitioning of nutrients to different parts of the plant, which will maintain plant health (Fick and Mueller 1989). Hall (1998) gives the plant developmental stages are as follows: germination, establishment, vegetative stage, flowering-bud stage, flowering stage and seed-pod stage. After the germination stage, the establishment stage is one of the most sensitive points as it is susceptible to herbicide, frost and insect and disease damage. After the germination process, the first plant structure that emerges from the soil is the two cotyledons Fig. 2A. The cotyledons provide nutrients for the seedling to grow until it can support itself by photosynthesis. These cotyledons start to shrivel as the nutrients are used up.

From the growth point the first leaf appears (Fig. 2B), called a unifoliate leaf because it only has one leaflet. As the epicotyl extends, trifoliate or multifoliate leaves emerge (Fig. 2C). Once four or five trifoliate leaves are present, the cotyledons disappear or the first secondary stem appears, and then the seedling can sustain itself because photosynthesis provides enough energy for the seedling to grow without the reserves of the cotyledons. As mentioned before, any factor delaying or preventing successful photosynthesis before the three trifoliate leaf stage, or before enough reserves can be stored, can prevent a successful stand establishment (Hall 1998, Meyer 1999, Undersander et al. 1997).

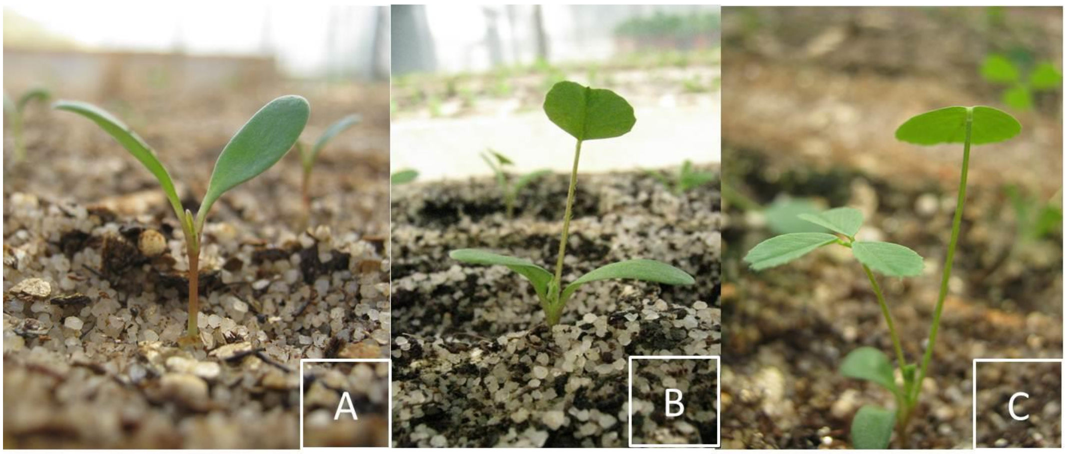

Fig. 2 The developmental stages of a lucerne seedling. A: two cotyledons, B: unifoliate leaf from the epicotyls and C: first trifoliate leaf that emerges from the epicotyls.

Following establishment, vegetative growth increases the biomass of all the above ground structures. This is a very important stage as yield is a parameter that directly influences profit margins. This is not, however, the only important stage as the bud, flowering and pod stages, defined in Table 11, are used to determine the average stage of the stand and to determine the time to harvest to optimise quality and quantity of the product (Fick and Mueller 1989, Hall 1998).

Table 11 Definitions of morphological stages of development for individual lucerne stems (Fick and Mueller 1989, Hall 1998)

Stage name	Stage definition
Early vegetative	Stem length ≤ 152.4mm; no buds, flowers, or seed pods
Mid-vegetative	Stem length from 152.4 mm to 304.8 mm; no buds, flowers, or seed pods
Late vegetative	Stem length ≥ 304.8 mm; no buds, flowers, or seed pods
Early flower bud	1 to 2 nodes with flower buds; no flowers or seed pods
Late flower bud	≥ 3 nodes with flower buds; no flowers or seed pods
Early flower	One node with one open flower; no seed pods
Late flower	≥ 2 nodes with open flowers; no seed pods
Early seed pod	1 to 3 nodes with green seed pods
Late seed pod	≥ 4 nodes with green seed pods
Ripe seed pod	Nodes with mostly brown mature seed pods

The point of harvesting is determined by the use of harvested components. Lucerne is known for forage which can be utilised in the form of grazing, hay and silage. This is, however, not the only use of lucerne as it can be produced for seed or for human consumption (Van Oudshoorn et al. 2001). As the quality and quantity is not directly related, the point of harvest must be where the best product and the highest quantity meet. Fig. 3 shows the relationship between quantity and quality of lucerne (Hancock et al. 2009). Finding the stage of development where harvesting does not damage the stand persistence will increase the stand production and cost efficiency. The longer the stand can persist, the higher the yield from the stand. Damage to stand persistence will have a negative impact on the yield at the next harvest and will impact the time it will take to reach the next harvest. Damage is mainly related to root reserves (Fig. 4), as the more root reserves there are at the point of harvesting, the more reserves are available for subsequent recovery. This is also a buffer for any event that may prevent the stand from obtaining optimal photosynthetic rate (Hancock et al. 2009, Lattimore 2008).

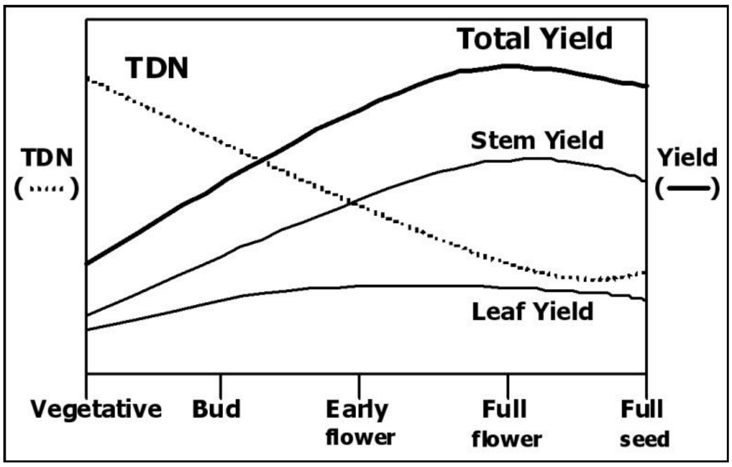

Fig. 3 The effect of maturity of the lucerne stand, shown as growth phases, on the total yield, leaf and stem yield and the Total Digestible Nutrients (TDN) (Hancock et al. 2009)

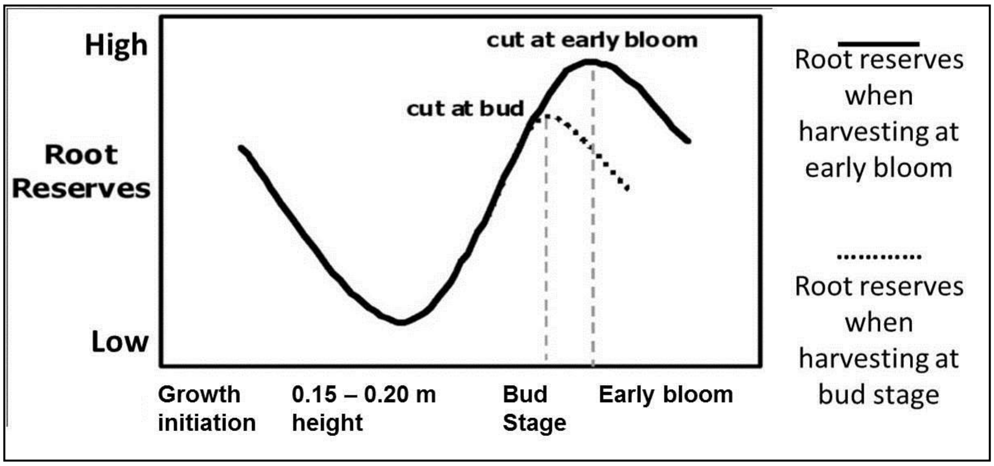

Fig. 4 The fluctuation of lucerne root reserves over the period between harvests (Hancock et al. 2009)

Cultivar selection is crucial to suit the production system of the individual farmer, as the position of the growth points in the crown vary with the winter dormancy class. Cultivars with lower winter, dormancy numbers (2 or 3), have deep crowns, protecting the growth points and are therefore more suitable for grazing (Van Oudshoorn et al. 2001).

Table 12 describes the different winter dormancy classes and what can be expected in terms of their growth, recovery and morphology. Selecting a cultivar that suits the area and the use the farmer requires, is made easier by these dormancy classes (Van Oudshoorn et al. 2001).

Table 12 A descriptive summary of the winter dormancy classification of lucerne (Van Oudshoorn et al. 2001)

Dormancy Class	Classification	Description
2 -3	Winter dormant	Can withstand very low temperatures for long periods Distinct dormant period Growth point below soil surface Suited for grazing
4-6	Semi-dormant to intermediately dormant	Limited growth in winter Faster growth in Autumn and Spring Leafy with broad crowns
7-8	Moderately active to winter active	Faster recovery after harvesting Slower growth in winter but does not stop growing Narrow crowns Lower leaf density due to longer internodes Bigger leaves Mostly suited for hay production
9	Highly winter active	Fast recovery after cutting Most productive in winter

The influence of water potential and salinity on emergence and development of lucerne

The coating components are designed not to inhibit germination (physiologically or mechanically), but should be soluble in water to become available to the seedling. This results in changes in the water quality surrounding the seed. The water quality can influence germination by exceeding osmotic potential thresholds or cause toxicities due to salinity or other solutes (Halmer 2004).

Water uptake by the seed from the substrate is considered to be passive and is driven by the difference between the water potential of the substrate ($\Psi_{substrate}$) and the seed (Ψ_{seed}). Lucerne seed water potential is mainly determined by the water potential of the seed components, while the quantity of water absorbed is relative to a higher protein content compared to the oil content of the seed components

(Bennett 2004). Seed water potential is determined by the osmotic cell water potential ($\Psi_{cell\ osm}$), matrix water potential (Ψ_m) and cell turgor (Ψ_T) (Equation 1).

$$\Psi_{seed} = \Psi_{cell\ osm} + \Psi_m + \Psi_T$$

Equation 1 The seed water potential components (Bewley and Black 1994, Hadas 2004)

Seed water potential is usually very low, ranging between about -50 MPa and -100 MPa. This low water potential does, however, not ensure success at reaching the *critical hydration level.* The *critical hydration level* can only be reached if the water potential of the substrate is above the *critical water potential*. Fig. 5 shows the water potentials at which the mentioned species will experience more than 50% inhibition of germination (McDonough 1975, Swagel et al. 1997, Uhvits 1946, Zhang et al. 2010). If the water potential of a soil is -1.5 MPa, germination of lucerne will be inhibited, and will therefore not be recommended in these areas (Bewley and Black 1994, Hadas 2004).

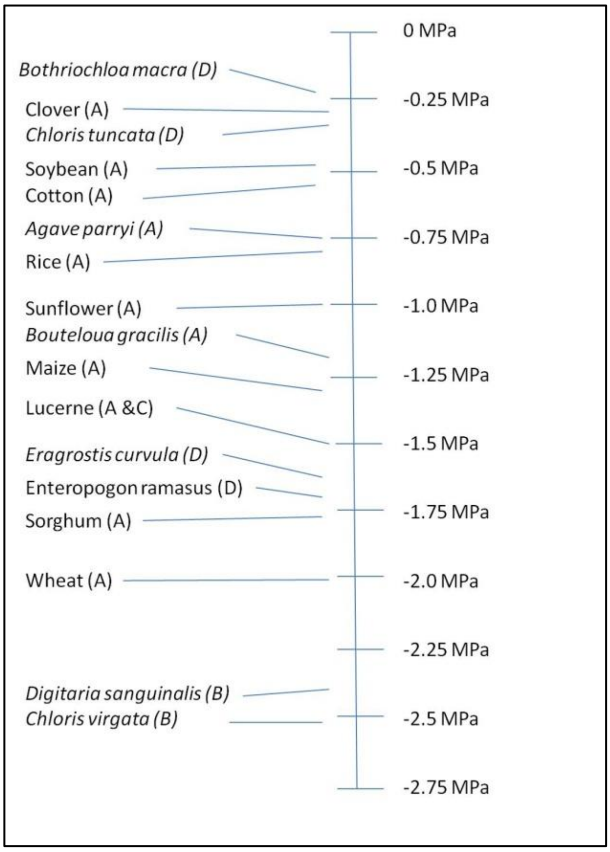

Fig. 5 The water potential threshold for agronomic and forage species below which significant germination inhibition will take place. 'A' refers to species from Swagel et al. (1997), 'B' refers to species from Zhang et al. (2010),'C' refers to species from Uhvits (1946) and 'D' refers to species from Maze et al. (1993).

The growth medium into which seed is sown acts as the water reservoir for the seed and seedling. Substrate water potential has similar factors contributing to it as seed water potential. It consists of an osmotic component (Ψ_{os}), matrix component (Ψ_m), mechanical overburden constraints (Ψ_e), and gravitational and hydrostatic components (Ψ_g and Ψ_p) (Equation 2). All of these factors have a large influence on the water potential, but the substrate matrix water potential (Ψ_m), which is largely influence by particle size and clay fraction, and substrate osmotic water potential (Ψ_{os}), determined by solutes in the growth medium, play the largest role in germination (Bewley and Black 1994, Hadas 2004). These two components are also influenced by the amount of water, and are therefore not easy to measure or predict, especially when the water quality also influences these components.

$$\Psi_{substrate} = \Psi_{os} + \Psi_{m} + \Psi_{e} + \Psi_{g} + \Psi_{p}$$

Equation 2 The substrate water potential components (Bewley and Black 1994, Hadas 2004)

Two causes of osmotic stress are salinity and drought effects. Salinity has become a big factor to consider in plant production due to the scale of the problem. According to Soleimani et al. (2011) 400 Mha of land are affected by salinity on a global scale. Some effects of salinity on plants are the delay in germination, non-uniform germination and emergence, reduction in growth and ultimately a decrease in yield (Soleimani et al. 2011). Osmotic stress due to salinity can be less severe than drought stress at the same water potential, due to the seed embryo's ability to absorb cations such as Na^{+}, maintaining a water potential gradient which supports water uptake (Kaydan and Yagmur 2008). This accumulation does, however, cause other problems such as ion toxicity, nutrient imbalances and cellular damaged caused by salt accumulation in intercellular spaces (Soleimani et al. 2011).

Conclusion

Practices such as species and cultivar selection, seedbed preparation and soil amelioration cannot be excluded from the cultivation process and good agricultural practice cannot be substituted with coated seed. Incorporation of seed coating with good agricultural practices can, however, assist farmers to decrease input costs and overcome challenges. Choosing the constituents used in seed coatings to facilitate seedling establishment is challenging. The different seed coatings are improved on a continuous basis and are adapted to overcome challenges created by agriculture, and other industries, like the mining sector.

The farmer can choose the species most suited for a specific environment and a cultivar suited to the production objectives. By taking the environmental challenges into account, the farmer can choose whether to use coated or non-coated seed in the production system. Knowing how the species will react, knowing how the coating reacts with the species and how the product reacts in different environments is important when choosing between coated and non-coated seed. This is just as important as knowing the soil and climatic environment of the production area, which has been recognized for a long time as the basis of most agronomic recommendations. Empowering specialists with the knowledge required, can put the specialist in a position to improve production, lower production costs and in some cases improve the environmental condition of the site.

Hypotheses

Germination:

H_1 = There is no difference between germination % of coated and non-coated lucerne for the cultivars SA Standard and SuperCuf

H_2 = There is no difference in germination % between coated and non-coated lucerne for the cultivars SA Standard and SuperCuf, when using two methods of determining germination %

H_3 = There will be no differences between germination % of coated and non-coated lucerne for the cultivars SA Standard and SuperCuf under NaCl salinity conditions.

H_4 = Differences observed between coated and non-coated seed germination is not due to osmotic potential of the saline conditions.

Seedling emergence:

H_1 = The seed coating will have no influence on the emergence percentage in different growth mediums

H_2 = The seed coating will have no influence on the emergence percentage when irrigated with saline water

Seedling growth:

H_1 = The seed coating will have no influence on root and shoot growth parameters.

Biomass:

H_1 = Using coated seed will have no influence on the yield of the stand when compared with non-coated seed

H_2 = The seed coating will have no influence on stem to leaf ratio

H_3 = Seed coating will have no influence on the dry matter production of stands sown at different sowing rates.

Aims of the research

The purpose of this study was to identify whether seed coating influences germination, emergence, seedling growth parameters and seasonal yield of lucerne plants. The interaction between coating, saline conditions and growth media was investigated to identify what the difference would be between ideal lucerne production conditions and suboptimal conditions, due to salinity for example. Furthermore, the yield obtained from stands established with coated and non-coated seed was analysed to determine whether seed coating will ultimately influence yield.

Germination: The objective of the germination trials was to identify the influence of seed coating on germination of lucerne under prescribed germination conditions and altered conditions, such as saline conditions.

Seedling emergence: The purpose of the seedling emergence trials was to identify the influence of seed coating on the emergence of seedlings in different growth media, taking into consideration that after germination, seedlings could be prevented from emerging and could have high mortality rates due to a higher incidence of abnormal seedlings.

Seedling growth: The objective of this trial was to determine if seed coating will cause differences between seedlings established with coated and non-coated seed. Salinity was included as a variable to determine whether salinity and seed coating will influence the growth and development of lucerne seedlings.

Biomass: The objective of the field trial was to determine whether the differences in germination, emergence and seedling growth caused by the seed coating treatment was extended to field conditions. The season when stands were established and the density of the stand were also used as variables.

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CHAPTER 2

Prepared according to the guidelines of Experimental Agriculture

THE EFFECT OF SEED COATING TREATMENTS ON THE GERMINATION OF LUCERNE (*MEDICAGO SATIVA* L.)

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ABSTRACT

Fast and uniform germination is one of the most important factors influencing stand establishment in lucerne. Seed treatments have been used to assist in fast uniform germination to ensure stand establishment and to overcome factors impacting on this process, such as soil salinity as a result of over fertilisation and poor irrigation water. The main purpose of coating lucerne seed is not to manipulate the germination process, but to facilitate germination. To determine if and to what extent the germination process is influenced, different growing conditions, such as salinity, must be taken into account. By comparing germination % obtained from using the Jacobsen apparatuses and petri dishes, the influence of a nutrient concentrated environment was compared with an environment where nutrient and water movement is possible. Using different saline conditions, it could be determined if salinity influenced the germination of coated seed, compared with non-coated seed. It was found that the coated seed of lucerne cultivar SuperCuf, was more sensitive to germination in petri-dishes than SA Standard. Results from SA Standard showed there was a higher germination % for the coated seed, suggesting that the coating overcame inhibition as a result of saline conditions. Seed coating does therefore influence germination of lucerne, but the interaction of the seed coating with the surrounding seed environment changes the effect on germination. The genetic differences between cultivars will also have an influence on how the coating will affect the germination process.

Keywords: *germination, seed coating, Jacobsen apparatus, water potential, electrical conductivity.*

INTRODUCTION

Germination is a complex process which is influenced by the environmental conditions surrounding the seed (Hadas, 2004, Bewley and Black, 1994). The onset of the germination process is either a genetic response or environmental conditions trigger the genetic make-up of the seed. The genetic responses and environmental conditions cannot be controlled under field conditions, but may be manipulated to improve the chances for successful establishment (Hadas, 2004).

Areas chosen for crop production usually have favourable growth conditions, but there is an increased amount of land that is being degraded by poor agricultural practices, mining activity, construction and other human activities. These conditions decrease the potential of successful pasture establishment. Identifying ways to successfully manipulate these growing conditions is important for the reclamation of these degraded areas.

The basic requirements for germination are water, temperature that facilitates cellular activity, an aerated substrate and a substrate that will not mechanically impede emergence (Hadas, 2004). These factors are, however, more complicated than just water and air. The amount and quality of the water is important because of the chemical reactivity and water potential caused by the chemical reactions (Hadas, 2004).

Knowing the response of plant species to environmental conditions is important for selecting the correct species or cultivar for a particular area, in addition to optimizing the sowing and establishment practices. According to Ashraf and Foolad (2005), knowing these responses are also important when selecting seed treatments, such as priming or coating of seeds.

Seed coatings are an ideal way to assist germination and establishment in poor conditions. Currently seed coating technologies are novel and new developments of the components involved in the technology are on-going. Many of the components are patented and each company involved in this technology, patents its technology or recipe (Halmer, 2004). Therefore an understanding of how the coating influences germination and establishment is restricted to a company's coating technology of a specific species. Not all technologies are successful and results should not be generalized for all coating technologies.

Seed coatings were originally used to change the shape and size of the seed. This allows for easy calibration of the seeders used (Halmer, 2004). The coating can, however, also carry nutrients (Hackett, 1965, Gherardi and Rengel, 2003, Gourley *et al.*, 1993, Haby *et al.*, 1999), plant growth regulators (Halmer, 2004), microbes, like *Sinorhizobium meliloti (Fraser, 1966, Fraser, 1975, Halmer, 2004, Höflich et al., 1994)* and *Mycorrhizal* fungi (Azcón *et al.*, 1991, Wu *et al.*, 2009), and agrochemical components, like fungicides and insecticides (Adkisson, 1958, Pike and Glazer, 1980, Pike *et al.*, 1993).

The coating components are designed not to inhibit germination (physiologically or mechanically), and should be soluble in water in order to become available to the seedling (Halmer, 2004). This leads to changes in the water quality surrounding the seed, which will in turn influence the water potential gradient that drives the passive uptake of water.

An increased concentration of salts and other solutes in the soil water or a decrease in soil water content can delay or prevent the onset of germination by creating an external osmotic potential that prevents water uptake or causes toxicities by Na^+ and Cl^- ions. This causes uneven germination over a prolonged period of time, resulting in varied plant sizes in the stand and reduced plant growth and final crop yield (Soleimani *et al.*, 2011).

Identifying parameters that will give an indication of the success that can be expected when using coated seed as opposed to non-coated seed is important, especially when the environment has favourable plant growing conditions or unfavourable degraded growing conditions. In an attempt to understand the influence of seed coating treatments on the germination of lucerne seed, two methods of germination testing were compared. In addition to this trial, the water quality surrounding the seed was manipulated. These trials would give an indication of the interaction between soil water quality and the coating and how these factors influence the germination of lucerne under similar environmental conditions. It was hypothesised that the seed coating would lower soil water potential surrounding the seed which would influence water uptake from the soil. In addition, if enough water was available to facilitate water and solute movement away from the seed, germination would not be inhibited by a lower water potential.

The hypotheses for these trials were:

H_1 = There is no difference between germination % of coated and non-coated lucerne for the cultivars SA Standard and SuperCuf

H_2 = There is no difference in germination % between coated and non-coated lucerne for the cultivars SA Standard and SuperCuf, when using two methods of determining germination %

H_3 = There will be no differences between germination % of coated and non-coated lucerne for the cultivars SA Standard and SuperCuf under NaCl salinity conditions.

H_4 = Differences observed between coated and non-coated seed germination is not due to osmotic potential of the saline conditions.

METHODOLOGY

A germination study was conducted at the Hatfield experimental farm, tissue culture lab and Phytotron D, Pretoria, South Africa (25°45' S 28°16' E), to evaluate the influence of seed coating treatments on the germination of lucerne (*Medicago sativa* L). Four trials were conducted, a conventional germination test, a trial comparing two germination test methods, a germination trial comparing germination % at different osmotic potentials and a trial comparing germination % at different salt concentrations. For these trials two cultivars (SA Standard and SuperCuf) were used, both cultivars had two identical seed treatments, namely coated and non-coated. The cultivar characteristics are described in Table 1. The coating is a commercial product containing lime, an insecticide, a fungicide, nutrients, rhizobia and polymers to bind these constituents. Each trial was conducted with a fresh batch of seed.

Table 1. SA Standard and SuperCuf characteristics

Cultivar	Dormancy	Origin	Characteristics
SA Standard	4-6	South African Landrace	Intermediate crown position Coarse to medium stems Exceptionally high resistance to root and crown-rot complex and other root and crown diseases
SuperCuf	9	Australia: Cuf101 cross with Sequel	Leafy stems Strong autumn and spring growth Strong regrowth after harvesting

Standard germination trials

A standard germination trial was conducted using the International Rules for Seed Testing (Anonymous, 2006). The 'top of paper' variation was used, using Whatman® No. 1 filter paper, placing 50 seeds per replicate, with 10 replicates. Each replicate was placed in a clear petri-dish (90 mm diameter) in a growth chamber at 21 ± 3 °C. Distilled water was used as the water source and all replicates received 2.5 ml water. The replicates were randomly placed in the growth chamber, all equal distance from the light source. The seeds were germinated under constant light intensity.

Seeds were considered to have completed germination when the radicle protruded from the seed by 1mm (Chon *et al.*, 2000). Germinated seeds were counted on day 4 and day 10 after the trial started. Germinated seeds were discarded after each count (Anonymous, 2006).

Comparison between the Jacobsen apparatus method and the petri-dish method of germination testing

The conventional method of using a petri-dish may not be suitable when testing coated seed. Under these conditions, the coating material stays in contact with the seed, changing the water quality of the small amount of water added to the petri-dish. This scenario does not represent what would happen in the soil, due to water movement in the soil. As a result a simulation of the Jacobsen apparatus (Figure 1) was used to facilitate water and nutrient movement. Trays containing water were used as a water reservoir and were then covered. A paper wick was placed in the water and through the cover and was held in place by a spacer. The spacer has a dual purpose of preventing the formation of a vacuum seal and to keep the wick in position. Filter paper was placed on the wicks, allowing water movement from the water source to the filter paper and *vice versa*. Fifty seeds were placed on each filter paper disc and then covered by plastic cups (with ventilation holes) to prevent the filter paper from drying out faster than water can be replaced from the reservoir (Figure 1E). Each treatment had 6 replicates.

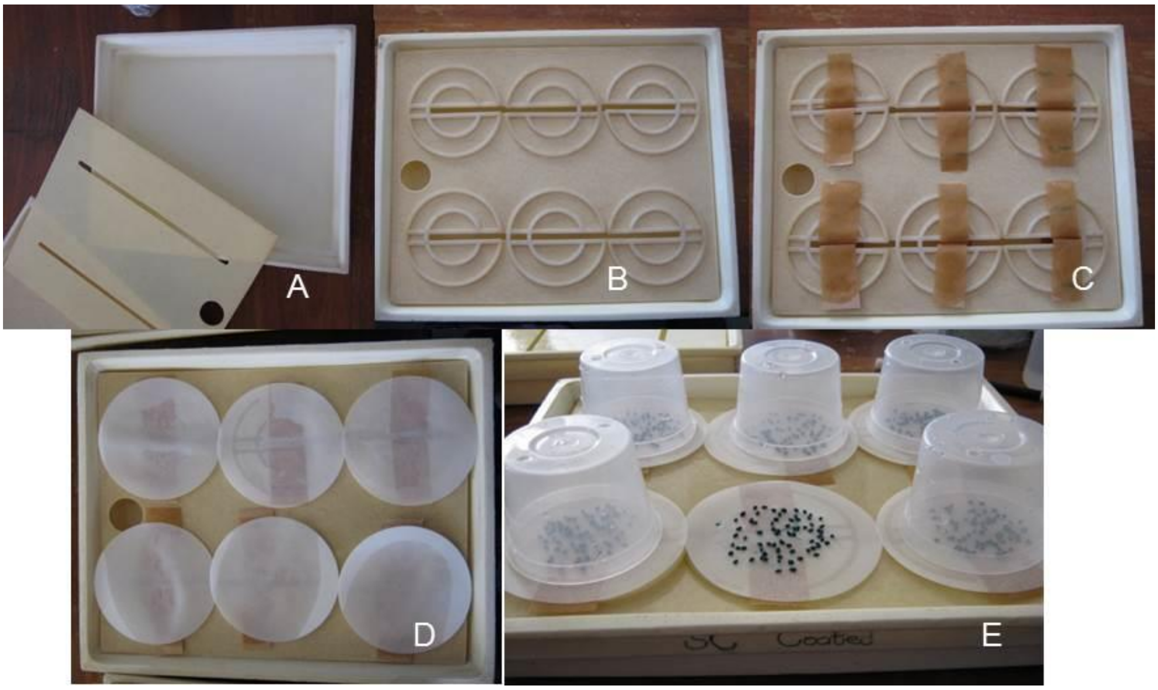

Figure 1. The Jacobsen apparatus used in the trial. 'A' is the reservoir and cover, 'B' is the spacer on the cover, 'C' shows the wicks in place, 'D' shows the filter papers in place, 'E' shows the seed in place and covered by the plastic cups.

The whole apparatus was placed in a growth chamber at 21 ± 3°C under constant light and at equal distances from the light source, as described above. Data were collected similarly as the standard germination trial. Germinated seed were counted on the 4th and 10th day of the trial. After each count the germinated seeds were discarded (Anonymous, 2006).

Comparing the germination of non-coated seed in response to solutions with different osmotic potentials.

Using the methodology described in Swagel *et al.* (1997), solutions with specific osmotic potentials were created using D-Mannitol. The D-Mannitol concentrations and subsequent water potential is shown in Table 2.

Each substrate plus a control (distilled water) was used in the germination trial. The trial was conducted in petri-dishes because no coated seed was used. Coated seed was excluded from this trial to determine the influence of osmotic potential on the germination of lucerne.

Table 2. Characteristics of the solutions used in the osmotic potential gradient germination trial

D-Mannitol (1 mol per l)	Osmotic potential (MPa)
0.04	-0.1
0.1	-0.25
0.2	-0.50
0.4	-1.0
0.6	-1.5
0.8	-2.0

This trial proceeded according to standard germination test guidelines (Anonymous, 2006). The 'top of paper' method was used with 50 seeds per replicate, with four replicates of each substrate. Petri-dishes were placed in a growth chamber at 21 ±3 °C under constant light. Germinated seeds were counted on day 10.

Comparing the germination % of coated and non-coated seed at different salt concentrations

A series of salt (NaCl) concentrations were created and were then evaluated in terms of their electrical conductivity (EC), using a Mettler Toledo SevenEasy™. Figure 2 shows the comparison of NaCl solutions and the EC of those solutions. From this curve (Figure 2), a solution with specific EC could be created prepared

(Equation 1). Table 3 provides the NaCl concentrations, the corresponding EC and calculated osmotic potential (MPa) of the solutions (Lenntech, 2012).

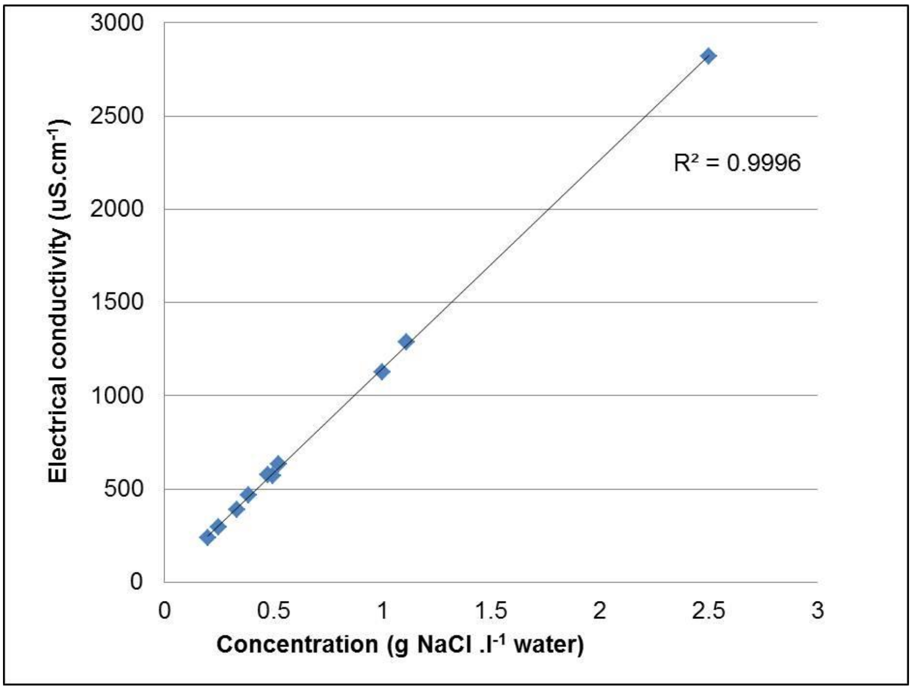

Figure 2. The concentration of NaCl solutions compared with the electrical conductivity of the solution. The trendline R^2 is also given.

$$y = 1119.7x + 24.748$$

Equation 1. Linear equation for the trendline of the concentration of NaCl solutions compared to the electrical conductivity of the solution.

The three solutions described in Table 3, with a control of distilled water, were used in the germination trial, together with the Jacobsen apparatus described previously (Figure 1).

Table 3. Solution characteristics used in the EC gradient germination trial

g NaCl. L^{-1} water ($g.L^{-1}$)	Electrical conductivity ($uS.cm^{-1}$)	Osmotic potential (MPa)
0.208	250	- 0.015
0.423	500	- 0.030
0.642	750	- 0.046

The data was collected as in the previous trials. Germinated seeds were discarded after counting on day 4 and day 10.

Statistical analysis

These trials were designed to conform to a completely randomized design (CRD). Analysis of variance (ANOVA) was determined for all trials using SAS Version 9.2 (PROC GLM) for Microsoft Windows. Data was transformed using arcsine transformation, because germination % was used in the data analysis. Least significant difference (LSD) was calculated at $P< 0.05$.

RESULTS AND DISCUSSION

The standard germination test

The standard germination test was conducted on coated and non-coated lucerne seed to determine the vigour and uniformity of the cultivars. Day 4 results can be interpreted as the predicted vigour for a lucerne seed lot, and should give an indication of uniformity. A seed lot with good vigour can be defined as a seed lot that will germinate and emerge quickly and uniformly under various environmental conditions, which can be found in field situations (Bennett, 2004). However, it is accepted that it is not the only or the best indication of vigour. According to Klos and Brummer (2000a), Klos and Brummer (2000b), seedling height is the best, or the most reliable trait to measure the vigour of a seed lot.

From Table 4 it is noted that there were no statistically significant differences in germination % between coated and non-coated SA Standard seed at day 4 or day 10, when germinated using the “top of paper” method. This suggests that the coating did not influence the germination of SA Standard. This is, however, not true for SuperCuf, where the non-coated seed treatment had a significantly higher germination % at both observations, than the coated treatment. This suggests that the SuperCuf cultivar is more sensitive to the coating technology, either causing physiological restrictions or delayed germination activities. As the germination assessment did not extend beyond 10 days, as specified by ISTA (Anonymous, 2006), it is unclear if the lower germination % is inhibition or delay. If this is due to a delay, an observation period stretching to 12 days would be required. This possible delay is significant in the management and possible selection of the seed coating for this cultivar. Further investigation should indicate if this is due to an osmotic effect or due to toxicity of the seed coating. The 10 day experimental period is, however, sufficient to meet the aims of the experiment and is the period prescribed by ISTA (Anonymous, 2006).

Table 4. The mean germination % of coated and non-coated lucerne cultivars, SA Standard and SuperCuf using the “top of paper” method

Cultivar	Coating	Day 4 (% ± std dev)	Day 10 (% ± std dev)
SA Standard	Coated	[a]89.3 ± 4.8	[x]92.1 ± 3.2
	Non-coated	[a]92.0 ± 3.6	[x]93.6 ± 2.7
SuperCuf	Coated	[B]88.3 ± 4.9	[Y]90.6 ± 4.8
	Non-coated	[A]96.8 ± 2.4	[X]97.6 ± 2.5

*Mean comparisons were done within cultivar and within days

Different letters within a cultivar and germination day indicate significant differences at $P \leq 0.05$

Comparison between the Jacobsen apparatus method and the petri-dish method of germination testing, comparing coated and non-coated seed.

The petri-dish method is flawed for predicting germination responses under field conditions, as the chemicals and the water have nowhere to go and remain in contact with the seed. In field conditions, water has different forces influencing it and its movement in the soil, allowing the movement of solutes away from the seed (Hadas, 2004). The Jacobsen apparatus theoretically allows for some water movement and chemical movement by diffusion. It stands to reason that the chemical potential of the surface where the seed is in the Jacobsen apparatus is less negative than it would be in a petri-dish.

SA Standard showed significant differences between coated and non-coated seed for both methods (Figure 3). The differences between coated and non-coated seed were less defined when the petri-dish method was used as compared to the Jacobsen table method at day 4. This is assumed to be due to the evaporation rate of the surface in the Jacobsen table. The coating material appears to act as a reservoir of water for the seed, while the non-coated seed did not have this reservoir and therefore imbibition was likely to occur at a slower rate in these seeds. This was, however, overcome during the subsequent days. Results at day 10 show that coated seed had a higher germination % than the non-coated seed. Between the methods, however, there were no significant differences between the coated and non-coated seed germinated in the petri-dish and the seed germinated on the Jacobson table.

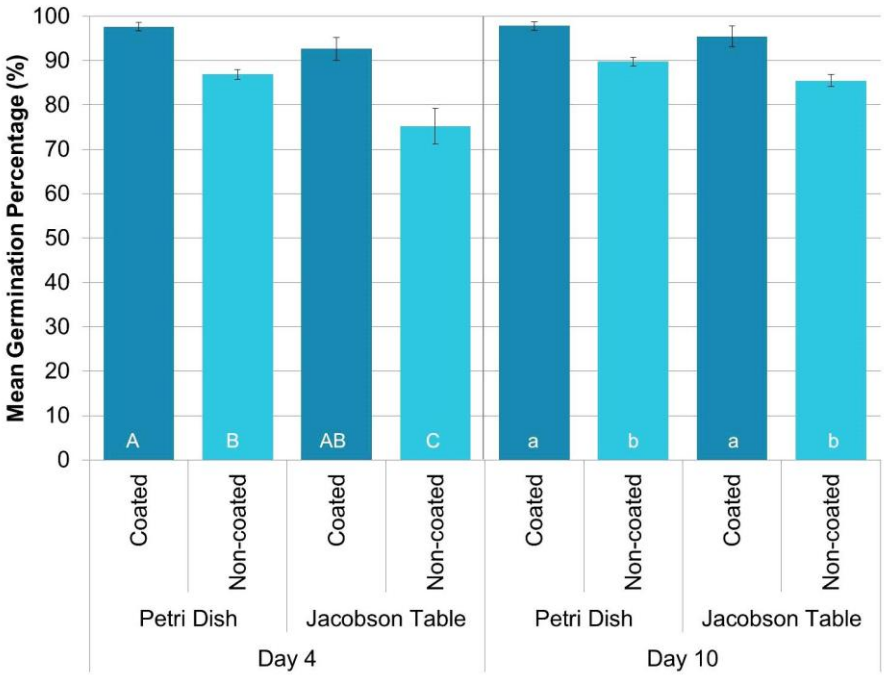

Figure 3. The mean germination % of SA Standard seed, comparing coated and non-coated seed reactions using two methods.

*Mean comparisons were done within days 4 and 10
*Same letters with the same case (A,B,C or a,b,c) are not significantly different.

Figure 4 shows the germination results obtained from SuperCuf seed. The results are different than that found for SA Standard, as the coated seed had a lower germination % for both methods and both days, but this difference was not significant.

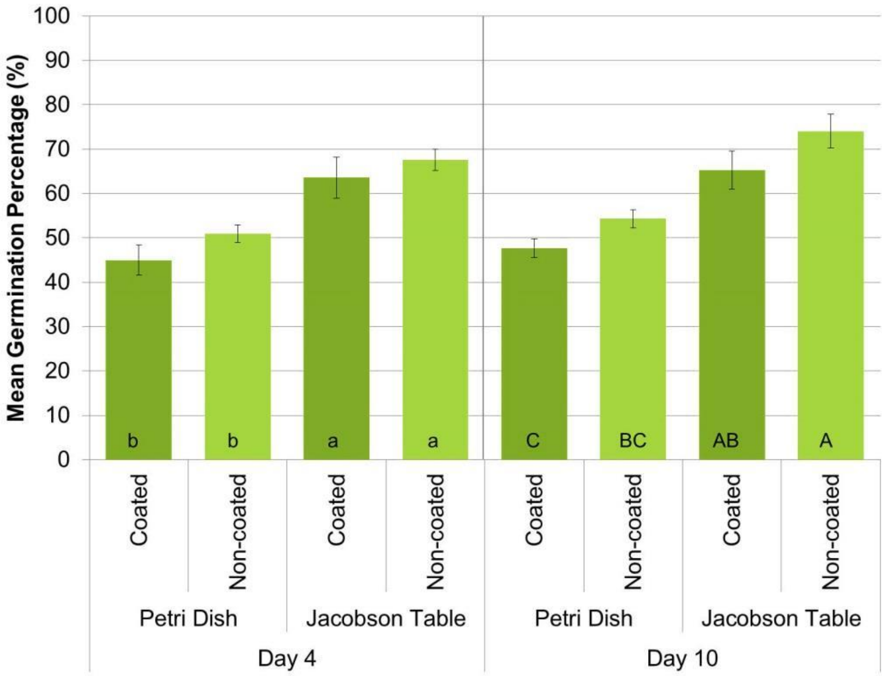

Figure 4. The mean germination % of SuperCuf seed, comparing coated and non-coated seed reactions using two methods.

*Mean comparisons were done within days 4 and 10
*Same letters with the same case (A,B,C or a,b,c) are not significantly different.

The difference in germination %s between the two methods at day 4 and day 10 indicate that the petri-dish method has a negative effect on the germination of SuperCuf. Further investigation is necessary to determine the reason why the petri-dish method influences the germination. Possible reasons might be due to root exudates from the seeds which germinated first, or lower oxygen concentrations due to temporary seals that form between the petri-dish base and lid (Dakora *et al.*, 1993, Hadas, 2004). It is, however, clear that the response of coated and non-coated seed to this method were the same.

It also appears that the coating adds to the inhibition. Diluting the inhibitor, when using the Jacobsen apparatus, increases the germination % for both coated and non-coated treatments.

The germination response of non-coated seed to solutions with different osmotic potentials.

Figure 5 is a representation of what was observed from the cultivars, SA Standard and SuperCuf in terms of germination %, when they are found in conditions of low water potential. A curve from literature was added to compare results from this trial.

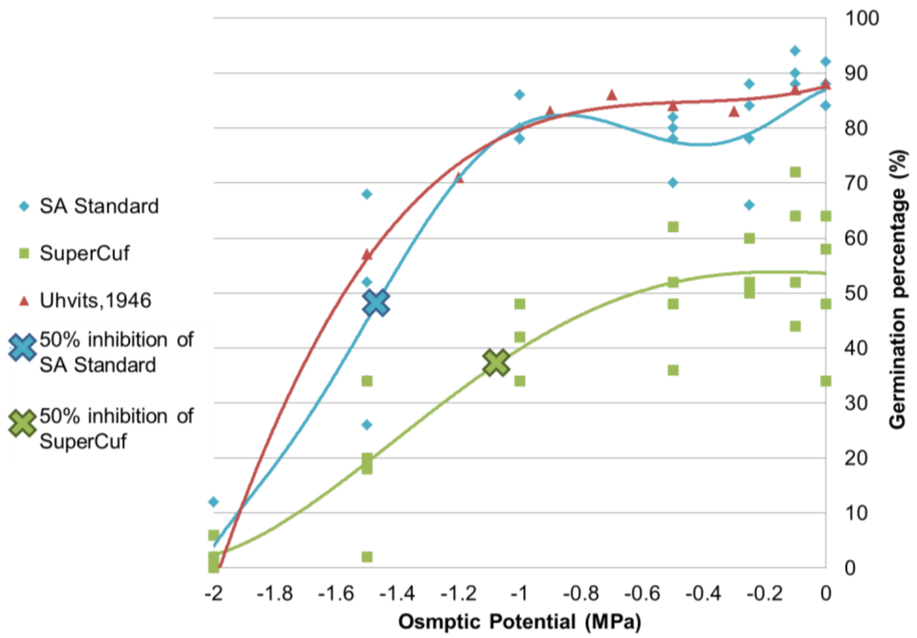

Figure 5. The germination response of SA Standard (R^2 = 0.912), SuperCuf (R^2 = 0.826) and Arizona Chilean, published by Uhvits (1946)**,** to solutions within a range of osmotic potentials, -2, -1.5, -1, -0.5, -0.25, -0.1 and 0 MPa. Markers show the 50% inhibition thresholds.

The SA Standard trendline (chosen for best fit) indicates that there is some variation between 0 MPa and -1 MPa. Neither SuperCuf nor the results from the Uhvits (1946) data show the same variation, and can therefore be due to very low germination percentages observed in two reps. The variation will therefore not be used to describe the osmotic influence on the germination of SA Standard.

From the trendline intercepts of the three curves, the 50% inhibition of germination points was calculated as -1.39 MPa for SA Standard and -1.1 MPa for SuperCuf. The SA Standard curve has a higher threshold for a low water potential than SuperCuf. The sensitivity of SuperCuf to low osmotic potentials is likely the reason why the seed germinated better in the Jacobsen apparatus than in petri-dishes. This threshold is similar to the data from Swagel *et al.* (1997) and Uhvits (1946). The critical water potential for both cultivars is about -2 MPa. It should, however, be stated that according to literature (Maze *et al.*, 1993, McDonough, 1975), the germination initiation could be extended indefinitely as induced dormancy can prevent germination. Parida and Das (2005) stated that osmotic stress, reversibly inactivates metabolic pathways, such as electron transport, due to contraction of intercellular spaces. This trial was only conducted till day 10, according to ISTA guidelines, and delays in germination, longer than 10 days, was excluded from this data (Anonymous, 2006).

Comparing the germination % of coated and non-coated seed at different salt concentrations

Figure 6 shows mean germination % of SA standard seed in response to an EC gradient. It is interesting to note that the substrates with low EC's (distilled and 250 uS.cm^{-1}) are not significantly different when comparing coated and non-coated treatments. It is also worth noting that the substrates with higher ECs (500 µS.cm^{-1} and 750 µS.cm^{-1}), had not only the highest germination %, but also the greatest difference between the coated and non-coated seed.

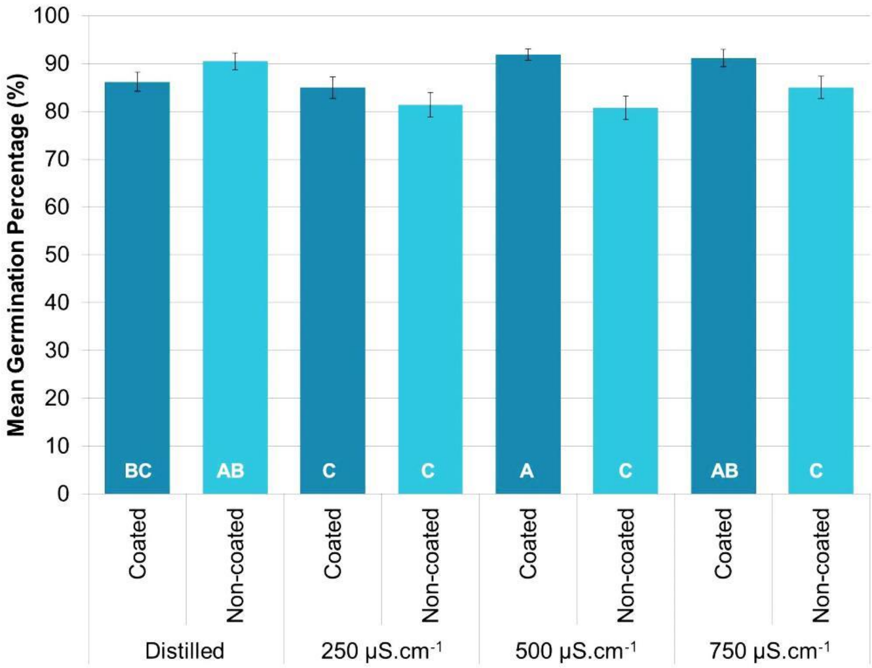

Figure 6. The mean germination % of coated and non-coated SA Standard, using the Jacobsen apparatus, using solutions with increasing electrical conductivity, measured at day 4.

*Bars with the same letters are not significantly different from each other

The influence of nutrients on germination and the interaction of these nutrients with saline conditions are highly complicated. According to Grattan and Grieve (1999), salinity can cause several salinity-induced nutritional disorders by affecting nutrient availability, competitive uptake and partitioning within the plant. Salinity dominated by Na^+ salts can cause deficiencies in Ca, K and P, while Cl^- reduces NO_3^- uptake. Supplementing Ca has shown to enhance germination of a number of species in saline conditions by reducing Na and Cl uptake (Grattan and Grieve, 1999, Cachorro *et al.*, 1994). Only the solutions with no added NaCl (distilled water) had lower germination % for the coated treatment than for the non-coated treatment. This was, however, only after day 4 and a germination delay caused by the seed coating may be the reason of the difference.

Figure 7 shows the impact of an EC gradient on the germination of SuperCuf. There are no significant differences between the coated and non-coated seed treatments and there is no significant difference between the substrates used after 4 days.

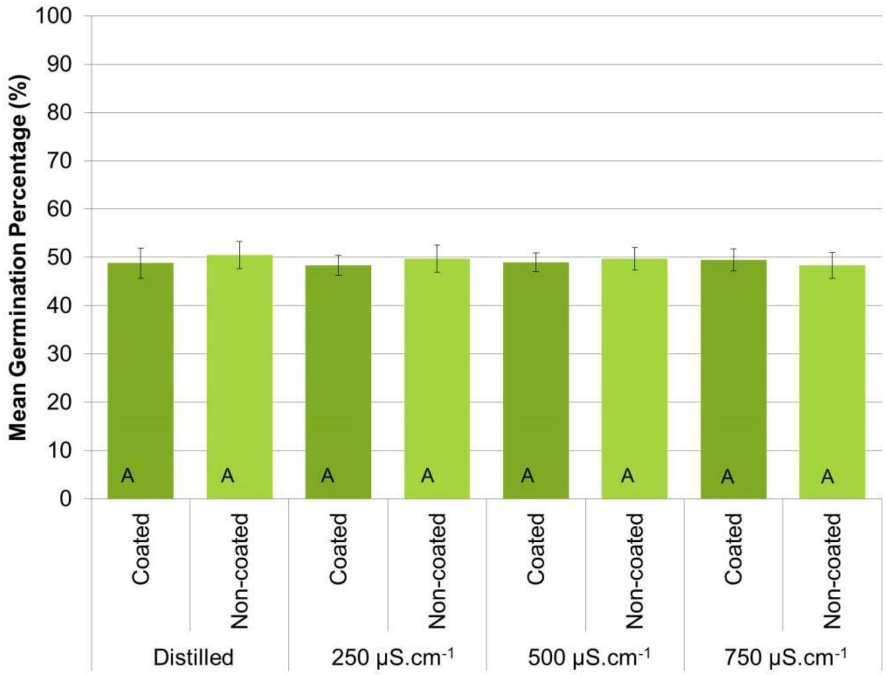

Figure 7. The mean germination % of coated and non-coated SuperCuf, using the Jacobsen apparatus, using solutions with increasing electrical conductivity, measured at day 4.

*Bars with the same letters are not significantly different from each other

The results for SA standard after 10 days show that with solutions of low ECs (distilled and 250 µS.cm^{-1}), there were no significant differences between coated and non-coated seed treatments or between the two treatments (Figure 8). There was, however, a significant difference between coated and non-coated seed for the 500 µS.cm^{-1} and 750 µS.cm^{-1} treatments, with coated seeds showing significantly higher germination % than uncoated seeds. There appears to be a reaction between the coating and the NaCl, which facilitates germination in these seeds. Further investigation is required to determine how different salts, such as sulphate containing salts, dominant in some mining areas, will react with the coating. According to Ungar (1978) and Uhvits (1946), NaCl salt causes ion toxicity in the seed and the decrease in germination % is partly due to this. These authors added that other salts, such as $MgSO_4$ and MgCl, showed higher toxicity for lucerne in isotonic solutions. It appears that the EC and the ion toxicity both play a role in germination %, but the interaction and subsequent results of other salts and the seed coating still needs to be investigated.

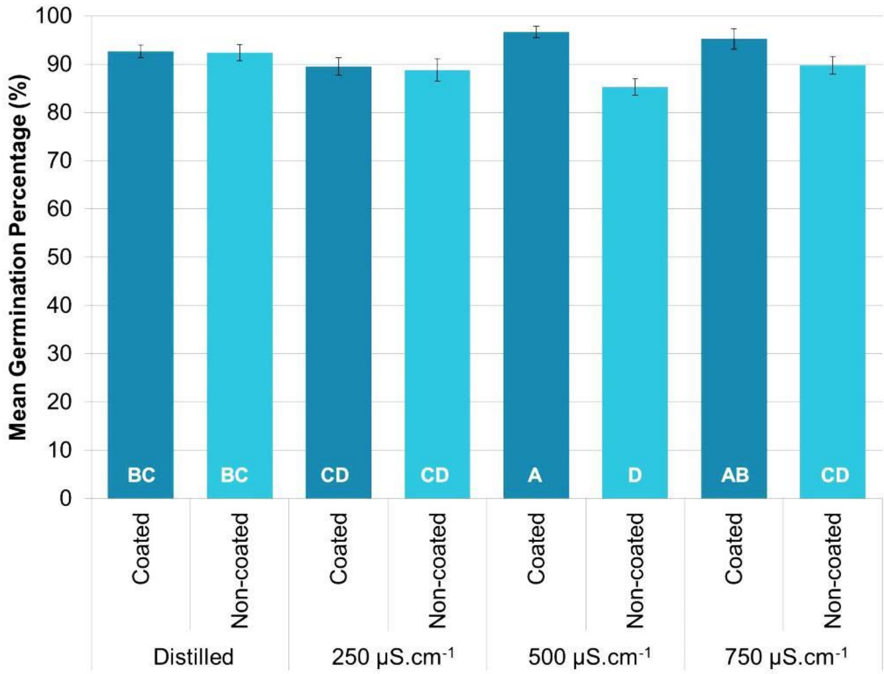

Figure 8. The mean germination % of coated and non-coated SA Standard, using the Jacobsen apparatus, using solutions with increasing electrical conductivity, measured at day 10.

*Bars with the same letters are not significantly different from each other

The results did, however, differ when SuperCuf (Figure 9) was compared with SA Standard (Figure 8). There were no significant differences between the salt treatments and also no significant difference between the coated and non-coated treatments. This suggests that, at the level tested in this study, salinity did not influence the germination. Depending on the adaptation of the cultivars, the reaction to salinity differs between cultivars, and should therefore be determined. SA Standard, the landrace, has a larger genetic biodiversity and might be more able to adapt to different environmental conditions than SuperCuf (Fairbanks and Andersen, 1999, Guines *et al.*, 2003, Katiċ *et al.*, 2004).

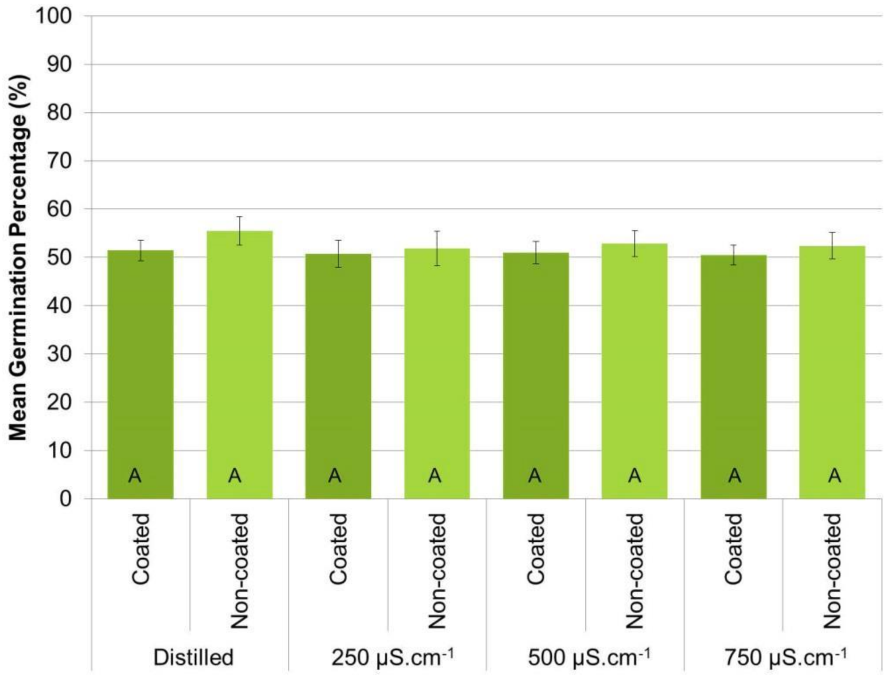

Figure 9. The mean germination % of coated and non-coated SuperCuf, using the Jacobsen apparatus, using solutions with increasing electrical conductivity, measured at day 10.

*Bars with the same letters are not significantly different from each other

CONCLUSION

The germination % of coated SuperCuf, was found to be significantly lower than the non-coated treatments, when germination % was determined using petri-dishes. Using a new seed lot, it was determined that the difference between the coated and non-coated treatments were not significant. However, a significantly higher number of seed germinated when using the Jacobsen table. Coated SA Standard reacted differently to SuperCuf in that the germination % was higher than the non-coated treatments for both methods tested. It is therefore clear that SuperCuf is more sensitive to its environment, due to the constituents of the coating or possible exudates during germination. This theory is also supported by the response of the non-coated seed to and osmotic gradient. SuperCuf reached the 50 % inhibition point at -1.1 MPa, while SA Standard reached this at -1.39 MPa. This difference can be explained by the dehydration of the seed during maturation and storage. The lower water potential inside the seed facilitates imbibition even at lower osmotic potentials surrounding the seed.

It was interesting to observe that the germination of SuperCuf showed no difference between coated and non-coated treatments over a range of saline conditions. This suggests that SuperCuf is not as sensitive to saline conditions as it is to high osmotic pressure. This also suggests that the conditions created by the Jacobsen table overcame the inhibition of germination and that seed coating does not add significantly to this inhibition.

SA Standard did, however, show more variation between coated and non-coated treatments under the influence of NaCl salinity. It was observed that the salt concentration influenced the non-coated SA Standard, but the coated treatments were not influenced. This suggests that the lime in the coating might counter the toxic effects of NaCl, but require further investigation.

From these trials, it can be concluded that the method of testing the germination % can have a significant influence on the results. This influence can vary depending on the cultivar. Further investigation is required to determine whether osmotic potential is the cause and whether exudates cause the change in osmotic potential and inhibition.

These observations also suggest that the coating and NaCl interacts, thereby reducing the negative influence the salinity can have on germination. The effect of salinity on the germination of different cultivars will determine the magnitude of the response of the coating to the conditions, as observed between SA Standard and SuperCuf.

ACKNOWLEDGMENTS

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CHAPTER 3

Prepared according to African Journal of Range and Forage Science guidelines

Seedling emergence of coated and non-coated *Medicago sativa* L. seed in different growth environments

Leana Nel and Wayne Truter

Abstract

Emerging seedlings, are very sensitive to factors such as drought, salinity and chemical damage from agro-chemicals. Poor or non-uniform emergence or survival of seedlings can cause a great increase in production costs with an increase in management requirements, such as weed control. A seed coating can be used to provide nutrients to seedlings, necessary to establish a strong plant. The interaction between the seed coating and the seed's environment must be investigated to determine if the seed coating has a beneficial effect and under which conditions these treatments are most beneficial. The interaction of growth medium composition and nutrient availability is a well-known concept. The growth medium as the reservoir of water and nutrients and the soil water quality, especially salinity, was investigated in this study. Salinity, caused by irrigation or over fertilization of cultivated lands, is one of the factors causing non-uniform emergence. Two trials were conducted to determine the influence of coating on the emergence of lucerne seedlings, using two cultivars, SA Standard and SuperCuf. These two cultivars will help in determining whether there is a significant difference in response between cultivars. Firstly, the emergence percentage was determined in different growth media, namely a commercial growth medium, a sandy loam and silica medium. To further explore the influence of salinity on the impact of seed coatings on the emergence of lucerne seedlings, a trial was conducted in a sandy loam soil, irrigated with different saline water treatments. It was determined that the influence of the seed coating on emergence of seedlings depended on the growth medium. The emergence of seedlings is mostly dependent on nutrients in the cotyledons. Growing conditions which caused lower emergence % for non-coated SA Standard seeds (commercial growth media) was, however, overcome by using coated seed. In the saline irrigation trial, it was found that the coating did not always influence emergence. There was an interaction between the sandy loam growth medium and the saline irrigation water, manifested in the emergence of lucerne. When distilled water and water with 750 $\mu S.cm^{-1}$ was used, higher emergence was observed compared with treatments irrigated with 250 and 500 $\mu S.cm^{-1}$ water. Coated SuperCuf had a higher emergence % than the non-coated treatments when irrigated with the 750 $\mu S.cm^{-1}$ water. It can therefore be concluded that seed coating does have an influence on emergence, but it depends on the growth medium quality and possible saline conditions present.

Keywords

Emergence, irrigation water quality, seed coating, salinity, electrical conductivity.

Introduction

Lucerne (*Medicago sativa* L.) germination and emergence from the soil is very important when considering the subsequent yield of a crop. This species can compensate for poor emergence, with tillering, but only to an extent. These compensations can, however, only be achieved under good management practices, but will require additional input costs which cannot always be justified. Extra input costs as a result of fertilizers and irrigation cannot be justified to overcome poor establishment due to poor germination and emergence (Finch-Savage 2004, Kaydan and Yagmur 2008).

An aspect related to emergence, which can be applied in management and planning of crop stands, is the plant density which has a strong correlation with total yield of a stand, plant size and plant uniformity. As the number of emerged seedlings per unit area increases, the total yield increases, but at the same time the density increases, the plant size decreases, eventually reaching a plateau at high plant densities (Figure 1, (Finch-Savage 2004)). Keeping these effects in mind, it is important to use cost analysis, which take yield, input costs and plant size (yield per plant) into account, when determining an appropriate sowing density. A non-uniform stand can add to the cost analysis equation, by affecting yield through non-uniform plant sizes at harvest (Finch-Savage 2004).

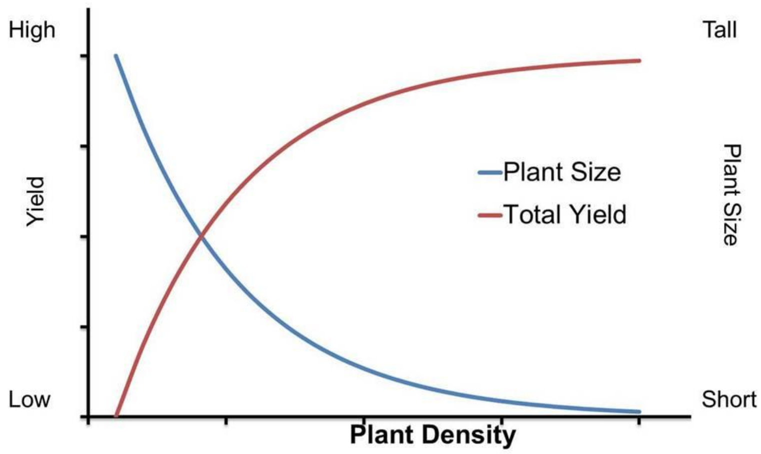

Figure 1: Schematic illustration showing the interactions between plant size and total yield as influenced by plant density, modified from Finch-Savage (2004)

In order to optimise successful lucerne stand establishment, it is important to understand the impact of environmental factors on seed germination and seedling emergence and to be able to apply this knowledge practically in the field. Factors, such as planting depth, seedbed preparation, soil characteristics and availability and quality of water all influence the germination and emergence of the seedlings and should be integrated in the establishment program.

When considering growth media as a three-phased system, the matrix comprises of solids, which are the weathered primary parent material, electrically charged clays, which are weathered from the primary particles, and organic matter. Due to the shape, size and charge of the solids, the pores formed between the solids are filled with water containing solutes from the soil or gases. The clay fraction of the growth medium exerts the greatest influence on the growth medium characteristics, as the chemical charges are responsible for cohesion and adhesion forces. The size of the clay fraction is related to pore sizes and therefore the bulk density of the growth medium. The characteristics of the solid phase consequently creates specific medium qualities such as soil strength, bulk density, water movement and the subsequent aeration regime (Hadas 2004).

It is clear that growth data and the growth environment should not be separately interpreted, as the one influences the other. When considering soil compaction as an example; compacted soils have a reduced number and size of pores, which decreases the infiltration rate of water, gaseous exchange and increases the resistance of penetration, which limits seed germination and seedling establishment (Hadas 2004). Penetration of roots into compacted soil is one of the many methods to alleviate soil compaction by its interactions with the environment, and has been shown to decrease water runoff and increase water infiltration (Mosebi 2010).

One of the important roles of a growth medium is to act as a reservoir for water for the plant. The amount of water available to the plant in the growth medium is related to the number and size (influenced by the clay fraction) of the pores and the forces that act on the water adsorbed, namely gravity, osmotic, hydrostatic and matrix forces and swelling from the soil clay-water interactions. The movement of water in its free form into a growth medium, as influenced by the forces mentioned above, is termed soil water potential. The availability of water to the seed for germination in the growth medium can similarly be defined, but gravitational and hydrostatic forces can be ignored (Brady and Weil 2002, Hadas 2004).

The difference between germination trials conducted in water and those done in growth media are that the water available to the seeds in growth media are at a negative pressure and requires metabolic energy for the uptake of water (Hadas 2004). The forces acting on the water around the seed are the focal points in management for optimizing the emergence of the crop. Seedbed preparation and planting depth can be considered as tools to manipulate matrix and envelope forces, whilst osmotic soil water potential can be manipulated by the amount of water and the quality of the water added through rain and irrigation (Hadas 2004). If the soil water potential can be managed above the base water potential at which germination will not take place, temperature becomes the most influential factor for successful germination and emergence (Bennett 2004).

The two main causes of osmotic stress, salinity and drought, have large effects on agricultural land and can cause non-uniform germination and emergence, resulting in non-uniform stand establishment and reduced yield (Munns and Termaat 1986, Kaydan and Yagmur 2008, Munns and Tester 2008, Soleimani*, et al.* 2011). Osmotic stress, due to salinity, can be less severe than drought stress at the same water potential, due to the ability of the seed embryo to absorb cations, such as Na^+, thereby maintaining a water potential gradient which supports water uptake. This accumulation does, however, cause problems such as ion toxicity, nutrient imbalances and cellular damage as a result of salt accumulation in intracellular spaces (Kaydan and Yagmur 2008, Soleimani*, et al.* 2011). Depending on the cause of the salinity, there are tools that can be used to manage this condition, such as reducing irrigation, or improving irrigation water quality (Brady and Weil 2002, Al-Busaidi and Cookson 2003, Aydinsakir*, et al.* 2013).

Seed treatments have been used to overcome some effects of salinity on the developing plants and subsequent yield (Ashraf and Foolad 2005). Seed pelleting or coating is a technology that was developed in the 1940's to manipulate the shape of the seed to improve handling ability and accuracy in planting. The material surrounding the seed can be species specific in order to enhance the effect of the coating on the success of establishment. Coatings may contain nutrients, growth enhancers and substances to provide energy to the seed and seedling, but also substances which can improve water holding capacity (Halmer 2004, Ashraf and Foolad 2005). Coatings, for species such as rice, can contain peroxides which can supplement oxygen in submerged environments (Ashraf and Foolad 2005). Seed coatings can therefore be very useful in improving conditions for germination and seedling emergence by interacting with the water, soil and plant.

The influence of water quality, growth media and seed coating on the emergence of lucerne can be used to understand and manage the establishment of lucerne and be able to troubleshoot from the data available. The research available on the seed coating of specific species and its interactions in the environment is limited. The aim of this trial was therefore to evaluate the impact of seed coating on the emergence of lucerne seedlings in different growth media and under saline conditions. We hypothesised that there would be no significant difference in emergence percentage between coated or non-coated seed. It was also hypothesised that the emergence would not be dependent on the growth medium into which the seed is sown. A third hypothesis was that there would be interaction between the salinity and seed coating resulting in a higher emergence percentage for coated lucerne seed.

Materials and methods

Two trials were conducted, to determine the influence of coating on the emergence of lucerne seedlings in a phytotron on the Hatfield Experimental farm (University of Pretoria). Two seed treatments namely coated and non-coated seed, of the cultivar

SuperCuf and the landrace, SA Standard were used in the trial. Table 1 shows the characteristics of these two cultivars. The seed coating contained lime, nutrients, an insecticide, a fungicide and binding polymers. The seed coating were the same for both cultivars. The same seed lots were used for each cultivar for both trials and for the coated and non-coated seed treatments.

Table 1: Lucerne cultivars SA Standard and SuperCuf characteristics

Cultivar	Dormancy	Origin	Characteristics
SA Standard	4-6	South African Landrace	Intermediate crown position Coarse to medium stems Exceptionally high resistance to root and crown-rot complex and other root and crown diseases
SuperCuf	9	Australia: Cuf101 cross with Sequel	Leafy stems Strong autumn and spring growth Strong regrowth after harvesting

Trials were planted in polystyrene seedling trays (7 x 15 pits) with a volume of 200ml per well and seedling. Trays were randomly arranged in the phytotron. Each well could freely drain, but to prevent excessive evaporative losses the trials were covered with plastic at 0.45 m above the trays. The treatments were watered every second day with equal amounts applied per plant (approximately 10ml per plant).

The influence of seed coating on seedling emergence and survival in different growth media

The objective of this trial was to determine if the growth medium has an influence on the emergence of lucerne seedlings and if the seed coating will have an impact on seedling emergence. For this trial, three growth media were used, namely an inert growth medium (silica sand), an ‘ideal’ growth medium (commercial seedling mixture) and an agricultural soil (sandy loam). The commercial seedling mixture (obtained from Hygrotech, Pretoria) is a combination of vermiculite, peat and nutrients, which would limit nutrient deficiencies and its water holding capacity creates a long lasting reservoir for developing seedlings. The agricultural soil was taken from an area (-25°44'55.8924", 028°15'32.3352") that was cultivated with lucerne, and was left uncultivated for a few seasons. The agricultural soil pH (H_2O) was approximately 6, the mean electrical conductivity (EC) was 100 µS.cm^{-1} and the results of a nutrient analysis are given in Table 2. None of the growth media were ameliorated. There were seven replicates for each combination of cultivar, seed treatment and growth medium. Seedling emergence was determined 20 days after planting.

Table 2: Soil nutrient analysis of the agricultural sandy loam soil

P Bray I mg.kg^{-1}	Ca mg.kg^{-1}	K mg.kg^{-1}	Mg mg.kg^{-1}	Na mg.kg^{-1}
23.4	222	128	67	4

The impact of seed coatings on seedling emergence as influenced by solutions with different Electrical Conductivities (EC)

The objective of this trial was to evaluate the influence of saline irrigation water on seedling emergence of coated and non-coated seed. Water with four different salt concentrations (NaCl) was used to create saline conditions in the growing media. NaCl was chosen as it is the most abundant salt in saline soils and is often used to determine salt tolerance in plants (Munns and Termaat 1986, Hu and Schmidhalter 2005, Riadh*, et al.* 2010). The saline solutions, as described in Table 3, were used to irrigate every second day through-out the trial period. The control was distilled water. This trial used the agricultural soil (sandy loam) as a growth medium to indicate interactions between growth medium and water quality, in order to identify the effects water quality have on seedling emergence under agricultural conditions.

Table 3: Saline solutions used to create an electrical conductivity (EC) gradient for the seedling emergence trial

Salt concentration (g.L^{-1})	Electrical conductivity (µS.cm^{-1})	Water potential (MPa)
0	1.75	-0.000
0.208	250	-0.015
0.423	500	-0.030
0.642	750	-0.046

Seedling emergence (the number of living seedlings at each counting date) was counted at three intervals, namely 5, 10 and 15 days after planting. This provides an indication of vigour and survival of these seedlings up to day 15 after planting.

Statistical Analysis

These trials were designed to follow the principles of a randomized block design. All emergence percentage data were transformed by using arcsine transformation and were statistically analysed using PROC GLM. Statistical analyses were performed using SAS Version 9.2 (SAS 2002-2008) for Microsoft Windows. Least Significant Differences were calculated at $P \leq 0.05$. The t groupings shown in the data figures are from the transformed data, while standard deviation bars are from untransformed data.

Results and discussion

The influence of seed coating on seedling emergence and survival in different growth media

The influence of seed coating and growth medium and the interaction of the two treatments on the emergence and survival of seedlings can have significant consequences on yield (Finch-Savage 2004) and stand efficiency in terms of water and nutrient use efficiency (Van Oudshoorn*, et al.* 2001). It is therefore essential to understand the effect of these two variables on plant growth when considering recommendations and management decisions.

Comparisons between growth media (Figure 2), revealed that seedling emergence in coated SA Standard seed was not affected by growth medium, but the non-coated treatment show significant differences, with a lower seedling emergence percentage in the commercial growth medium, as compared to the sandy loam ($P \leq 0.0132$) and silica sand ($P \leq 0.0129$) media. Seedling emergence was significantly higher in the commercial growth medium when the seed was coated, as opposed to non-coated seed. This data suggests that the commercial growth medium, even though it would not be used for lucerne cultivation, is not ideal for non-coated lucerne (SA Standard) emergence and that the cause of the lower emergence and survival is overcome by the coating technology. Possible explanations for this difference in emergence are the concentration or composition of nutrients in the growth medium, such as the ratio between cations (Na^+, Ca^{2+}, K^+, Mg^{2+}) which influences plant growth by acting on biophysical and metabolic components (Hu and Schmidhalter 2005). There was, however, no significant difference in the seedling emergence percentage of SuperCuf coated and non-coated seed in the commercial growth medium.

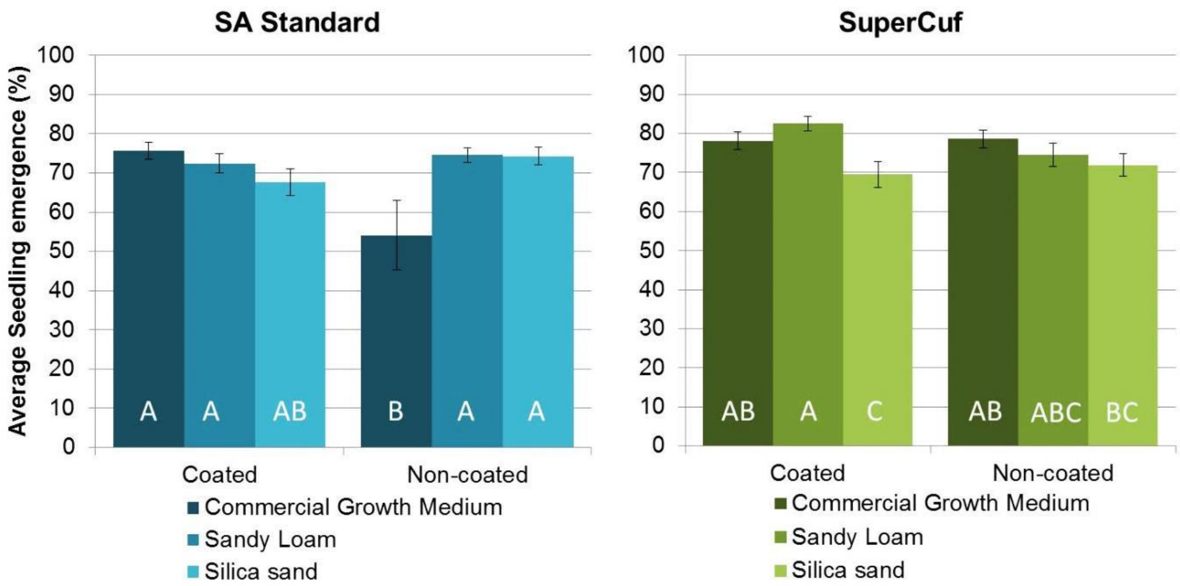

Figure 2: Influence of seed coating on emergence of SA Standard and SuperCuf at 20 days after sowing in different growth media, namely a commercial growth medium, sandy loam, and silica sand.

*Mean comparisons were done within the cultivar treatments
*Same letters are not significantly different

There were no significant differences in seedling emergence of non-coated SuperCuf seeds in different growth media (Figure 2). However, the seedling emergence from coated seed was significantly lower in silica sand than the sandy loam growth medium ($P \leq 0.0046$). The highest seedling emergence was observed for coated seed in the sandy loam growth medium ($P \leq 0.0403$).

It is interesting to observe that the inert silica growth medium did not show significant inhibition of seedling emergence in the non-coated treatments. This suggests that the nutrients required by the seedling for emergence is supplied by the reservoir in the cotyledons and under these conditions the coating material did not benefit the emergence of lucerne. This is likely due to the limited area for chemical reactions on the silica particle surface, as it is an inert material (Brady and Weil 2002). The difference between SA Standard and SuperCuf, in terms of seedling emergence in different media, clearly shows that the two cultivars are not influenced to the same extent by the growth media and seed coating. The commercial growth medium shows inhibited emergence in non-coated SA Standard, but did not for SuperCuf, whilst emergence of coated SuperCuf in the silica sand medium is significantly lower than the other growth mediums.

The emergence of coated and non-coated seed in response to saline water treatments

The influence of saline conditions, created by continuous irrigation with saline irrigation water, on seedling emergence percentage is shown in Figure 3. It is evident that salinity had a greater impact on seedling emergence than the growth medium, but the interaction between the growth medium and salinity does play a role in determining the role of the seed coating in seedling establishment.

At 10 days after sowing there were no differences between coated and non-coated SA Standard treatments for the individual water treatments. There were, however, significant differences, due to lack of emergence, between the distilled water control and the 250 $\mu S.cm^{-1}$ ($P \leq 0.0032$; $P \leq 0.0011$) and 500 $\mu S.cm^{-1}$ ($P \leq 0.001$; $P \leq 0.0005$) treatments, but no significant differences between the control and the 750 $\mu S.cm^{-1}$ ($P \leq 0.287$) treatment for both the coated and non-coated seed, respectively.

This trial showed that coated and non-coated SA Standard seedlings emerged similarly when treated with a range of saline solutions. It is, however, interesting to note that emergence was highest where distilled water and 750 $\mu S.cm^{-1}$ water was used. Emergence was significantly lower at 250 $\mu S.cm^{-1}$ and 500 $\mu S.cm^{-1}$ for both coated and non-coated treatments. The similar reactions between the distilled treatment and the 750 $\mu S.cm^{-1}$ can possibly be explained by the Ca loving nature of lucerne. Calcium plays an important role in germination and emergence in saline conditions by protecting the tissue from Na and Cl uptake and subsequent damage (Cachorro*, et al.* 1994, Grattan and Grieve 1999, Vaghela*, et al.* 2009). According to Brady and Weil (2002), the Na^+ cations are easily displaced by other cations from the clay particles. If the soil solution contains a high concentration of Na, more strongly adhered cations may be displaced, making it more available to plants. If this hypothesis is true, Ca will become more available when 750 $\mu S.cm^{-1}$ water is applied as irrigation. Calcium is readily available to the seedling in distilled water and do not have to compete with other cations, therefore reacting similarly to 750 µS.cm^{-1}. This hypothesis, however, has not been confirmed with soil analysis.

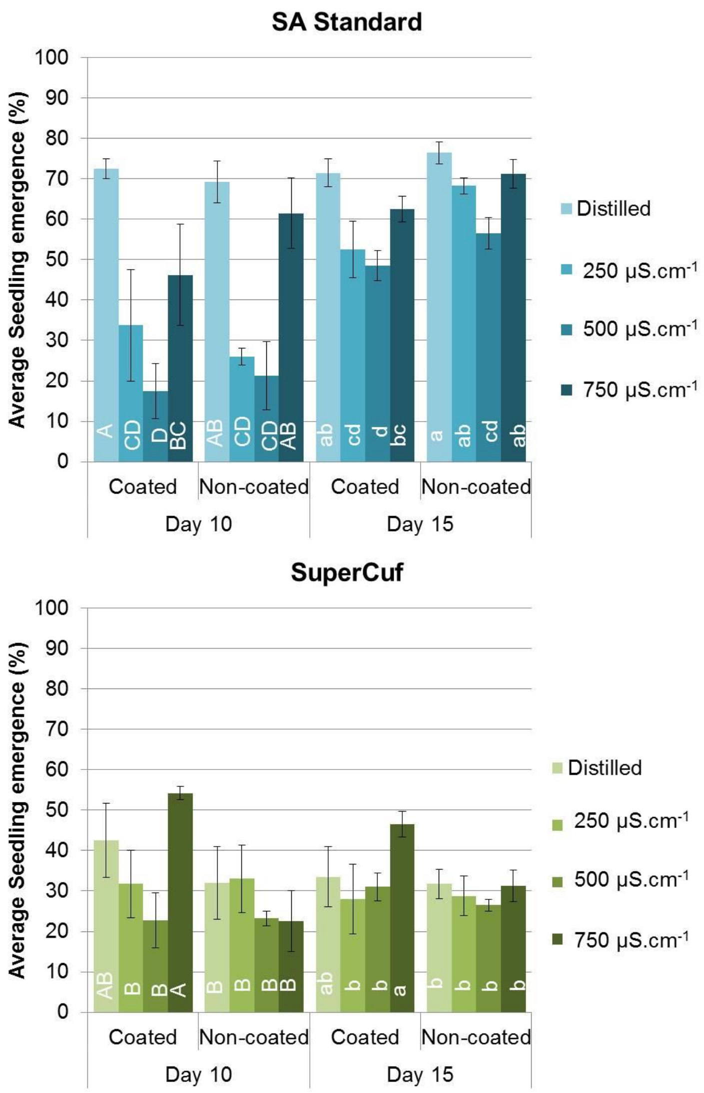

Figure 3: Influence of irrigation water quality on emergence of lucerne seed coating in SA standard and SuperCuf varieties grown in an agricultural sandy loam soil 10 days after sowing.

*Mean comparisons were done within days 10 and 15
*Same letters with the same case (A, B, C or a, b, c) are not significantly different

SA Standard coated seed did, however, show significant variation when 250 µS.cm^{-1} ($P \leq 0.0123$) was used as irrigation at 15 days after sowing. The lag in emergence at 250 µS.cm^{-1} and 500 µS.cm^{-1} is overcome, even though it remains lower than the distilled and 750 µS.cm^{-1} water treatments. It is not known if the difference would have been overcome if the experiment continued to day 20 or 25.

The non-coated SA Standard seed showed a greater recovery in the emergence of the seedlings after 15 days than the coated seed. The greatest recovery was observed in the 250 µS.cm^{-1} treatment, with an increase of 42%. This recovery is the main reason for the variation between coated and non-coated seed emergence at 250 µS.cm^{-1}. The limited recovery of coated seed suggests there might be more abnormal seedlings (as defined by ISTA ((Anonymous 2006)) due to chemical damage or metabolic interference at the 250 µS.cm^{-1} and 500 µS.cm^{-1} irrigation water treatments.

There were also significant differences between emergence of non-coated seed when comparing 500 µS.cm^{-1} water irrigation with distilled water ($P \leq 0.0009$), 250 µS.cm^{-1} ($P \leq 0.0469$) and 750 µS.cm^{-1} ($P \leq 0.0118$) irrigation treatments. This suggests that the 500 µS.cm^{-1} water treatment had the greatest influence on emergence of SA Standard. This observation is true for emergence of both coated and non-coated seed.

The responses of coated and non-coated SuperCuf to salinity treatments were different to those of SA standard. For the initial measurements (10 days after planting), significantly higher emergence was observed for the coated treatments as compared with non-coated seed at the 750 µS.cm^{-1} water treatment ($P \leq 0.0038$). In addition, there was a significant difference in the emergence of coated seed subjected to 750 µS.cm^{-1} irrigation water and all the other irrigation treatments. At this time there were no significant differences in seedling emergence for non-coated seeds.

Emergence of seedlings 15 days after sowing, was similar to day 10, where treatments watered with 750 µS.cm^{-1} water showed significantly higher emergence percentages for coated than non-coated seed ($P \leq 0.0406$). Even though the survival of seedlings from coated seed was lower at 750 µS.cm^{-1} water, there was very little change in the emergence percentages in the other treatments and the emergence percentage was still significantly higher in the 750 µS.cm^{-1} treatments. Seedling emergence in non-coated seed revealed no significant differences between any irrigation treatments.

This suggests that coating has the highest influence on SuperCuf emergence at 750 µS.cm^{-1}. At this high saline content, it appears the coating stimulated emergence, as this observation is even higher when irrigated with distilled water (P≤ 0.0816). Further investigation is required to understand this reaction.

Conclusions

The results from this study indicate that the two cultivars react differently to the coating, growth medium and saline conditions and the interaction effects. It is however clear that the chemical composition of the growth medium and the surface area where chemical reactions can take place is important. The commercial growth medium proved to have negative effects on emergence for non-coated SA Standard, while this was not found for non-coated SuperCuf. The negative effect observed in the commercial growth medium for non-coated SA Standard was not observed for the coated treatment. This indicates that under those conditions the coating provided protection or nutrients to overcome the inhibition caused by the growth medium. Growth medium characteristics which cause poor growth for lucerne and its symbiotic inoculant, such as clay content more than 35%, should not be overlooked when planting with coated seed, as these conditions will still have a major influence on the growth of lucerne.

The quality of the irrigation water played a large role in the emergence of the seedlings. Even though the two cultivars did not show similar results, the seedling emergence of both cultivars was influenced by the saline conditions. The interaction of the water quality and the coating appeared to have a limited effect on the emergence. Differences were observed on day 15 for SA Standard irrigated with 250 µS.cm^{-1} and SuperCuf irrigated with 750 µS.cm^{-1}. The water qualities with the greatest influence on SA Standard emergence are both the 250 µS.cm^{-1} and 500 µS.cm^{-1} water treatments, whilst the highest emergence was recorded for distilled water and 750 µS.cm^{-1}. The non-coated SuperCuf treatments showed limited variation between water quality treatments, while coated treatments showed significant variation between the 750 µS.cm^{-1} and the other water quality treatments.

Even though there was no clear trend to highlight the importance or the influence of the variables, it is clear that further investigation is required to determine under which growth conditions the coated seed would have a significant benefit to the emergence of lucerne. The individual observations where coating was beneficial to emergence was a result of complex growth conditions with unknown parameters, preventing clear conclusions to be made.

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Chapter 4

Prepared according to Agricultural Water Management guidelines

The physio-morphological characteristics of Lucerne (*Medicago sativa* L.) seedlings, influenced by seed coatings

Leana Nel, Wayne Truter and Nicolette Taylor

Abstract

Using seed coatings to change edaphic conditions, in order to optimize seedling growth and establishment, has been the challenge of many scientists and seed companies. The interaction of these changes with saline conditions may be advantageous to growth and the establishment of plants, or it may just provide what is required for the plant to adapt to these saline conditions. A pot trial was conducted to determine differences in physio-morphological characteristics (stem height, leaf area etc.) of lucerne caused by saline conditions, when treated with municipal (180 µS.cm^{-1}), 500 and 750 µS.cm^{-1} irrigation water. Two lucerne cultivars, SA Standard and SuperCuf, with two seed coating treatments, coated and non-coated, were used in this trial. The physio-morphological characteristics were highly correlated. Differences in the correlation were found between coated and non-coated seed treatments, when irrigated with 500 µS.cm^{-1} water. When comparisons were made between irrigation water treatments within the coated seed treatment, it was found that the correlation between the shoot dry matter yield and the other parameters measured was low when irrigated with 500 µS.cm^{-1} water. When the same comparisons were made for the non-coated seed treatment, a low correlation was evident between the leaf area and the other measured parameters, when irrigated with 500 µS.cm^{-1} water. It was concluded that the tolerance mechanism for salinity for plants irrigated with 500 µS.cm^{-1} water, caused more differences than the other water treatments.

Keywords: stem height, leaf area, dry matter yield, root growth

1. Introduction

Soil salinity is a global concern as it influences food security by decreasing productivity of many crop and forage species (Ahmad et al., 2010; Ashraf and Harris, 2004; Brady and Weil, 2002; Cheeseman, 1988; Parida and Das, 2005; Riadh et al., 2010). Arid and semi-arid regions are especially affected by salinity as precipitation usually does not overcome evaporative losses, which leaves dissolved salts behind in the soil. Irrigation aggravates the situation by adding salts to the soil system (Brady and Weil, 2002; Munns and Termaat, 1986; Munns and Tester, 2008). Irrigation and land clearing, causes the water table to rise, resulting in an accumulation of salt in the root zone (Munns and Tester, 2008). A third cause of soil salinity is the movement of salt containing water, through the soil by gravity and a water potential gradient. Low-lying areas are often more afflicted by salinity due to this movement (Brady and Weil, 2002; Munns and Tester, 2008).

Soil salinity is described as an accumulation, or concentration, of soluble mineral salts in volume or weight units, while sodicity specifically describes Na^+ ion concentration (Al-Busaidi and Cookson, 2003). Sodium chloride is the most abundant salt released from parent rocks and is also the most soluble salt, prompting plants to develop mechanisms to regulate the uptake and accumulation of Na^+ and Cl^- in its tissues (Munns and Tester, 2008; Parida and Das, 2005). Most plants actively exclude these ions while absorbing water from the soil (Munns and Tester, 2008; Parida and Das, 2005). The extent of this exclusion is dependent on the adaptation of the plant (Munns and Tester, 2008). Halophytes such as salt bush (*Atriplex amnicola*) are highly efficient in exclusion of salt and will still be able to grow at salt concentrations greater than seawater (Munns and Termaat, 1986; Munns and Tester, 2008). Lucerne (*Medicago sativa*) is very salt tolerant and will only stop growing when salt concentration reach levels between 400 and 500mM NaCl (Munns and Termaat, 1986; Munns and Tester, 2008). The production and tolerance of crops in saline conditions is shown in Table 1. In this Table lucerne is listed as not being very salt tolerant, but it should be noted that tolerance varies greatly between cultivars (Lattimore, 2008).

According to Munns and Tester (2008), there are two kinds of plant stresses associated with saline soils, namely osmotic stress, which is due to an osmotic effect at the soil and root interface, and ionic stress, which is due to toxicity of the salt ions to plant tissue. Osmotic stress is rapid and also has the greatest effect, which usually manifests as a decline in shoot growth. Dicotyledon plants such as lucerne, show a notable decreases in size of individual leaves in response to osmotic stress. Ionic stress develops slower, due to the time required for salt to accumulate in the plant tissue. Symptoms of ionic stress are usually observed by the senescence of older leaves. If growth is stunted to the extent that new leaves can't replace the dying leaves fast enough, photosynthesis will not be able to supply carbohydrates to the developing leaves, creating a cycle which would ultimately result in the death of the plants (Munns and Termaat, 1986; Munns and Tester, 2008; Riadh et al., 2010). Detailed responses to salinity stress and the selection of tolerant plants are discussed in reviews by Munns and Termaat (1986), Parida and Das (2005), Munns and Tester (2008) and Riadh et al. (2010).

Table 1

The salt tolerance of some crop and pasture species (Lattimore, 2008)

Tolerance	Species	Soil salinity limit* (µS.cm^{-1})
Most Tolerant	Puccinellia	16000
↕	Saltbush	12000
	Barley	8000
	Canola	6500
	Wheat, Millet, Berseem clover	6000
	Perennial ryegrass	5600
	Strawberry clover	2700
	Lucerne, Paspalum, Soybeans	2000
Least Tolerant	Subterranean clover, White clover	1200
*Salinity limit in the root zone causing 10% yield loss		

Growth parameters can change easily when faced with environmental challenges, as an evolutionary tactic, to adapt and overcome the limitations of a sessile lifestyle. This enables plants to explore its surroundings for resources such as light, water and nutrients (Forde and Lorenzo, 2001). Some common growth parameters, such as plant height, number of leaves and mass of the plant, can be used as indicators of the severity of salinity stress during the plant development, provided that the other growth conditions are consistent. There is, however, developmental stages that are defined, even though these stages are not strictly time bound, as it is influenced by temperature and water availability (Forde and Lorenzo, 2001).

Germination ends with radicle emergence, at which seedling development starts. Firstly the cotyledons emerge from the soil in an epigeal way. The cotyledons have limited photosynthetic ability, but they provide most of the resources for initial seedling growth from nutrient reserves. From the epicotyl growth point the first leaf develops, which is a unifoliate leaf. From then on the next leaves to develop are trifoliate or multifoliate leaves. When the third trifoliate leaf opens, the seedling becomes autotrophic, satisfying all energy requirements with current photosynthesis. From the axillary bud of the unifoliate leaf a secondary stem develops with trifoliate or multifoliate leaves (Hall, 1998; Undersander et al., 1997).

While shoot development continues, the root system develops and explores the surrounding soil for edaphic resources like water and nutrients. Under ideal temperatures, water and soil texture conditions, the biomass of the roots will reach approximately 80% of the above ground biomass (Forde and Lorenzo, 2001; Hall, 1998). Contractile root growth, which can start as soon as one week after emergence, pulls the cotyledon node below ground, which protect the growth points of the secondary stems against frost. After about 6 weeks of growth, these growth points are below the soil surface, but may last as long as 16 weeks after emergence (Hall, 1998; Undersander et al., 1997). The depth of the cotyledon nodes will vary depending on the dormancy rating of the cultivar, with deeper nodes having a lower the dormancy rating.

Manipulating edaphic conditions with coated seed, to improve seedling growth and establishment, has been studied by a number of scientists and seed companies. Resulting changes in the soil may be in nutrient status (Allen et al., 1961; Ashraf and Foolad, 2005; Scott and Blair, 1988) or by preventing plant disease caused by insects and fungi (Harman, 1991; Koch et al., 2005; Lewis and Clements, 1998; Ruben et al., 2008), to improve the biological status of the soil by adding micro-organisms to the soil (Deaker et al., 2004, 2007; Jung and Mugnier, 1982), or to change the effect temperature and moisture has on germination (Vyn and Murua, 2001; Willenborg et al., 2004). Interaction of these changes with saline conditions may be advantageous to growth and the establishment of plants, or it may just provide what is required for the plant to adapt to these saline conditions (Ashraf and Foolad, 2005). Investigations into these questions will result in answers specific to the seed coating technology, species and varieties and the environmental conditions, including soil conditions (Forde and Lorenzo, 2001).

The objective of this trial was to determine if seed coating will cause differences between seedlings established with coated and non-coated seed. Salinity was included as a variable to determine whether salinity and seed coating will influence the growth and development of lucerne seedlings. We hypothesised the seed coating will have no influence on root and shoot growth parameters under a range of saline irrigation treatments.

2. Methods and materials

A pot trial was conducted in a phytotron at the Hatfield Experimental Farm, Pretoria during November and December 2012. A sandy loam soil, from the Hatfield Experimental Farm (-25°44'55.8924", 028°15'32.3352") was used. Each pot received the equivalent of 50 kg $P.ha^{-1}$ and 250 kg $K.ha^{-1}$ and was mixed to a depth of 25 cm (of 30 cm deep pots). Five seeds were planted per pot and was reduced to one seedling after one week. Two coating treatments, coated and non-coated, where applied to two cultivars, the landrace SA Standard and SuperCuf, as described in Table 2. The coating contains lime, nutrients, an insecticide and fungicide and is inoculated with *Sinorhizobium meliloti* which is bound with a polymer. An irrigation treatment was included by adding 0.6 l per pot of different water qualities, as described in Table 3, every second to third day, depending on the evaporation loss due to the heat in the phytotron.

Table 2

Lucerne cultivars SA Standard and SuperCuf characteristics

Cultivar	Dormancy	Origin	Characteristics
SA Standard	4-6	South African Landrace	Intermediate crown position Coarse to medium stems Exceptionally high resistance to root and crown-rot complex and other root and crown diseases
SuperCuf	9	Australia: Cuf101 cross with Sequel	Leafy stems Strong autumn and spring growth Strong regrowth after harvesting The crown is positioned higher than SA Standard

Table 3

Irrigation water characteristics used in the pot trial

g NaCl. L^{-1} water ($g.L^{-1}$)	Electrical conductivity ($uS.cm^{-1}$)
0	180
0.28	500
0.52	750

At 20 days after planting, most seedlings had two trifoliate leaves and destructive harvests were initiated so that each combination treatment would have four replicates. Four harvests were conducted at five day interval, which resulted in a total of 240 pots harvested. At each harvest the length of the primary stem, the number of trifoliate or multifoliate leaves per plant, the leaf area root length and dry matter of the shoot material and root material per plant was determined. Primary stem length was measured, using a self-retracting measuring tape, from the growth point to the point where the cotyledons were attached to the stem. Primary root length was determined by measuring from the point where cotyledons were attached to the tip of the root as harvested from the pot.

The leaf area was determined by harvesting leaves and placing it on a white surface. An object with a known size was placed with the leaves and a digital image is taken of the leaves. Adobe® Photoshop® was used to determine leaf area using the method described by Dahab et al. (2003), using the object's size to convert pixels to real size. Fig. 1 is an example of a photo used to determine leaf area.

Biomass ($kg.ha^{-1}$) was determine by separating the shoot and root just below the point where the cotyledons are attached and drying the shoot and root samples for 24 h in Labotech ovens at 65 °C. Biomass was determined by using a Mettler Toledo PB 3002-S scale.

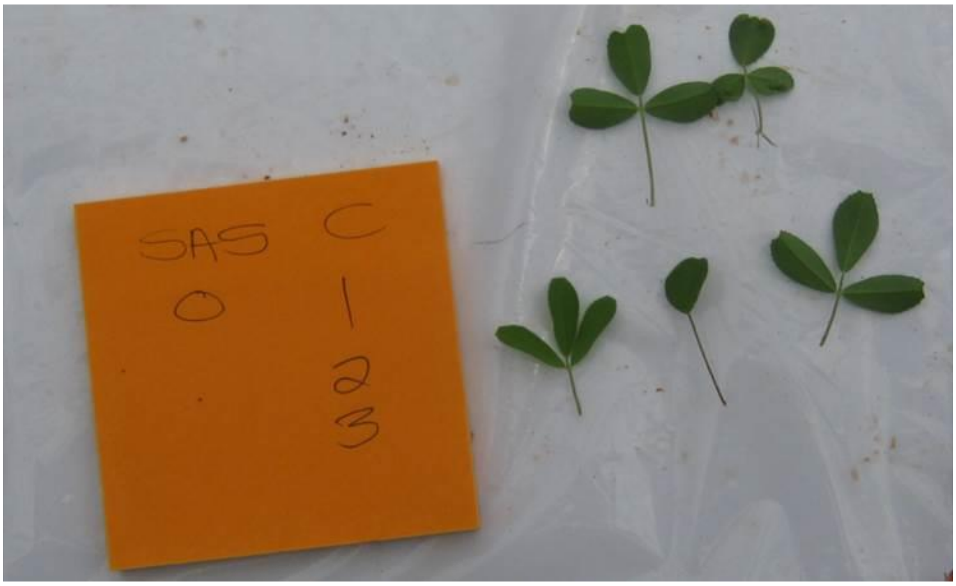

Fig. 1. Illustration of how the leaf area was determined using Adobe® Photoshop®. This digital image is of the third replicate of coated SA Standard at 25 days after sowing, irrigated with water containing no salt.

Statistical Analysis

This trial was designed to conform to a randomized block design (RBD). Statistical analysis on the measured parameters, were done using SAS Version 9.2 software for Microsoft Windows. Analysis included General Linear Model (PROC GLM) and correlation analysis (PROC CORR), and was done within cultivar and within harvests. The correlation analysis was done only for shoot physio-morphological characteristics as root measurements were complicated with root loss during harvesting, causing uncertainty in the root measurements. The Least Significant Differences (LSD) were calculated at P≤ 0.05 for both correlation analysis and analysis of variance.

3. Results and discussion

According to Scott and Blair (1988) the early growth of seedlings compared with weeds is most important in determining how successful the establishment of the crop stand will be. Seedling height, internode length and growth rate are positively correlated with each other and can be used as an indicator of how well a plant is adapted to its growing conditions (Katic et al., 2004). For both SA Standard and SuperCuf there were limited differences between coated and non-coated seed treatments for average stem height, as seen in Fig. 2 and Fig. 3. There were, however, significant differences in average stem height between coated and non-coated SA Standard treatments irrigated with 500 µS.cm^{-1} water, at the 30th day after planting (P= 0.009).

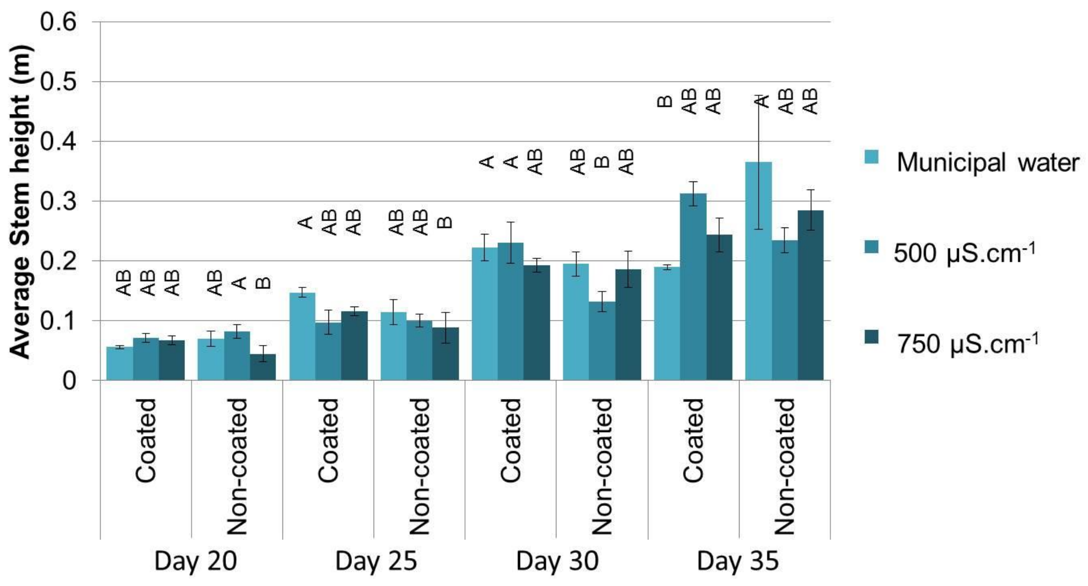

Fig. 2. The average stem height comparison of coated and non-coated SA Standard at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

Even though the observation at day 35 was not significantly different, the coated SA Standard treatment had longer stems than the non-coated treatment. A difference was also noted between coated and non-coated SA Standard seed treated with municipal water at day 35, with plants grown from non-coated seed having significantly longer stems than the plants grown from coated (P= 0.025). Whether this difference would persist if the trial continued is unclear, as the differences observed were not present in the previous harvest.

When considering the influence of the saline irrigation water on the seedling height, no significant differences between coated SA Standard treatments irrigated with the different water qualities, were observed. There was, however, a difference between non-coated SA Standard treatments at the first harvest, where seedlings irrigated with 500 µS.cm^{-1} had longer stems than seedlings irrigated with 750 µS.cm^{-1} water. The difference is likely due to stress caused by slower hydration of tissue as a result of a higher osmotic potential in the soil, thereby causing a reduction in cell expansion (Chon et al., 2004).

There were statistically significant differences in stem height between coated and non-coated treatments irrigated with 750 µS.cm^{-1} at the beginning of the trial (P= 0.0120), where plants from non-coated treatments had taller stems. A significant difference was also observed between coated and non-coated treatments at day 30 when irrigated with untreated municipal water (P= 0.0035), where coated treatments had taller stems. However, 5 days later at the next harvest there were no differences in stem height.

The influence of saline irrigation water on the seedling height of SuperCuf seedlings showed significant differences at day 30 for coated treatments irrigated with municipal and 500 µS.cm^{-1} water (P= 0.0286). Even though this difference was not observed in the following harvest, this is still an indication that there was an impact of the saline water on the growth of the seedlings, which is most likely due to a reduction in cell expansion. However, the treatments of 750 µS.cm^{-1} did not show significant differences between the municipal water and the 500 µS.cm^{-1}, although they did have shorter stems. At the first harvest plants from non-coated seeds, irrigated with municipal water had shorter stems than the treatments irrigate with 750 µS.cm^{-1} water. The longer stems might be linked with the higher root mass (Fig. 16) of the seedlings irrigated with 750 µS.cm^{-1}. Correlation between root and shoot (Dry Matter) DM is described by Alshammary et al. (2004); Cook et al. (1996) and is highly correlated.

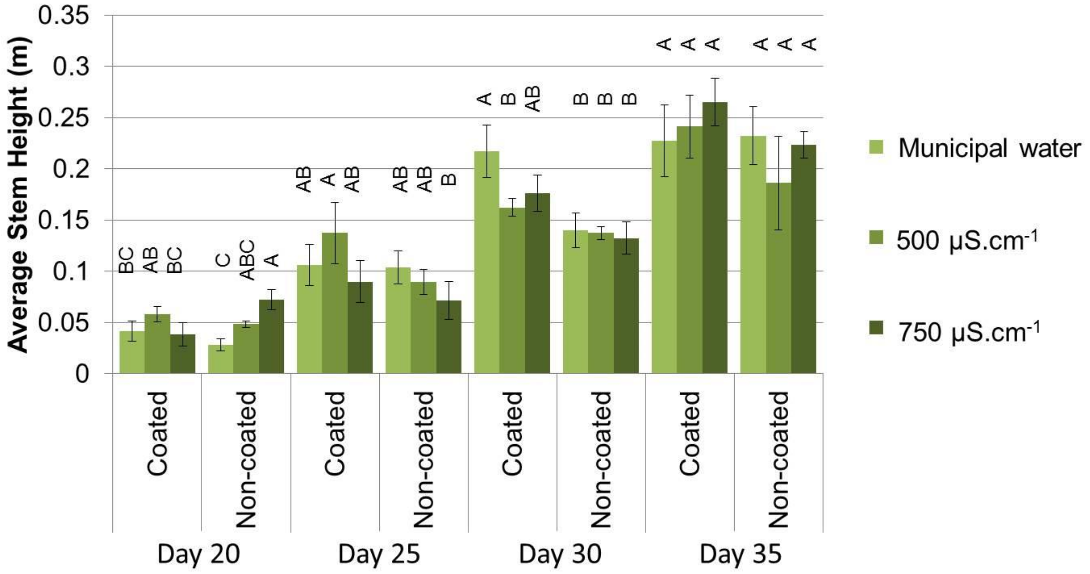

Fig. 3. Comparison of the average stem height of coated and non-coated SuperCuf at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

These isolated differences in seedling stem height are likely due to an interaction between cations and its interaction with salts added by saline irrigation water in the soil. According to Fenner and Lee (1989), the optimal balance of available nutrients required by a developing seedling can change due to its environment. The salt environment can cause temporary deficiencies or immobility of nutrients, causing the treatments under higher water salinity to have shorter stems. Teixeira et al. (2004) stated that nutrient availability, with reference to regrowth, would influence the size of leaves and length of stems, but not influence the number of leaves or stems. Using this principle, nutrient immobility and saline conditions might be the cause of shorter stems. The effect is short lived and does not repeat in following harvests.

Continuing with this principle (Teixeira et al., 2004), no variations are expected in the number of leaves. The isolated variations observed are therefore not due to carbon or nitrogen deficiencies even though growth and development depend on these two building blocks. This, however, does not eliminate deficiencies of other nutrients form causing slower development (Fenner and Lee, 1989). Fig. 4 shows significantly higher number of leaves per plant for the plants grown from coated SA Standard seed than those grown from non-coated seed, at the first harvest, when irrigated with 750 $\mu S.cm^{-1}$ water (P= 0.0062). At day 30, similar results were observed between plants irrigated with 500 $\mu S.cm^{-1}$ water (P= 0.0417). At the final harvest, however, the plants grown from coated seed had significantly lower number of leaves than the plants from non-coated seed irrigated with municipal water (P= 0.0213).

Differences in number of leaves per plant, caused by the saline irrigation treatments, were observed at the first harvest, where plants grown from coated seed and irrigated with 750 $\mu S.cm^{-1}$ water, had significantly more leaves than the treatments irrigated with 500 $\mu S.cm^{-1}$ (P= 0.0320) and municipal water (P= 0.0062). At the final harvest there was a significantly lower number of leaves for plants grown from coated seed irrigated with municipal water, compared with plants irrigated with 500 $\mu S.cm^{-1}$ water (P= 0.0062). These differences appear to be caused by an interaction between the seed coating, the soil and the saline conditions, due to the lack of significant differences between the non-coated treatments and the saline conditions.

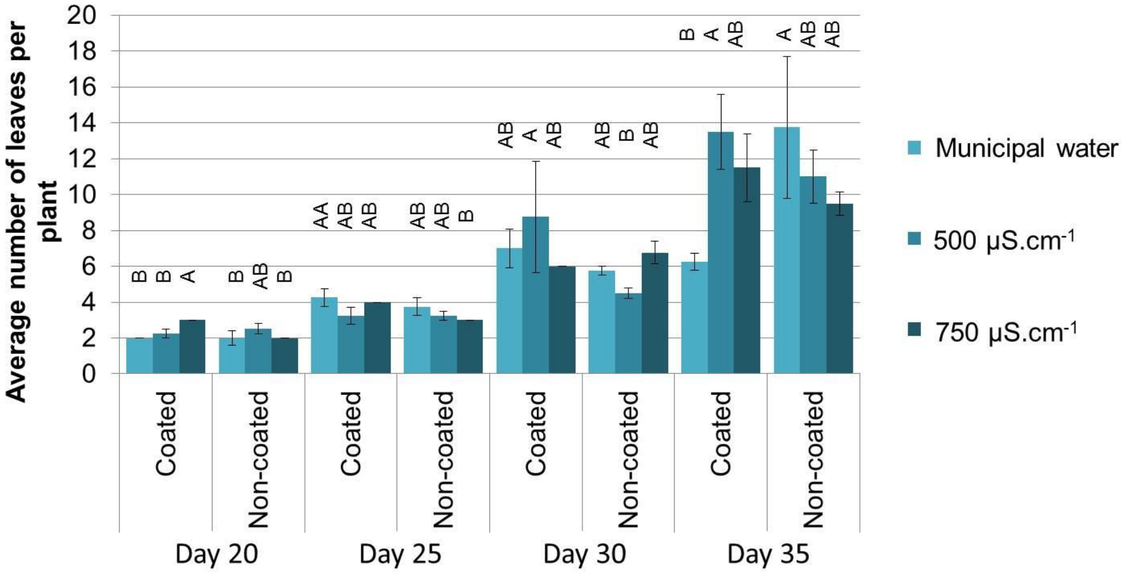

Fig. 4. Comparison of the average number of leaves per plant, of coated and non-coated SA Standard at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval
*Same letters are not significantly different

For SuperCuf there were very few significant differences in number of leaves between treatments (Fig. 5). At the first harvest there were differences between coated and non-coated treatments irrigated with 750 µS.cm^{-1} water (P= 0.0201). At the 30th day after sowing, there were differences in the number of leaves between the coated treatments irrigated with municipal water (P= 0.0228) and 750 µS.cm^{-1} water (P= 0.0430).

SuperCuf plants grown form coated seed, irrigated with 500 µS.cm^{-1} water had lower number of leaves per plant than plants irrigated with 750 µS.cm^{-1} water (P= 0.0430) at the 30th day after sowing. Similar to SA Standard, there were no differences observed between non-coated treatments influenced by saline conditions, in terms of number of leaves per plant.

It is hypothesised that these individual observations of differences are caused by short delays in development caused by a diversion of plant resources to adapt to the saline conditions. According to Humphries and Aurricht (2001), salt tolerance in lucerne is associated with maintaining lower concentrations of salt ions in the leaves and is accomplished by exclusion of salt by the roots. Work done by Noble et al. (1984) showed the root concentrations of ions stayed the same and were not influenced by the concentration of the ions in the soil, supporting the previously stated theory where roots exclude salt. Proline is one of many osmoprotectants produced by lucerne, which rapidly increases in shoots when under osmotic stress, but takes longer to increase in roots (Humphries and Auricht, 2001). The rapid production of proline can therefore cause a temporary sink for plant resources, causing a short delay in development. According to Munns and Tester (2008), these osmoprotectants can have a large requirement for ATP and can therefore have a significant influence on the growth of the plant.

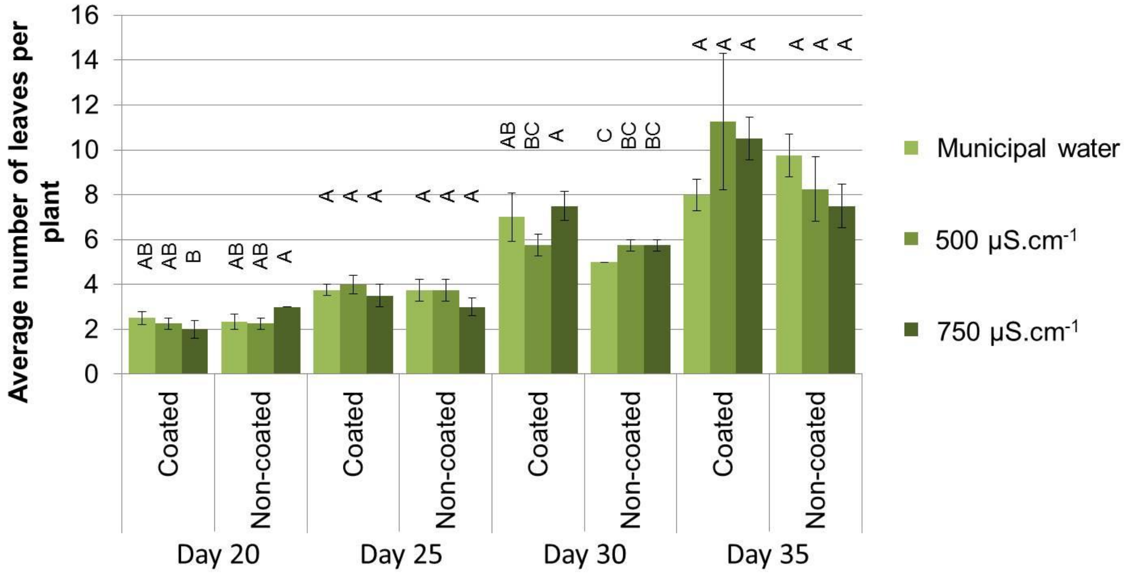

Fig. 5. Comparison of the average number of leaves per plant, of coated and non-coated SuperCuf at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

Leaf area per plant determines the amount of radiation intercepted and is one of the important factors determining photosynthetic rate of the plant. The photosynthates can then be used for growth and development, but can also be used to repair cell damage caused by high saline conditions (Riadh et al., 2010). Seedlings would benefit from higher photosynthetic levels as photosynthates can be beneficial to establish faster or acquire reserves to recover, if damage occurs to shoots. Faster growth would also mean less time to the first harvest (Fick and Mueller, 1989; Van Oudshoorn et al., 2001).

Data from this trial (Fig. 6) illustrated higher leaf areas for coated SA Standard than for non-coated seed irrigated with 750 $\mu S.cm^{-1}$ water (P= 0.0037) at the first harvest. According to Munns (2002), leaf expansion is influenced by saline and dry conditions and evidence suggests that these effects are due to hormonal signalling rather than water relations are the dominant controller of leaf expansion. Another difference between coated and non-coated SA Standard seed, was observed at the last harvest, where non-coated treatment had a larger leaf area than the coated seed treatment irrigated with municipal water. This is a consequence of leaf number (Fig. 4) as the average leaf area per leaf is not different, where the coated treatment had an average of 0.027 $m^2.leaf^{-1}$ and the non-coated treatment had an average leaf size of 0.028 $m^2.leaf^{-1}$. The delay in development of the plants grown from coated seed at day 35 is evident in all shoot parameters measured. Further investigation is required to determine whether this lag is due to experimental error or if the seed coating caused a reduction in growth. The lack of evidence in previous harvests, however, suggests that these differences are due to experimental error, created by slight differences in the environment, such as air flow differences closer by the vents and the door to the enclosure.

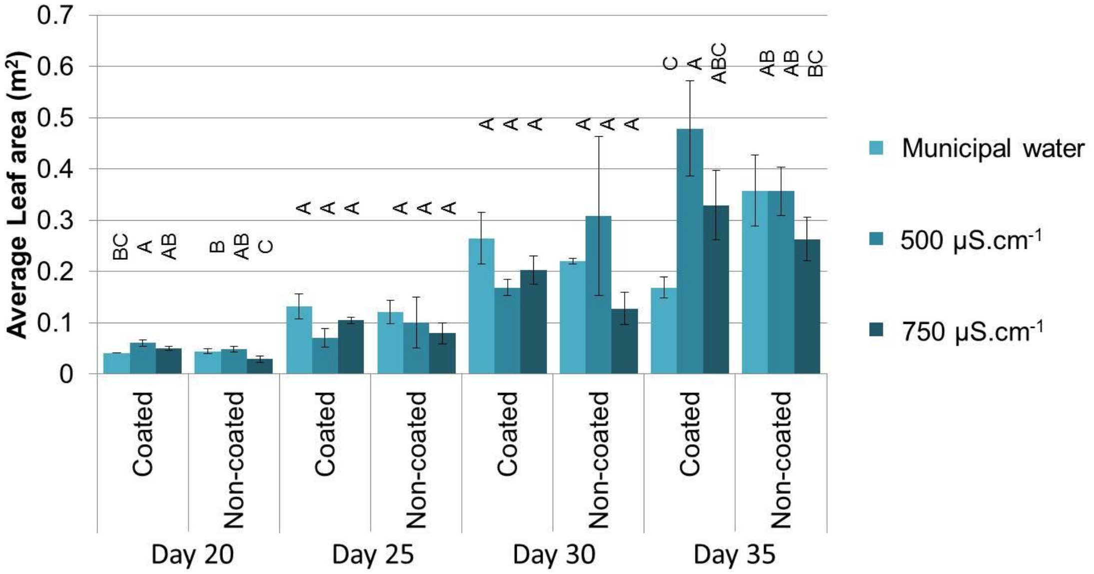

Fig. 6. Comparison of the average leaf area of coated and non-coated SA Standard at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

When considering the influence of the saline irrigation treatments on the coating treatments, differences were observed between coated treatments at the first harvest, where treatments irrigated with 500 µS.cm^{-1} water had higher leaf areas than treatments irrigate with municipal water (P= 0.0089). The same was observed at the last harvest (P= 0.0021). The water quality also influenced the non-coated SA Standard treatments at the first harvest date, where treatments irrigated with 750 µS.cm^{-1} water had significantly lower leaf areas than treatments irrigate with municipal water (P= 0.047) and with 500 µS.cm^{-1} water (P= 0.0054). Under these conditions a slight increase in salinity does not decrease the leaf area, but can stimulate the expansion of the leaves of lucerne plants. When the saline conditions become more extreme, however, a decrease in the leaf area can occur, as in the case of the 750 µS.cm^{-1} water treatment.

For SuperCuf higher leaf areas were observed at the first harvest for the coated seed treatments compared with the non-coated seed treatments, irrigated with municipal water (Fig. 7). There was also a difference between coated and non-coated treatments at day 30, irrigated with 500 µS.cm^{-1} water. Again the coated treatment had the higher leaf area (P= 0.0273). These observations suggest that the seed coating can encourage the expansion of leaves, allowing the plants to establish faster (Teixeira et al., 2004). Considering the influence of the water treatments on the coating treatments of SuperCuf, there were no significant influence observed.

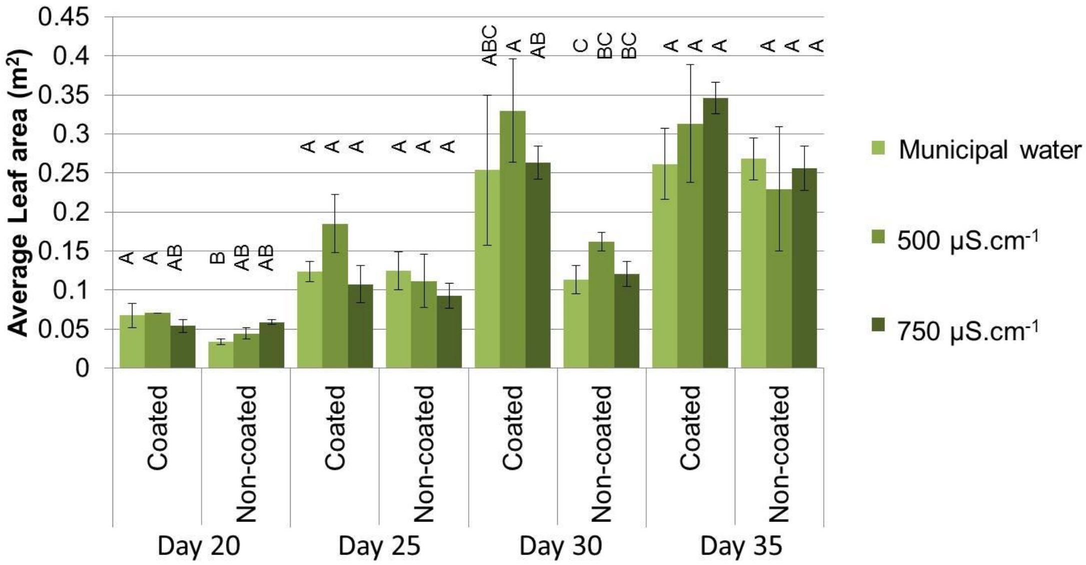

Fig. 7. Comparison of the average leaf area of coated and non-coated SuperCuf at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

Shoot dry matter yield has implications for seedling survival; success of the stand establishment and future yield. Seedling growth rate is linked to shoot dry matter, which influences the time to first harvest and if the seedlings are healthy, the successful regrowth after the harvest. Dry matter yield is a good parameter to use to determine how well a seedling has adapted to its growth conditions (Katic et al., 2004).

Differences in shoot dry matter between coated and non-coated SA Standard seed treatments (Fig. 8) were observed at the first harvest, where the non-coated seed treatment had higher shoot dry matter than coated seed treatment irrigated with municipal water, at the first harvest (P= 0.0482). Even though there were no significant differences between treatments in terms of stem height, the number of leaves per plant and the leaf area per plant, the cumulative differences of these parameters are the reason for the difference in dry matter. There was also a significantly higher shoot dry matter yield for the coated treatment irrigated with 500 µS.cm^{-1} water, when compared to its non-coated counterpart (P= 0.0182) at the third harvest. This difference is also significant when comparing stem height and the number of leaves. This suggests that the 500 µS.cm^{-1} water treatment enhance the growth of the SA Standard seedlings and even though the differences are not statistically significant in the other harvests, differences are notable at days 25 and 35. At the first harvest, the coated treatment irrigated with 750 µS.cm^{-1} water was also significantly higher than the treatment irrigated with municipal water (P= 0.0355). No differences between water treatments were observed for non-coated SA Standard treatments.

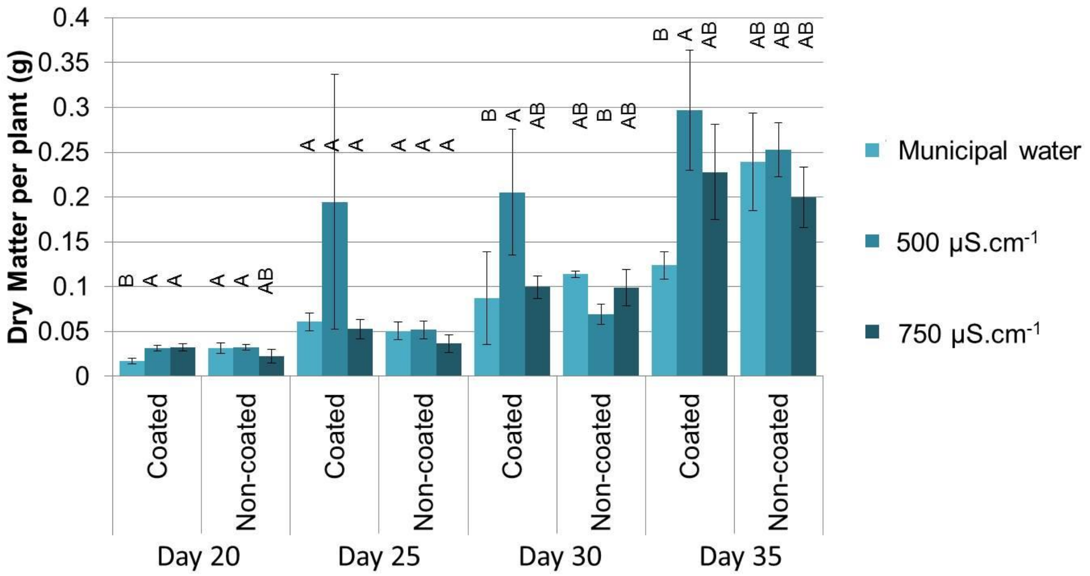

Fig. 8. Comparison of the shoot dry matter yield per plant, of coated and non-coated SA Standard at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

SuperCuf showed variance at the first harvest between the coated seed treatments irrigated with 750 µS.cm^{-1} water (Fig. 9), where the non-coated treatment had higher shoot yield than the coated treatment (P= 0.0091). At day 30 the non-coated treatment had higher yield than the coated treatment, irrigated with municipal water (P= 0.0062). The influence of the water quality appears to be negligible on the coating treatments of SuperCuf, as there were no differences observed between coated treatments irrigated with the different water qualities. There was, however, a difference in dry matter yield between the non-coated treatments at the first harvest, where the seedlings irrigated with 750 µS.cm^{-1} water had higher dry matter yield than those irrigated with municipal water. The stem height was also significantly higher for the seedlings irrigated with 750 µS.cm^{-1} water and contributes to the difference in dry matter production.

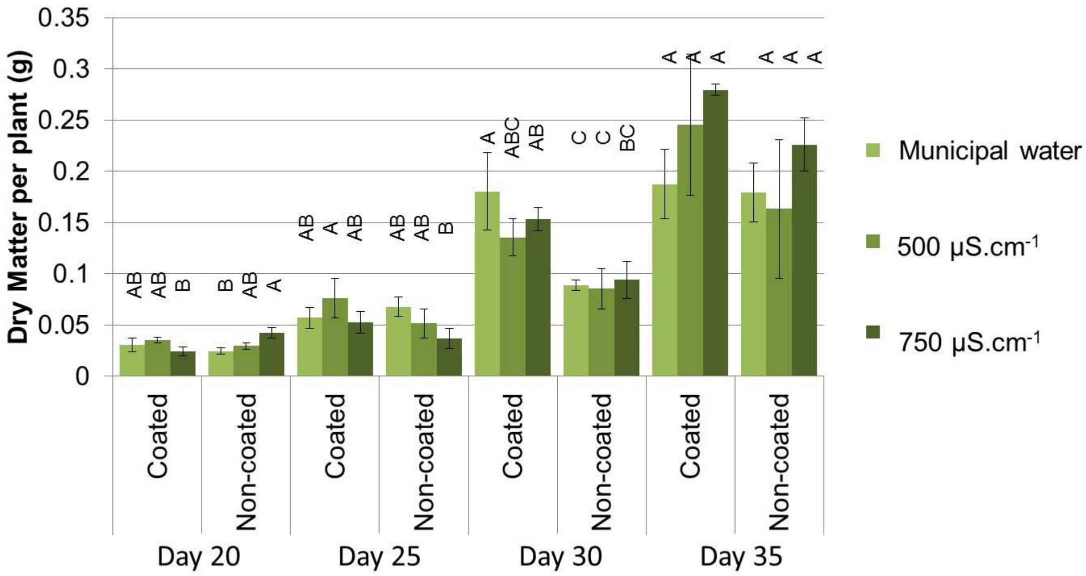

Fig. 9. Comparison of the shoot dry matter yield per plant, of coated and non-coated SuperCuf at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

The correlations between shoot physio-morphological characteristics are highly correlated as seen in Fig. 10. The red line represents the level below which correlations would not be statistically significant. For SA Standard, these correlations are disrupted when coated and non-coated treatments are irrigated with 500µS.cm^{-1}. The correlation between shoot dry matter yield data and the data from the other shoot parameters measured (stem height, number of leaves and leaf area) were low for the coated seed treatments. The correlation between leaf area data and the data from the other parameters measured (stem height, number of leaves and shoot dry matter yield) also were low. The irregular observations for seedlings grown from coated seed can be due to the appearance of secondary stems with many smaller leaves developing from the lateral buds, as seen in Fig. 11. This suggests that the overall production will be higher for coated treatments when irrigated with 500 µS.cm^{-1} water.

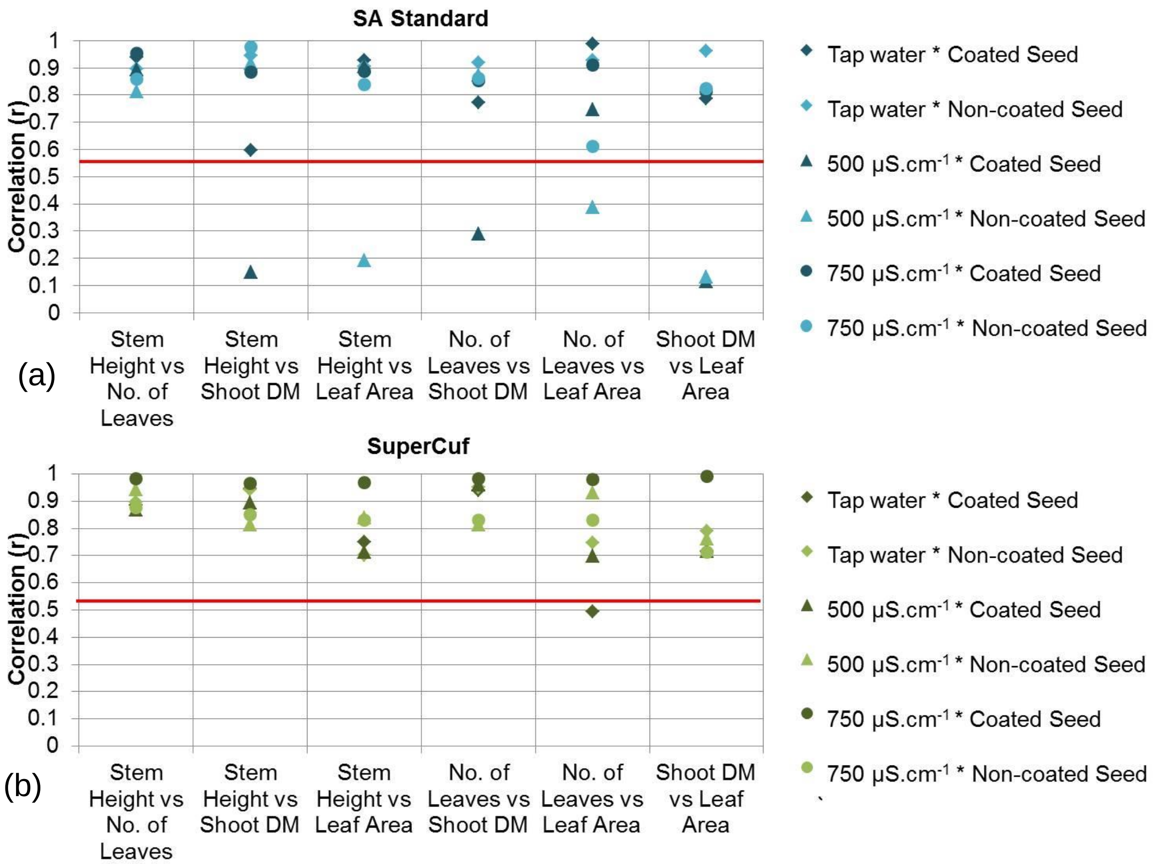

Fig. 10. Correlations between shoot morphological characteristics of coated and non-coated seedlings of SA Standard (a) and SuperCuf (b) irrigated with different water qualities, for the four harvest times.

Fig. 11. A coated SA Standard plant irrigated with 500 µS.cm^{-1} water.

The non-coated observations not conforming to the correlation data can be due to larger individual leaf areas and shorter internodes, as seen in Fig. 12. According to (Katic et al., 2004), shorter internodes with a high leaf % will likely result in slower growths and higher quality forage.

Fig. 12. A non-coated SA Standard plant irrigated with 500 µS.cm^{-1} water.

Root length (Fig. 13 and Fig. 14) and root dry matter yield (Fig. 15 and Fig. 16) did not appear to be influenced by the seed coating technology or water quality. This might not be true for plants grown in the field, since artificial preferential water flow and pot bound growing conditions can provide different results. Only infrequent differences were observed for both SA Standard and SuperCuf.

According to Fig. 13, there were significantly longer roots for coated SA Standard when irrigated with municipal water compared to when they are irrigated with 500 µS.cm^{-1} water (P= 0.0177) at day 30. This is the only observation of the difference between root lengths for SA Standard.

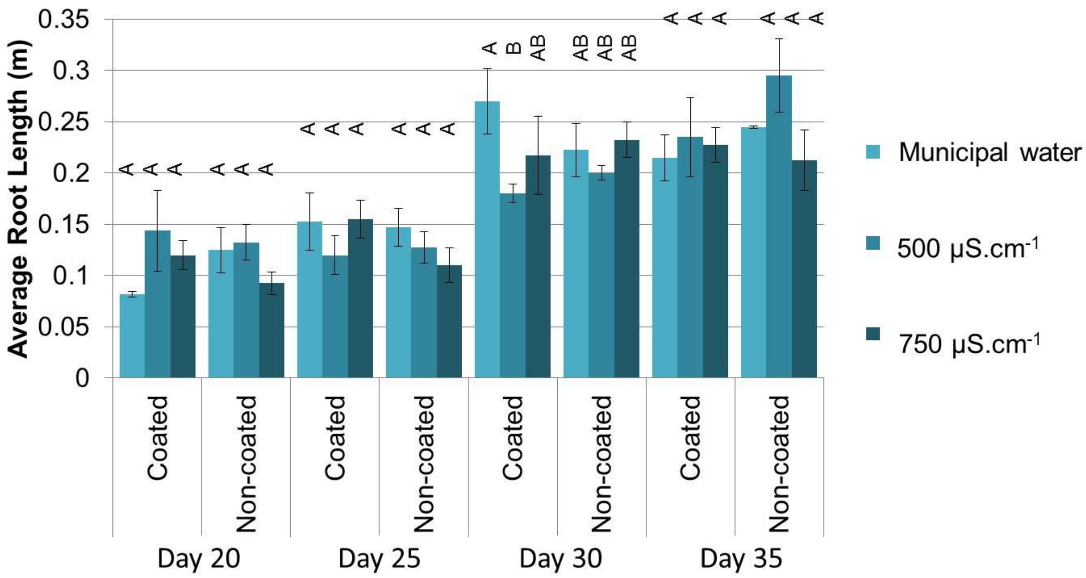

Fig. 13. Comparison of the average root length of coated and non-coated SA Standard at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

Fig. 14 shows the root length of the coated and non-coated SuperCuf as it is influenced by various irrigation water qualities. There was a significant difference after 30 days between plants grown from coated SuperCuf seed and those grown from non-coated SuperCuf seed, irrigated with 750 µS.cm^{-1} water (P= 0.0116). It is noted that coated seed treatments had longer roots than the non-coated seed treatments. This suggests that the root growth is encouraged by the presence of the seed coating when irrigated with 750 µS.cm^{-1} water. The difference was, however, not evident at the following harvest. At the final harvest, significantly shorter roots were observed for plants grown from non-coated seed, irrigated with municipal water compared with 500 µS.cm^{-1} water (P= 0.0075) and 750 µS.cm^{-1} water (P= 0.0053). These differences in root length are possibly due to Na^+ exclusion which causes a higher root cell turnover, therefore replacing cells rather than accumulating cells for growth. This mechanism allows for very fast recovery of roots in saline conditions (Munns and Tester, 2008).

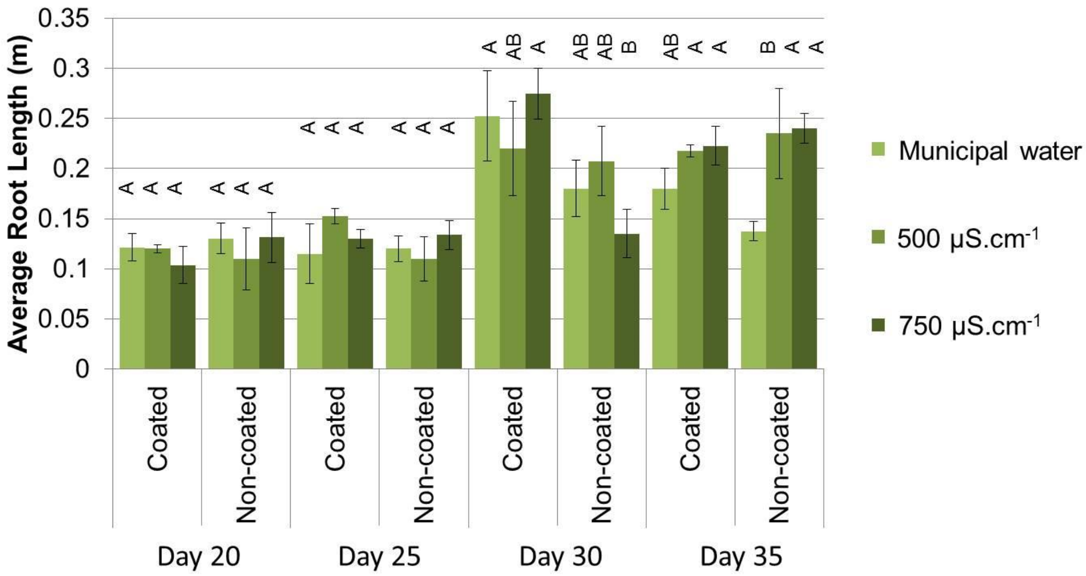

Fig. 14. Comparison of the average root length of coated and non-coated SuperCuf at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

According to Fig. 15, there were no significant differences in root dry matter yield between plants grown from coated and non-coated SA Standard seed. There was, however, higher root dry matter yields observed for plants grown from coated seed when irrigated with 750 µS.cm^{-1} water, compared with municipal water (P= 0.0228), at day 20. At day 30, the plants irrigated with 500 µS.cm^{-1} water had higher root dry matter yield than plant irrigated with 750 µS.cm^{-1} water (P= 0.0441), but the standard error is large.

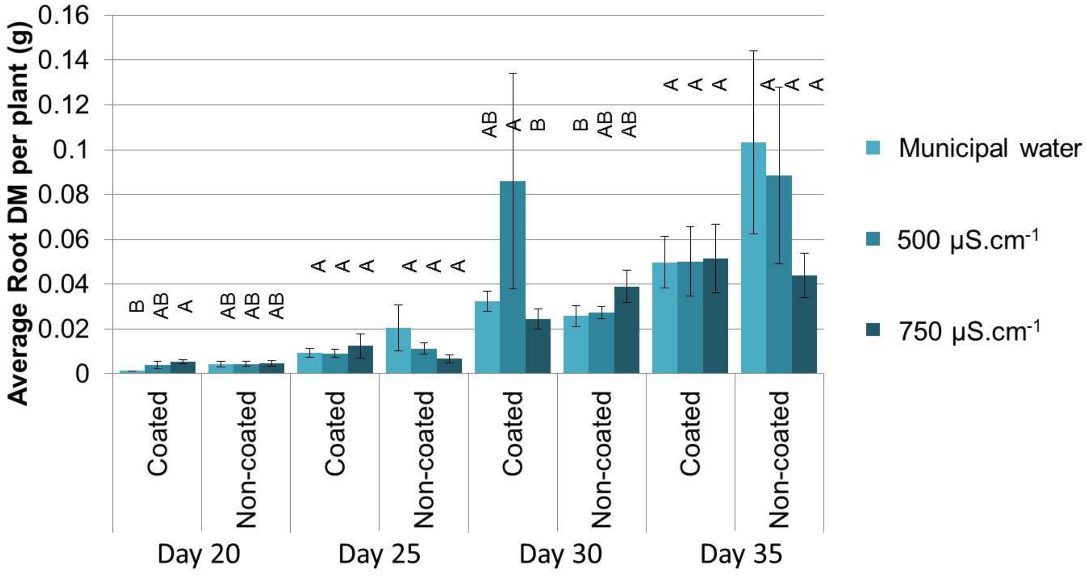

Fig. 15. Comparison of the average root dry matter per plant of coated and non-coated SA Standard at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

Fig. 16 illustrates the average root dry matter yield of SuperCuf plants, as it is influenced by the different saline irrigation treatments. At day 30, the plants grown from coated seed had higher root dry matter yields than the plants grown from non-coated seed, when irrigated with 750 µS.cm^{-1} water (P= 0.00341). When considering the influence of the saline irrigation water treatments on the growth of the plants, a difference was found between plants grown from non-coated seed at day 25. Treatments irrigated with municipal water had higher root dry matter yield than plants irrigated with 750 µS.cm^{-1} water (P= 0.0378).

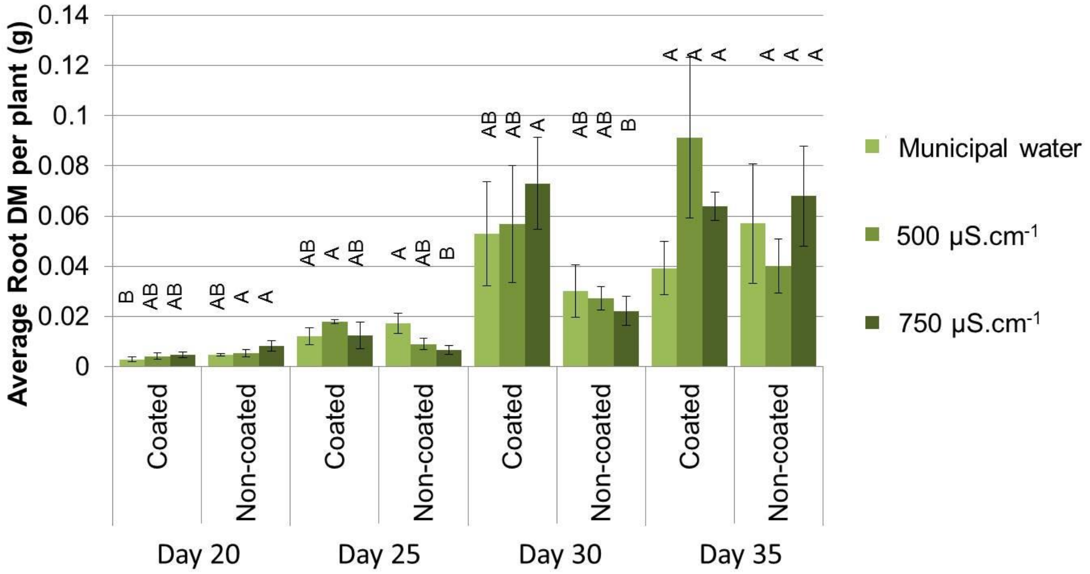

Fig. 16. Comparison of the average root dry matter per plant of coated and non-coated SuperCuf at various irrigation water qualities, at 5 day intervals from day 20 after sowing to day 35 after sowing.

*Comparisons were made between coated and non-coated seed within each harvest interval

*Same letters are not significantly different

It appears that root length or depth and root dry matter is not highly correlated. These individual differences in root growth in terms of dry matter yield are likely due to experimental error caused by the difficulty in harvesting all root material. According to Munns and Tester (2008) and Parida and Das (2005), root characteristics are not as sensitive to saline growth conditions as shoot characteristics. Severe saline conditions will overcome the plants tolerance for salinity, but leaf death will occur before root growth is inhibited. According to Munns and Tester (2008), the difference in sensitivity is an evolutionary mechanism which allows the plant to conserve soil water by reducing the water requirements of the plant, while keeping the ability to obtain the water.

4. Conclusion

The two cultivars used in this trial did not respond to the coating and saline irrigation treatments in the same way. SA Standard showed variation in correlation between shoot physio-morphological characteristics, which indicates that mechanism of tolerance to the saline conditions, either changed or became more prominent. It is also interesting to note that the changes observed were different between plants grown from coated and non-coated seed.

The small number of differences observed for the physio-morphological characteristics, between the irrigation treatments and the coated and non-coated seed treatments, indicates that the tolerance mechanisms are not overpowered by the conditions created in this trial. The genetic ability of the seedling would have more influence on how the seedling reacted to the conditions, than would the conditions it was in, as the conditions created by the treatments were within the tolerance limits of the seedlings. The differences in number of leaves and stem and stem height was likely due to short delays in development which can be the result of osmotic stress or the production of osmoprotectants which caused temporary syncs for energy.

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Chapter 5

Prepared according to the guidelines of Grassland Science

The yield differences of lucerne (*Medicago sativa* L.) grown from coated and non-coated seed

L. Nel and W.F. Truter

Abstract

Lucerne is South Africa's most popular forage legume and provides good quality fodder for many livestock species. Many extension documents exist, giving instructions on seedbed preparations, establishment, maintenance and when to harvest lucerne, which makes this crop more attractive for more farmers. The use of coated seed to establish a stand can simplify the establishment process even further by pre-inoculation with *Sinorhizobium meliloti* and increasing the seed size. Three trials were conducted to determine whether there will be significant differences in yield and quality of a stand when established with coated or non-coated seed. Trials entailed a first autumn planting first (established in 2009) and second spring planting (established in 2010) which were sown at 25 kg.ha^{-1} coated seed, while a third spring planting (established in 2010) was sown at 5 different sowing densities, namely 80%, 90%, 100%, 110% and 120% of recommended sowing density (25 kg.ha^{-1}). These sowing densities were selected to identify if the use of coated seed would influence the yields and adaptations of the plants at different sowing densities. Two cultivars were used, namely a landrace, SA Standard and the cultivar SuperCuf. Both of these cultivars had two seed treatments, namely coated and non-coated. These trials were established with supplemental irrigation. It was found that the pasture stands established with coated and non-coated SA Standard seeds, did not show many differences and were only present in the second season, where the non-coated seed treatments had significantly higher dry matter yield than the coated seed treatments. The quality, in terms of stem to leaf ratio, also did not differ between coated and non-coated seed treatments. Data collected from the stands established with SuperCuf, showed more significant differences and it was found that the non-coated seed treatments had higher yields in the first season. It was, however, found that the stands established with coated seed had lower stem: leaf ratio's, indicating a better quality fodder can be produced. When considering the influence of sowing density on the stands, it was found that at 90% and 120% of the recommended sowing density, the non-coated seed treatments had higher yields than the coated seed treatments, while the other densities showed no difference between coated and non-coated SA Standard seed treatments. The lack of significant differences between the two coating treatments and the sowing density treatments, suggests that these treatments have a limited influence on the lucerne yield. Either the number of plants per area was the same, caused by seedling mortality during the high growth rate in the early growing stage, or the morphological characteristics, such as number of stems per plant and number of leaves per stem, and adapted to result in similar yields and quality.

Keywords: Production, yield, quality, stem to leaf ratio

Introduction

From basic genetics it is known that the plants or organisms expression of its genetic potential is influenced by its environment (Fairbanks and Andersen, 1999). A quantitative observation, such as yield of a crop has continuous variation and multiple genes influencing its expression, together with the environmental conditions the plant is grown in, complicates the expression even further (Fairbanks and Andersen, 1999). Crop variation between species is understandably diverse, but cultivar differences can be just as variable (Guines et al., 2003).

Lucerne cultivars are classified into dormancy classes, which not only refer to their adaptation to cold winters, but also the architecture of the plant, such as the depth of the crown of the plant (Van Oudshoorn et al., 2001). Apart from the dormancy rating, cultivar selection objectives have also contributed to the large variety between cultivars. The ultimate use and country of origin also contribute significantly to the variability as the selection objectives are different for these factors (Van Oudshoorn et al., 2001, Katic et al., 2004, Lamb et al., 2006).

After the cultivars are selected, appropriate to the region to be planted in and the use planned by the farmer, there are still many environmental factors which can influence the plants genetic expression, such as biological, physical and chemical soil characteristics (Dexter, 2004, Fairbanks and Andersen, 1999). The complexity of the soil environment and its influence on the plant is extensive and is a field of study on its own. Poor physical soil quality may manifest in many ways, such as hard-setting, which leads to poor infiltration of water and causes an increase in erosion, poor workability and restricted root penetration. All these factors will ultimately influence the plant production (Dexter, 2004). The chemical quality of soil includes factors such as nutrient content and balance, soil pH and pollutants such as salts, heavy metals and other toxins. These factors are closely related to the soil water content, as water acts as a vehicle for these elements involved (Dexter, 2004, Hillel, 1982, Viets, 1962, Peters et al., 2005).

The quality and availability of the water to the plant largely influences yield. Many researchers have done field and greenhouse trials investigating the irrigation method, induced salinity and salinity-fertilization relationships (Helalia et al., 1996, Montazar and Sadeghi, 2008, Rogers, 2001), but the effect on plant growth also extends to microbial growth and activity, influencing the symbiotic relationship between legumes such as lucerne and rhizobia, thereby influencing nitrogen (N) availability (Rietz and Haynes, 2003, Singleton et al., 1982).

Field trials endeavour to standardize as many of these mentioned variables as possible in order to identify individual influences on yield and quality. Extension documents distributed by Universities and associations provide guidelines for production, not only to ensure yield and quality in the product, but also a persistent stand (Undersander et al., 2011, Laboski et al., 2006, Cosgrove et al., 1996, Lattimore, 2008, Van Oudshoorn et al., 2001). It is well understood that stand establishment is one of the most important factors influencing success and the previous chapters have discussed this aspect. The harvest interval and maintenance of the stand is clearly explained in these agricultural extension documents and can be adapted to some extent to suit the production system and infrastructure of the producer (Van Oudshoorn et al., 2001, Undersander et al., 2011, Laboski et al., 2006, Cosgrove et al., 1996, Lattimore, 2008).

The aim of these trials was to determine whether the use of coated seed to establish a lucerne stand will influence the establishment and yield of the stands. It was hypothesised that the use of coated seed will have no influence on the yield of the stand when compared with non-coated seed and the inoculation of coated seed is just as efficient as the conventional method of inoculation. It was also hypothesised that the seed coating will have no influence on stem to leaf ratio and that seed coating will have no influence on the dry matter production of stands sown at different sowing rates.

Materials and methods

Three field trials were conducted between 2009 and 2012, namely a trial established in autumn 2009, in spring 2010 and a sowing density trial established in spring 2010 as seen in Figure 1. All three trials were designed to conform to a completely randomized design.

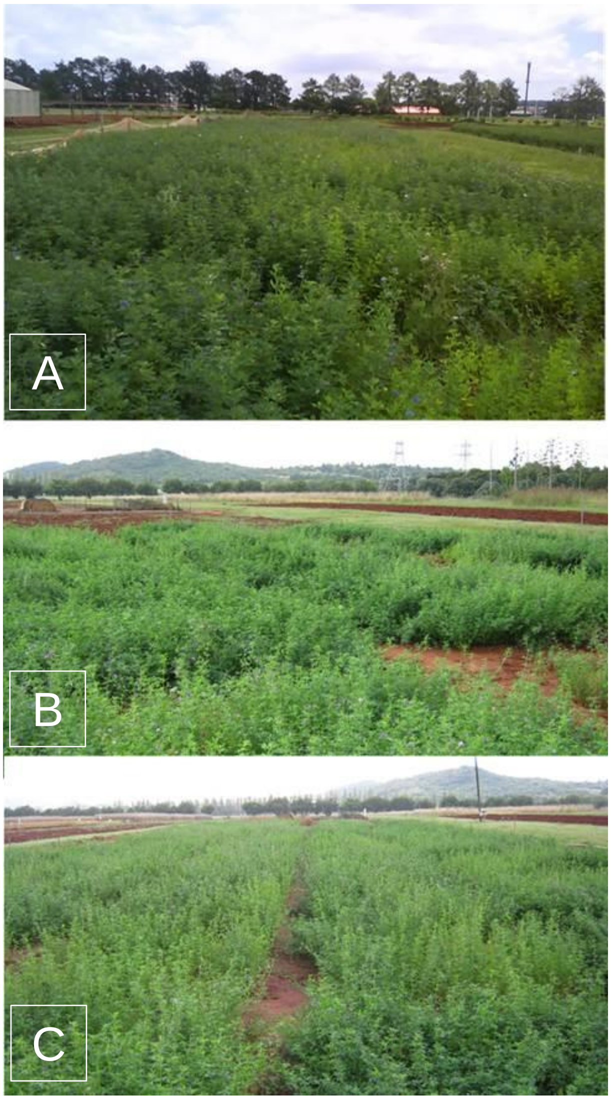

Figure 1 The trial established in autumn 2009 (A), spring 2010 (B), and the sowing density trial established in spring 2010 (C)

Two lucerne cultivars were used in these trials, which are described in Table 1. Each cultivar had two seed treatments, namely coated and non-coated, where the coating contained lime, nutrients, an insecticide, a fungicide and binding polymers and the symbiotic inoculant, *Sinorhizobium meliloti*. Non-coated treatments were inoculated in the conventional manner with *Sinorhizobium meliloti,* by coating the seed before planting with a mixture of lime and inoculant. The objective of the trials, was to determine whether there would be significant differences in yield between stands using coated and non-coated seeds.

Table 1 Lucerne cultivars SA Standard and SuperCuf characteristics (Anonymous, 2007, Anonymous, 2012a, Anonymous, 2012b)

Cultivar	Dormancy	Origin	Characteristics
SA Standard	4-6	South African Landrace	Intermediate crown position Coarse to medium stems Exceptionally high resistance to root and crown-rot complex and other root and crown diseases
SuperCuf	9	Australia: Cuf101 cross with Sequel	Leafy stems Strong autumn and spring growth Strong regrowth after harvesting The crown is positioned higher than SA Standard

In the trial established in Autumn 2009, a second treatment was included, namely adding sufficient N to half of the plots to suppress inoculation (50 $kg.ha^{-1}$) (Van Oudshoorn et al., 2001), while leaving the other half dependent on the N resulting from the symbiotic relationship of *Sinorhizobium meliloti* with *Medicago sativa* L. (lucerne).

The sowing density was manipulated so that within each cultivar similar amounts of seed were used for both coated and non-coated treatments, rather than similar seed masses. The recommended sowing density for the coated seed treatments is 25$kg.ha^{-1}$. The applied sowing densities are described in Table 2. The trials established in autumn 2009 and spring 2010 used the recommended sowing density as baseline. Each treatment had five replicates of 2m x 5m. Similar soil conditions were created for the sowing density trial, in terms of fertilization and irrigation practices. The stands were fertilized, a week before sowing, with Superphosphate (50kg P. ha^{-1}) and Potassium chloride (250 kg K. ha^{-1}) so that P and K were not growth limiting factors. The stands received supplemental irrigation. Table 2 shows the sowing densities of the density trial. The five sowing densities vary on a 10% weight difference and the recommended sowing density is used as 100% reference base.

Table 2 Mass distribution of sowing densities.

% of Recommended Sowing Rate	Coated seed per hectare ($kg.ha^{-1}$)	Non-coated seed per hectare ($kg.ha^{-1}$)
80%	20	10
90%	22.5	11.3
100% Recommended Rate	**25**	**12.5**
110%	27.5	13.8
120%	30	22.5

The trials were harvested when the plants in the stand developed to the 10% bloom stage, as described by Fick and Mueller (1989) and Hall (1998). Samples were harvested by cutting plants in 1.5 m^2 quadrants, 0.07 m from the soil surface. Samples were stored in a cold storage room while harvesting continued and before the samples were transported to a ventilated glass house. The dry matter was determined by drying the samples in a ventilated glass house for 14 days. For these trials the dry matter production and stem to leaf ratio was determined for each treatment, which would provide information on the yield and a quality parameter of the treatments. Some stem to leaf ratios of harvests where excluded from analysis where significant leaf loss occurred, as a result of frost damage in August 2010 and cold storage failure in January 2011.

The dry matter production and stem leaf ratio data were subjected to statistical analysis using PROC GLM in SAS Version 9.2 software (SAS, 2002-2008). Analyses were done within cultivar and within harvests and not between harvests. For the sowing density trial, the interaction between the coating and the sowing density were also determined. The LSD's were taken at $P \leq 0.05$, but for the dry matter production the LSD was extended to $P \leq 0.1$ to identify treatments that might be significant under practical conditions.

Results and Discussion

Trial established in autumn 2009

Establishing a lucerne stand in autumn reduces the competition with weeds and ensures that more harvests can be achieved during the next growing season (Van Oudshoorn et al., 2001). Figure 2 illustrates the dry matter production of SA Standard established in autumn 2009. The data shows no significant difference between the coated and non-coated treatments for the first season (2009 – 2010). In the second season (2010 – 2011), however, there were significant differences between coated and non-coated treatments at the fifth harvest, observed for treatments which received N (with N $P \leq 0.026$) where the non-coated treatment had a higher dry matter yield than the coated treatments. This difference caused a difference in the total seasonal yield of the second season between stands planted with coated and non-coated seed ($P \leq 0.0936$).

When N treatments are compared, there were no significant differences for any treatment at any harvest over the two seasons. The low number of significant differences between N treatments and the coating treatment suggest that the stand was successfully inoculated, as the consequence of failed inoculation would result in no available N to the plant. It also suggests that inoculation of the plants, fertilized with N, was effective after the N was depleted, as the N level was enough to suppress initial infection, but not enough to sustain production (Van Oudshoorn et al., 2001). When comparing the Least Square Means (LSM's) the infection of the plants in the N treatments with *Sinorhizobium meliloti* had already started by the third ($P \leq 0.1088$) and fourth harvest ($P \leq 0.1005$) of the first season, as these two harvests show the highest variance, even though these differences are not significantly different.

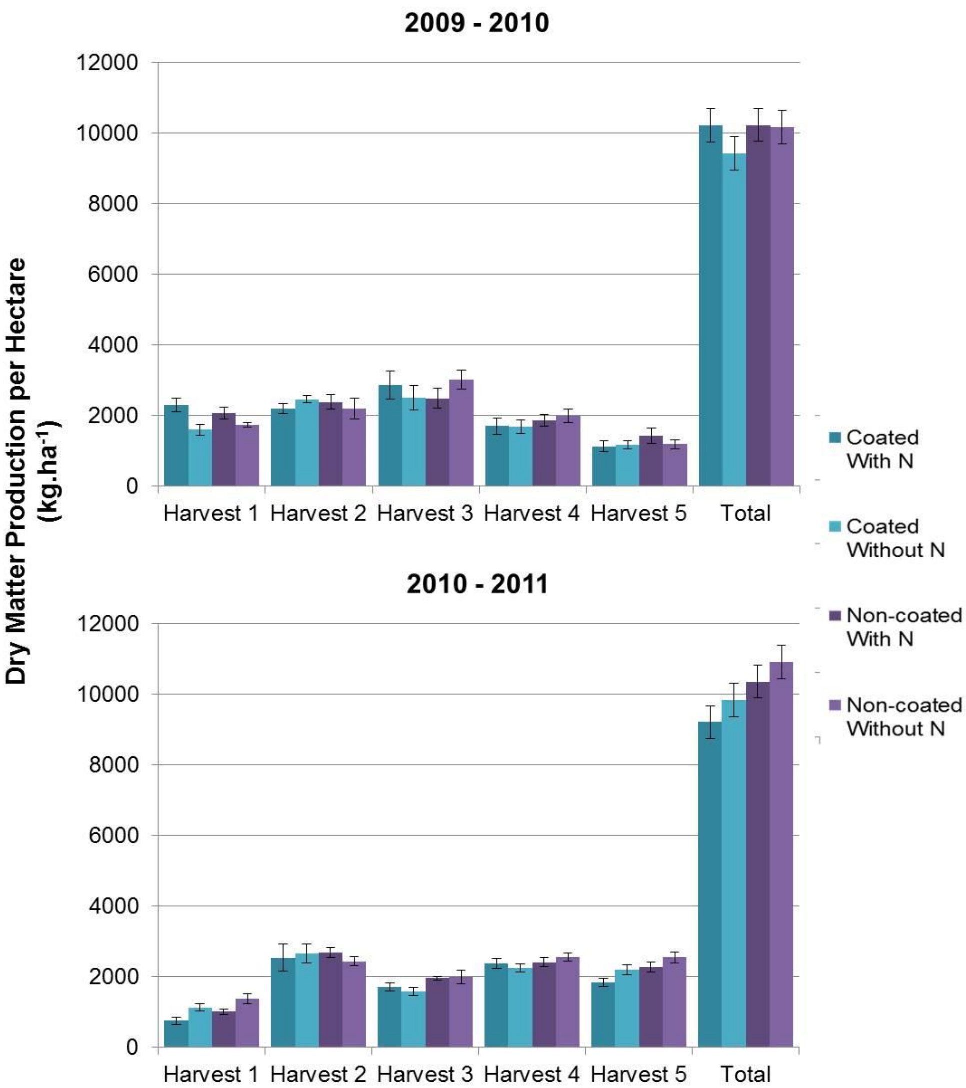

Figure 2 The dry matter production of SA Standard established in autumn 2009, including treatments established with N and without N.

With respect to the SuperCuf cultivar little effect is noted between N treatments. Figure 3 illustrates the dry matter production of SuperCuf as influenced by the seed coating and the nitrogen application. When comparing the means of the coated and non-coated treatments, the data shows very little difference between yields of stands established with coated and non-coated seed. The cumulative effect of the total seasonal yield, however, shows larger differences in dry matter production between the yields of plants from coated seed compared with non-coated seed, for treatments that did not receive any N fertilizer ($P \leq 0.0915$).

When comparing the nitrogen treatments, there were significant differences in the first season, but not in the second season. This suggests that the inoculant had successfully infected the treatment where N was applied by the start of the new growing season. For the coated treatments the difference was seen in the second harvest (P ≤ 0.0092). For the non-coated treatment the difference already started at the first harvest (P ≤ 0.0255) and was also present at the second harvest (P ≤ 0.019. This trend was, however, not evident for the beginning of the second season, where there were very little differences between N treatments. The seasonal totals for SuperCuf showed cumulative differences between the N treatments for stands established with both coated (P ≤ 0.0172) and non-coated seed treatments (P ≤ 0.0115) and in both cases the stands that did not receive N fertilizer had higher yields.

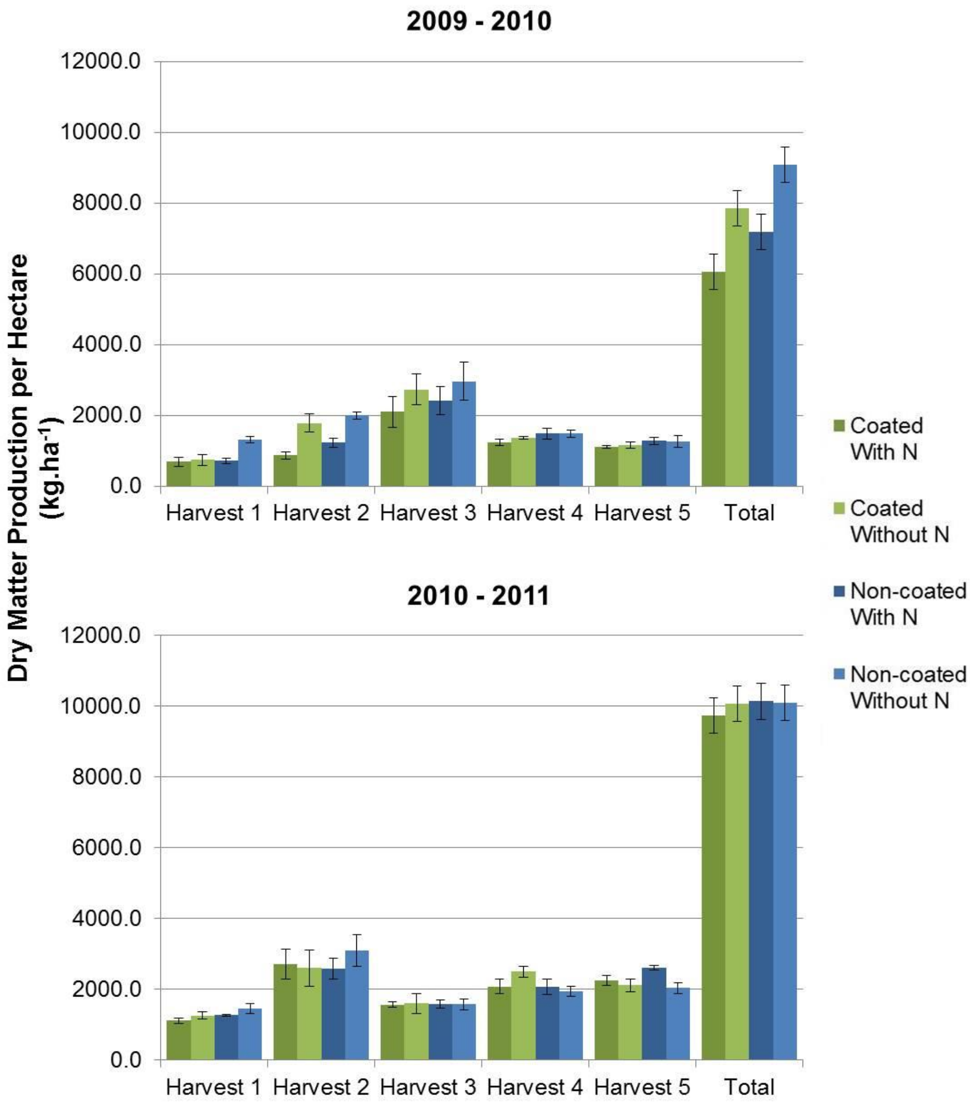

Figure 3 The dry matter production of SuperCuf established in autumn 2009, including treatments established with N and without N.

The difference in response to N applications between the coated and non-coated treatments', suggest that the inoculant in the coating takes longer to infect SuperCuf than the conventional method, which might be due to a slower colonisation of the rhizosphere. According to Brockwell (1962) a delay in germination or a delay in the release of the inoculant from the coating, can cause lower infection of the root hairs. Another explanation for the slower infection of the coated SuperCuf is that there is a lower compatibility between the inoculant and the plants as described by Cooper (2004). The response of SA Standard and SuperCuf to applied N, shows that SuperCuf is more sensitive to N deficiencies as can be seen in the dry matter production. The response of SuperCuf to seed coating follows the same pattern, in that it is more sensitive to the effects of the coating, affecting the yield.

Figure 4 shows the stem to leaf ratio of SA Standard and SuperCuf as influenced by the seed coatings. According to the data, the seed coating did not influence the stem to leaf ratio for SA Standard, but did, however, show significant differences for SuperCuf, not only for the first, but also the second season. For the first harvest ($P \leq 0.0022$), the third harvest ($P \leq 0.001$) and the forth harvest ($P \leq 0.0038$) of the first season and the second harvest of the second season ($P \leq 0.0432$), the coated seed treatments had lower stem to leaf ratio than the non-coated treatments, indicating that there are more leaves than stems at these times. These harvests correspond with harvests where yield was also lower. This suggests that the lower yields are due to shorter internodes or shorter stems with the same number of leaves and or larger leaves than the non-coated seed treatments. These results are similar to work done by Teixeira et al. (2004), where the amount of available reserves, influenced the leaf size and stem length rather than the number of leaves or stems.

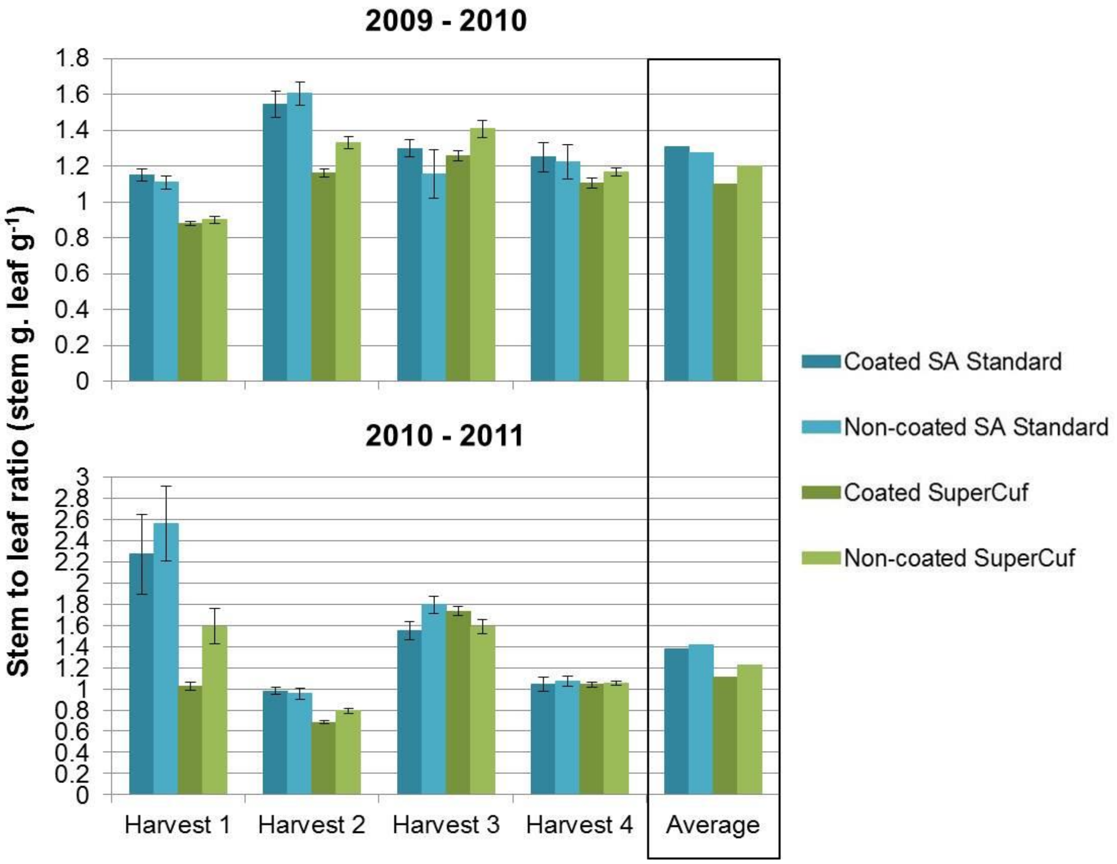

Figure 4 Stem to leaf ratio of SA Standard and SuperCuf established in autumn 2009.

Trial established in spring 2010

Establishing a lucerne stand in spring has the advantage of more favourable soil water conditions without irrigation and more seedlings are likely to survive frost damages, compared with seedlings that have to survive through winter (Van Oudshoorn et al., 2001). Figure 5 shows the dry matter production for the trial established in spring 2010. This data shows difference between stands sown with the coated and non-coated seed treatments for SA Standard at the first two harvests (first harvest ($P \leq 0.0067$), second harvest ($P \leq 0.0118$). The seasonal total yield of SA Standard is very similar between stands established with coated and non-coated seed. Additionally, SuperCuf showed no notable differences between the stands established with coated and non-coated seed treatments, in contrast to the trial established in autumn 2009. The ultimate yields of the seasons are similar for both trials (autumn 2009 and spring 2010).

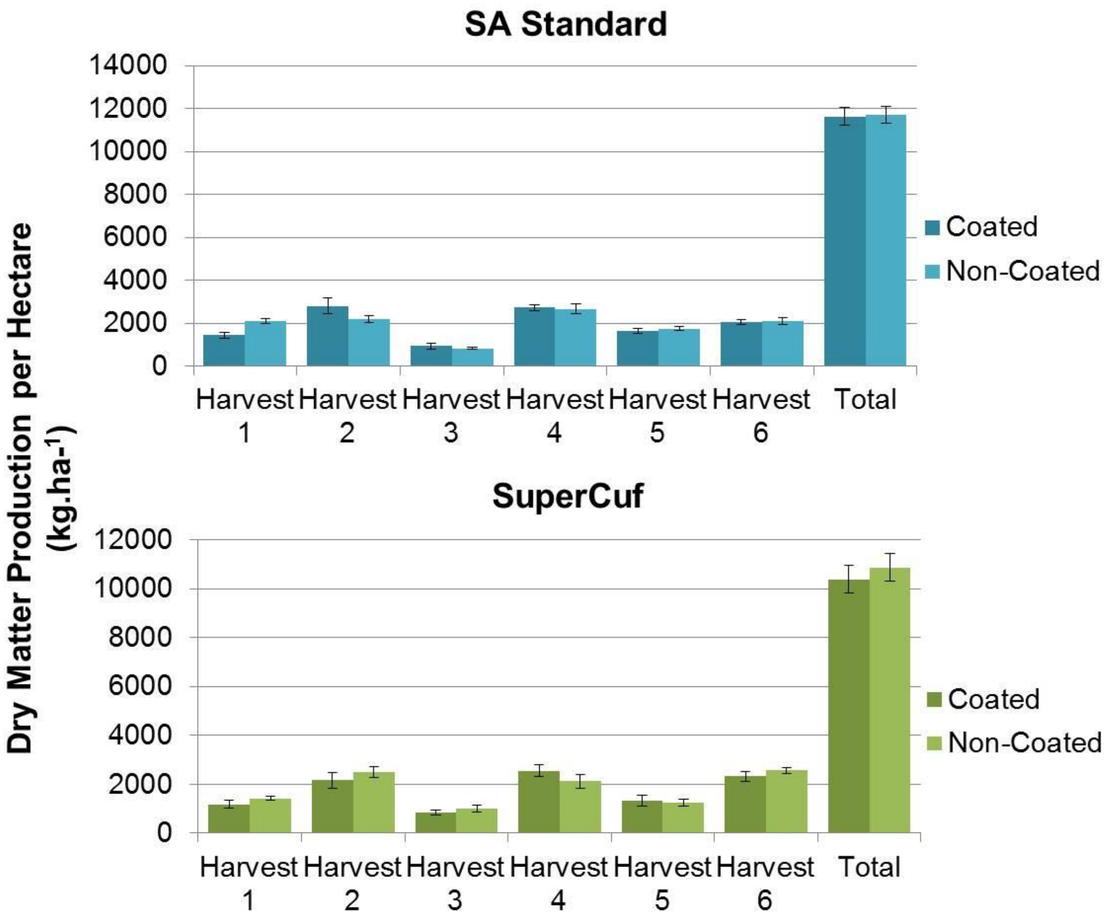

Figure 5 The dry matter production of SA Standard and SuperCuf established in spring 2010

Figure 6 presents the data of the stem to leaf ratio of the trial established in spring 2010. For this trial there were no remarkable differences between coated and non-coated seed treatments for both cultivars. There was, however, a difference, even though not significant ($P \leq 0.05$) for SuperCuf from the second harvest ($P \leq 0.0784$). Again this compares to the data collected for the autumn 2009 trial. However, in the spring 2010 trial the non-coated seed treatment had the better quality in terms of stem to leaf ratio, but can't be statistically supported.

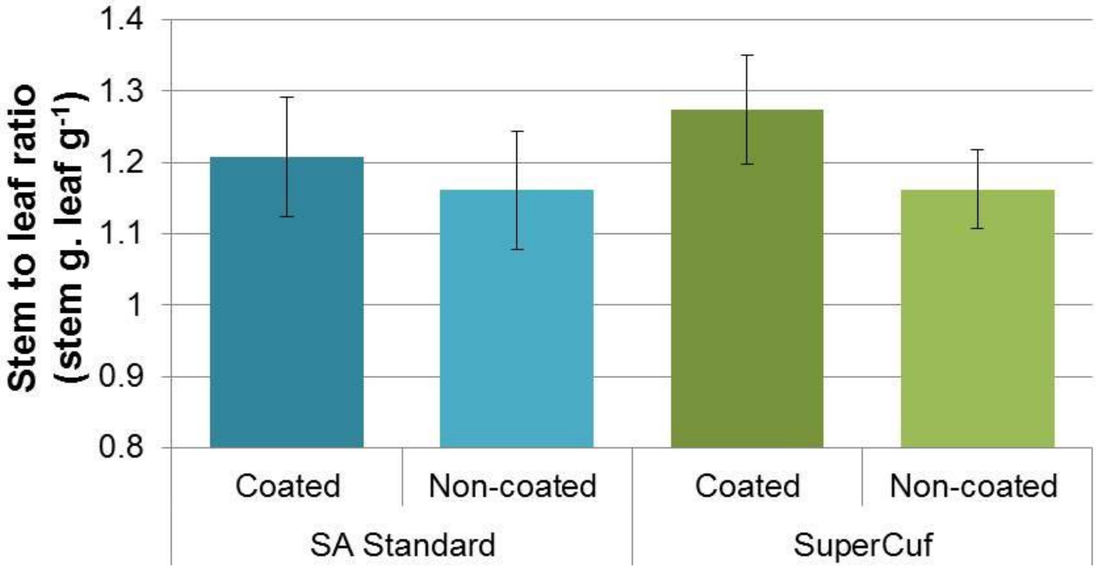

Figure 6 Average stem to leaf ratio of SA Standard and SuperCuf established in spring 2010.

Sowing density trial established in spring 2010.

When considering the data from the sowing density trial, it is expected that there would be an increase in yield with the higher sowing densities (Finch-Savage, 2004), but data shown in Figure 7, however, show no such trend. According to Figure 7, there were no significant differences between plants grown from coated and non-coated seed at the different harvests. There were, however, differences between these treatments at the first harvest for plants sown at 90% to 120%, where the plants grown from non-coated seed had higher yields. These differences were mostly balanced out by the subsequent harvests and the total seasonal yield (Figure 8) which only show significant differences between 90% ($P \leq 0.0452$) and 120% ($P \leq 0.0039$) of the recommended sowing density. It is evident that the non-coated treatments (sown at 12.5 $kg.ha^{-1}$) had higher cumulative yields than the treatments established with coated seed (sown at 25$kg.ha^{-1}$).

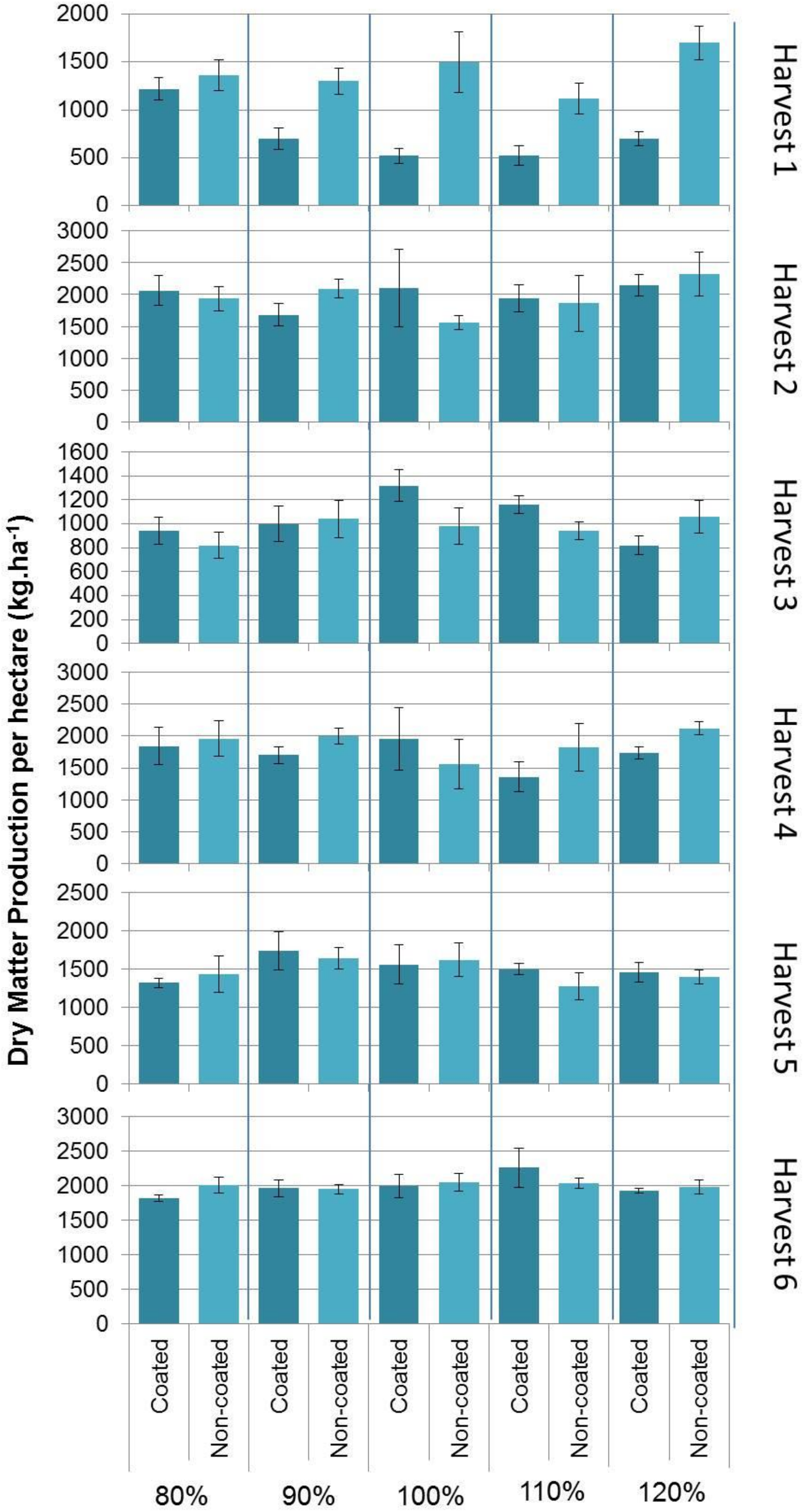

Figure 7 Dry matter production of SA Standard, showing the coating treatments at a range of sowing densities using the recommended sowing density as reference.

According to the data presented in Figure 8, there is no significant difference between the sowing density treatments (at 80% to 120% of recommended sowing density) of plants grown from coated seed. There were, however, significant ($P \leq 0.1$) differences between treatments sown with non-coated seed. Stands sown at 120% of recommended sowing density had a higher dry matter production than stands sown at 110% ($P \leq 0.0137$), 100% ($P \leq 0.034$) and 80% ($P \leq 0.0872$). It is unclear why the stands sown at 90% and 120% of recommended sowing density had the highest dry matter production, while the stands sown at 110% of recommended sowing density had the lowest yield. The difference in response for non-coated treatments, as compared to the coated treatments, suggests that the coated treatment could help overcome limiting factors associated with higher planting densities, such as competition for water and nutrients.

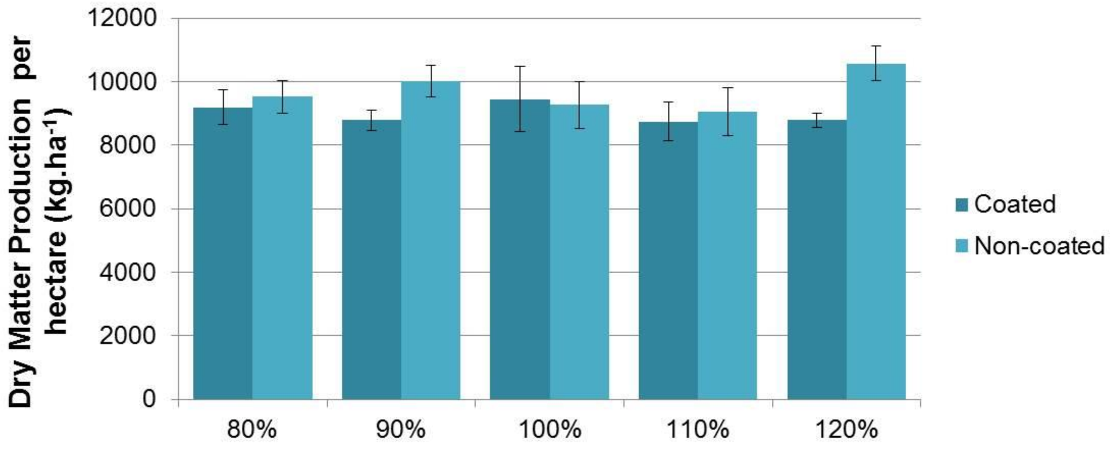

Figure 8 The seasonal total dry matter production of SA Standard at a range of sowing densities

From this data, it is clear that the seed coating and the sowing density have limited influence on the yield of the stands. Difference in sowing density would be overcome by physio-morphological characteristics such as the size of the crowns, number of stems per plant or number of stems from axillary buds (branching) (Meyer, 1999). These characteristics were, however, not measured and require further investigation. According to Gosse et al. (1988), it could also be reasoned that the number of plants per area decreased during the summer when growth rates are the highest, with parallel increases in the size of the crown or number of stems per plant. Therefore, the number of plants and the size of the crown ended up the same resulting in similar yields.

Gosse et al. (1988) also suggests that dry matter yield is not a parameter that would present a clear indication of the competition effect for light, therefore filling spaces to intercept light can easily overcome effects caused by a lower number of plants per area. Competition for N, water and nutrients would have a larger effect on yield than the competition for light. Following this reasoning, it stands to reason that the coated SA Standard seed treatments either showed restrictions in the higher sowing densities and or adaptation or filling at lower densities to overcome the lower number of plants per area. There were also no observable deficiencies of water, nutrients and nitrogen, causing differences in yield between densities. The individual differences observed in the non-coated seed treatments could however be attributed to this deficiency.

Literature suggests that regrowth after winter is influenced by the quantity of carbon and N stored in the perennial organs (roots and crown) (Teixeira et al., 2004). Additionally Van Oudshoorn et al. (2001) and Berg et al. (2003) emphasizes the importance of potassium in the winter hardiness of plants, as it plays an important role in the production of root reserves. According to Undersander et al. (1997), the winter dormancy is also influenced, or activated by climatic factors and is clearly a complex situation with additional factors influencing the genetic character of the cultivars. Clear reasoning on the influence of coating technology on root reserves and winter dormancy is not known, and requires further investigation.

SuperCuf does not show the same variation in winter dormancy (dormancy rating 9). The data in Figure 9 and Figure 10 shows that none of the variables, coating and sowing density, caused any significant differences between yields of stands sown between 80% and 120% of the recommended sowing density. There were, however, differences in yield between stands established with coated and non-coated seed, at the second harvest. The stands established with coated seed had higher yields than the stands established with non-coated seed at 110% and 120% of recommended sowing density. This displays that, at these higher densities, there was some competition between the plants and the plants grown from coated seed had the required resources to grow well.

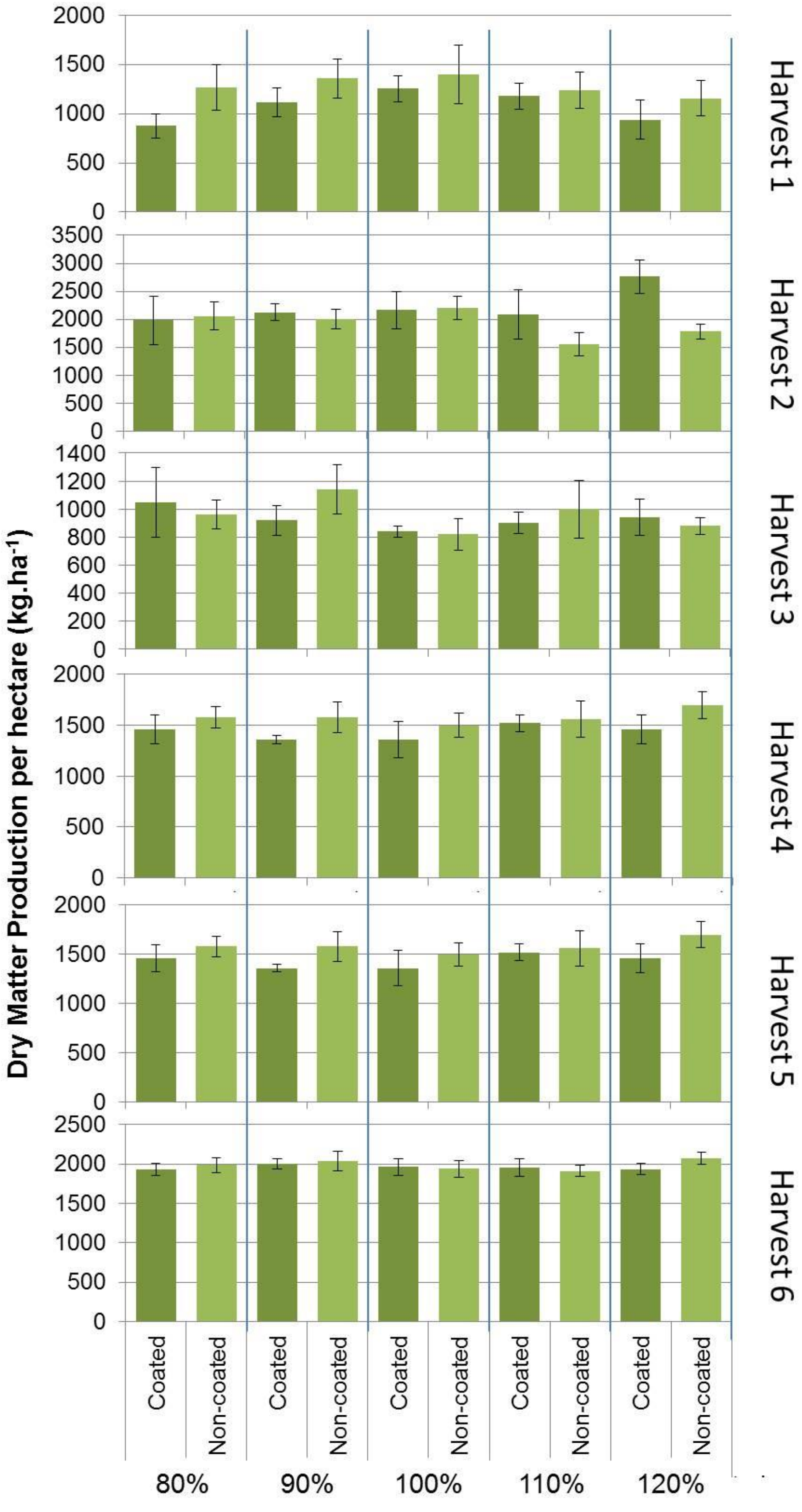

Figure 9 Dry matter production of SuperCuf, showing the coating treatments at a range of sowing densities using the recommended sowing density as reference.

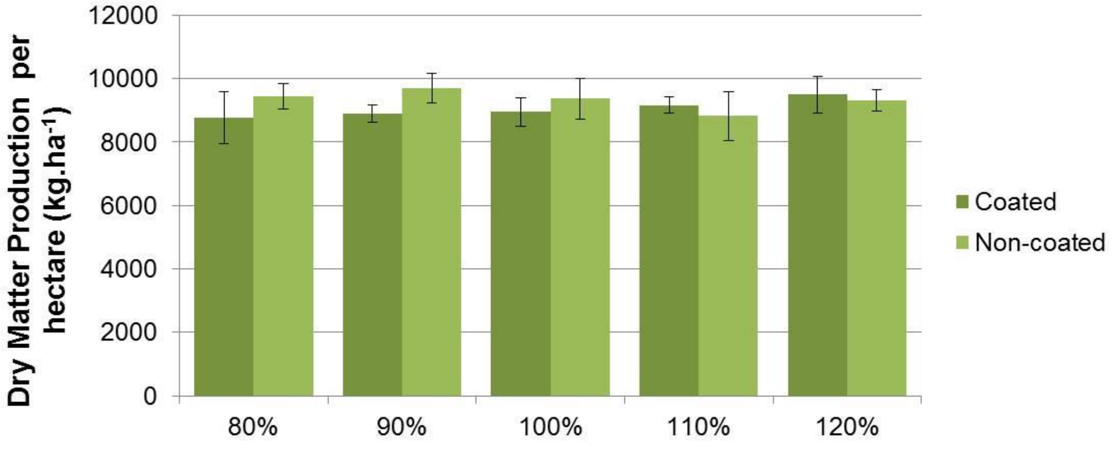

Figure 10 The seasonal total dry matter production of SuperCuf at a range of sowing densities

Even though there is no significance within each sowing density's stem to leaf ratio between stands grown from coated and non-coated SuperCuf seed treatments, there is a difference between the non-coated treatments sown at 80% and 120% of the recommended sowing densities, where 120% had the lower ratio or better quality (Figure 11). This suggests that these treatments are adapted and that the differences in morphological characteristics balance the yield responses.

SA Standard also shows limited differences between coating and density treatments in terms of stem to leaf ratio. There is, however, a difference between coated and non-coated treatments at 80% of recommended sowing density. There is also a significant difference between non-coated treatments at 80% and 100% of sowing densities.

The similarity of the data between coated and non-coated seed treatments and the sowing densities, suggests that there was no trend in terms of stem to leaf ratio, but that morphological adaptations of SA Standard and SuperCuf results in the same quality between the treatments. Higher plant densities, could for instance have less stems per plant, but more leaves per stem and longer stems to accommodate them, as the morphological characteristics of lucerne are significantly correlated with yield and quality (Katic et al., 2004). According to Lamb et al. (2006) the density of the plants evens out and is facilitated by seedling death and survival. Whatever the adaptations, there is limited effect in both yield and quality in terms of stem to leaf ratio at various planting densities.

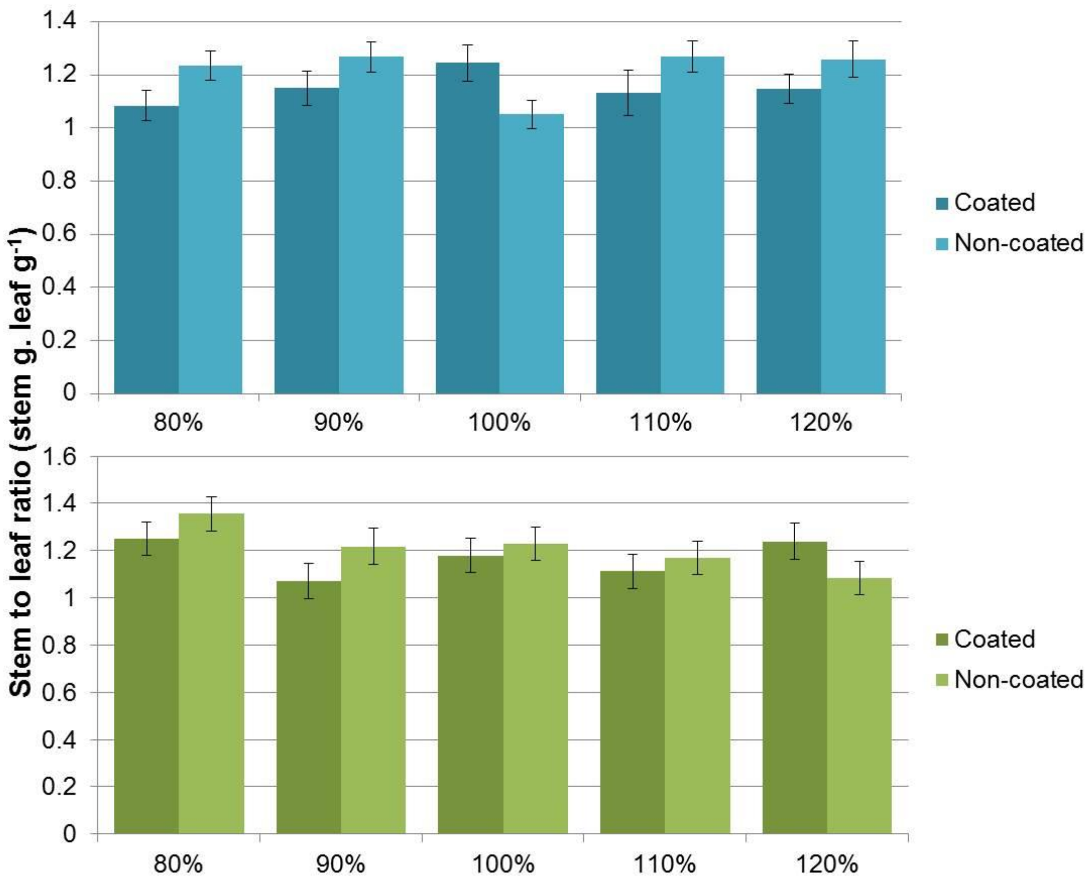

Figure 11 Stem to leaf ratio of SA Standard and SuperCuf at a range of sowing densities established in spring 2010.

Conclusion

It can be concluded from all these trails that the influence of seed coating on the seasonal yield, is limited. As the growing conditions are manipulated, with sowing density or N application to inhibit Rhizobial inoculation, the stands established with coated seed, compared with non-coated seed, react differently. SA Standard showed higher yields for non-coated seed treatments were used compared with coated seed treatments, when stands were established with N fertilizer in the second season of the trial established in autumn 2009. SuperCuf showed significant differences between coated and non-coated seed treatments in the first season of growth, but the influence is more significant between N treatments. There was also no significant difference in the average stem to leaf ratio between coated plants grown from coated and non-coated SA Standard seed. The same similarity was found in the trail established in spring 2010, where the seasonal yields and quality (stem to leaf ratio) was non-significant.

Seed coating has a limited effect on yield for SA Standard and SuperCuf at sowing densities between 80% and 120% of recommended sowing densities (10- 22.5 kg non-coated seed per hectare). This statement is, however, dependent on successful stand establishment. Seed coatings are convenient ways to inoculate a stand as it is effortless for the farmer.

The effect of seed coating on the quality in terms of stem to leaf ratio is irregular and it is more likely a combination of multiple factors that lead to differences in the stem: leaf ratio. Yield and stem to leaf ratio differences are influenced by the selection of the cultivar, the management of the stand, the climatic conditions and the maturity of the stand. The last two factors most likely contributed to the differences observed between harvests. This data can, however, be understood better the more mature the lucerne becomes and the longer the trial is monitored.

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CHAPTER 6

General Conclusions and Recommendations

1. The effect of seed coating on the germination of Lucerne

The basic requirements for germination are water, temperature that facilitates cellular activity, an aerated substrate and a substrate that will not mechanically impede the emergence of the radicle (Hadas 2004). Seed coatings should therefore not interfere with these requirements, and should rather promote the access of these components to the seed. Determining the impact of the seed coating on the germination of lucerne, under optimal and suboptimal germination conditions, would enable the farmer to make a well informed decision concerning the use of coated seed.

There were significant differences in germination vigour of coated and non-coated SA Standard seed, where coated seed had higher germination percentage at day 4 of the experiments than the non-coated seed. These differences were still present at day 10 of the experiment and were found when using both petri-dishes and the Jacobsen table to determine germination percentage. When the germination was tested in a range of saline water conditions, it was found that SA Standard did not show differences between coated and non-coated seed treatments when using water with a fairly low electrical conductivity (EC). However, when using water with a higher saline content (500 and 750 $\mu S.cm^{-1}$), there was a pronounced difference between coated and non-coated seed treatments, where the coated seeds had higher germination than the non-coated seed. These observations leads to the conclusion that SA Standard is able to utilize the seed coating to benefit germination, especially under saline conditions, where the coating helps to overcome limiting factors created by the saline conditions. The inhibition of germination caused by the salinity is likely not caused by a low osmotic pressure (Chapter 2, Table 3), as the saline water treatments' osmotic potential did not fall in the range which would cause significant inhibition of germination (Chapter 2, Figure 5). Equipping the seed with the nutrients to better adjust to the saline conditions can increase the success of establishing lucerne in salt afflicted conditions. This can be due to a precipitation reaction of the NaCl or the elements in the coating are counteracting the NaCl effects on the seed. Common deficiencies caused by NaCl are Ca, K, P and NO_3^-, which can be supplied by the seed coating. The supplementation of Ca in saline conditions has shown to improve the germination response of a number of species (Cachorro et al. 1994; Grattan and Grieve 1999).

SuperCuf had no differences between coated and non-coated seed treatments in terms of vigour or germination percentage. There was, however, a significant difference in the results between using a petri-dish and Jacobsen table (Chapter 2, Figure 4), where the use of the Jacobsen table showed higher germination %. This difference suggests that there might be a substance exuded from the seed which causes autotoxicity. No literature was found to confirm that this type of autotoxicity exists and the water surrounding the seed was not analysed to determine the cause of the reduced germination. Further investigation is therefore required.

There were no significant differences between coated and non-coated seed treatments when germination percentage was determined using different saline water conditions. There were also no differences between the germination of the seed between the saline conditions. This suggests that the coating does not influence the germination of SuperCuf and that SuperCuf is more adapted to saline conditions than SA Standard. Even though there is no evidence to support this theory, it is possible that SuperCuf was selected to be more tolerant of saline conditions, especially considering the significant problems Australia is experiencing with dryland salinity.

Other than the better handling ability of coated seed, it can be recommended that coated seed be used in environments prone to salinity, especially when NaCl is prevalent in the soil. When determining the germination percentage of coated seed, it would be better to use a method where there is some dilution in the immediate vicinity of the seed, simulating the conditions in the soil.

To answer the new questions that emerged from these trials, some genetic information is required about the cultivars. This will help to determine whether the impact of seed coatings is cultivar specific and whether cultivars tolerant to salinity will act similar to the seed coating. Analysis of the water from the germination trials, will determine if SuperCuf seed leaches autotoxic chemicals from the seed and whether these chemicals also inhibit germination of other cultivars and species.

2. *The influence of seed coating on seedling emergence of Lucerne in different growth mediums*

The influence of seed coating and growth medium and the interaction of the two treatments on the emergence and survival of lucerne can have significant consequences for yield (Finch-Savage 2004) and stand efficiency in terms of water and nutrient use efficiency (Van Oudshoorn et al. 2001). Identifying effects associated with the interaction between seed coating and the growth medium will help identify possible situations where the seed coating will be more effective in assisting the seedling to establish.

As with the germination trial, the two cultivars used did not show the same differences in emergence between coated and non-coated seeds. Non-coated SA Standard had significantly lower emergence than the coated seed, when planted in a commercial growth medium. It appears that the composition of the commercial growth medium inhibits the emergence of SA Standard, but the coating can overcome the inhibition. Identifying the instrumental factor/s in the growth medium which influence seedling emergence will help identify the constituent in the seed coating which overcome this inhibition.

When the emergence percentage was determined under different saline irrigation treatments, it was found that the emergence was highest when irrigated with distilled water. It was, however, interesting to observe that the plants irrigated with 750 $\mu S.cm^{-1}$ water had higher emergence than the two lower salt treatments. This data is similar to the germination data, where the seed treated with 500 and 750 $\mu S.cm^{-1}$ water had higher emergence percentage than the other water treatments.

SuperCuf did not show any difference between the seed coating treatments when planted in different growth media. It appears that the growth medium does, however, influence the emergence of SuperCuf seedlings (Chapter 3, Figure 2), where the silica sand growth medium had lower emergence than the other growth media. In contrast to the results found for SA Standard, SuperCuf appears to be more influenced by the silica sand treatments. This is likely due to the easy draining nature of the growth medium, causing significant wet and dry phases, due to the lower water holding capacity. When the seedlings were irrigated with different saline water treatments, it was clear that the coated treatments provide assistance to the seedling under higher saline conditions (750µS.cm^{-1}). This protection is possibly as a result of the Ca from the lime, which protects the tissue from Na and Cl uptake and subsequent damage due to accumulation of these salts (Cachorro et al. 1994; Grattan and Grieve 1999).

From this data it can be concluded that the use of coated seed can improve the emergence of SuperCuf in saline conditions. It would be of great value to analyse the growth media before and after the trials. This would determine how much of the coating is left in the soil after the trial period and how the irrigation changed the salinity of the soil during the trial period. It would also be of interest to analyse some leachate from the soil during the trial, which would determine what nutrients are soluble and whether these nutrients are dissolved from the coating or from the soil.

3. *The effect of seed coating on physio-morphological characteristics of Lucerne*

According to Scott and Blair (1988) the strong early growth of seedlings compared with weeds is most important in determining how successful the establishment of the crop stand will be. The early growth of seedlings is mostly determined by the quality of the seed, resulting in early emergence and strong growth, attaining photosynthetic independence early. Physio-morphological characteristics of seedlings which can act as an indicator of how well a seedling is adapted to its environment, are seedling height, internode length and growth rate (Katic et al. 2004). These factors will also influence the yield of a plant and stand. According to the data, both cultivars and seed treatments were well adapted to the growing conditions created with different saline irrigation treatments, as there were very few differences observed. The differences in stem height observed were mostly found when irrigated with high salinity water, causing slower development for young seedlings by decreasing the nutrient availability in the soil and influencing the water uptake by the plant. The slower development also resulted in lower number of leaves per plant and shoot dry matter production. The effects are, however, short lived and the plants recover from the stress by possibly maintaining lower concentrations of salt ions in the leaves and exclusion of salt by the roots. This is achieved by creating osmoprotectants which requires a large amount of energy, diverted from the cellular pathway which delivers to developing cells with energy (Humphries and Auricht 2001; Munns and Tester 2008; Noble et al. 1984).

The amount of light intercepted, as influenced by the leaf area per plant, is very important for plants grown in saline conditions, as metabolites created by photosynthesis can be used in the recovery of cells damaged by saline conditions (Riadh et al. 2010). The data from this trial illustrated that during the observation period, a limited amount of difference occurred between plants (SA Standard and SuperCuf) grown from coated and non-coated seed. For most of the observations where differences were observed, plants grown from coated seed had greater leaf areas than the plants grown from non-coated seed. When considering the influence of the water quality on the expansion of leaves, it was found that with a slight increase in salinity, plants have larger leaf areas, while a higher salinity generally resulted in smaller leaves. It is known that leaf expansion is negatively influenced by higher saline conditions and evidence suggests that hormonal signalling controls the expansion of the leaves (Munns 2002).

From a producer's perspective, one of the most important parameters to be measured is dry matter production. Seedling shoot dry matter yield also has implications for seedling survival and successful stand establishment. The data collected from this trial suggests that no predictions can be made whether plants grown from coated or non-coated seed (both SA Standard and SuperCuf) will result in differences in shoot dry matter yield as there were no consistent differences between the treatments. Considering the influence of the water quality on the yield of the seedlings, it was found that SA Standard seedlings irrigated with 500 $\mu S.cm^{-1}$ had higher yields than when the other water treatments were used. This suggests that the water and the coating constituents stimulated the growth of the seedling, as the stem height and number of leaves were significantly higher. The effect of water quality on SuperCuf appears to be negligible. This is consistent with data from Chapter 2 and Chapter 3, showing that the saline conditions do not influence SuperCuf seedlings.

Other than the above ground physio-morphological characteristics measured, data from this trial showed that root parameters are not influenced by the seed coating and water quality. However, harvesting the roots proved to be problematic and the differences in the root parameters might only be discovered when an in-depth root study is done.

When an area is afflicted by salinity, it can be recommended that lucerne be established there. The most appropriate cultivar for these saline conditions is SuperCuf because there are limited differences between the physio-morphological parameters when irrigated with the different saline water treatments. In areas with less severe saline environments, the use of coated seed will result in higher leaf areas, which will enable seedlings to recover from possible damage.

4. The yield differences of lucerne grown from coated seed treatments

Many extension documents are available from reputable sources, which give clear instruction on how to establish a lucerne stand, the quantities of nutrients are required and extracted per harvest and when to harvest to optimise the quality, yield and regrowth of a stand. Two of the most important parameters measured in a lucerne production system are yield and quality of the stand. From the field trials it was detected that the two cultivars do not have similar yields or quality. These two parameters appear to have a negative correlation with each other. SuperCuf has lower stem: leaf ratios and also lower yields, while SA Standard has lower quality than SuperCuf, but higher yields, under the same management conditions.

Using pre-inoculated coated seed is a very easy way to simplify the establishment process. The bigger seeds make the calibration of equipment easier, while the colour of seed makes it easy to determine whether the placement of the seed is at the correct depth. If the coated seed is stored and transported under the correct conditions, the whole inoculation process can be eliminated from the establishment process. This is corroborated by the data obtained from the trial established in autumn 2009 (Chapter 5, Figure 2 and Figure 3). From this data it was also clear that SuperCuf was more sensitive to the N treatment and performed better when inoculated, rather than being dependent on N fertilizer (Chapter 5, Figure 3).

When considering the sowing density of lucerne, it appears that the sowing densities in this trial resulted in no notable differences. This is either due to morphological differences in the plants, which balances the yields obtained from the different stands, or the number of plants that survived is the same. The density of the seedlings decreases during the establishment process, when the growth rates are the highest, resulting in a higher competition effects between plants. If the same number of seedlings develop into mature plants, their structural development would be parallel in terms of the size of the crown or the number of stems per plant (Gosse et al. 1988).

5. Recommendations

It is concluded at this stage that the determination of the germination percentage of coated seed, would be higher when a method is used where there is some dilution in the immediate vicinity of the seed, simulating conditions surrounding the seed in soil. Analysis of the water from the germination trials, will determine if SuperCuf seed leaches autotoxic chemicals from the seed and whether these chemicals also inhibit germination of other cultivars and species. It would also be of interest to analyse some leachate from the soil during the emergence and pot trials, which would determine what nutrients are soluble and whether these nutrients are dissolved from the coating or from the soil. Analysis of the growth media would also provide valuable information. This would determine how much of the coating is left in the soil after the trial period and how the irrigation changed the salinity of the soil during the trial period.

When an area is afflicted with salinity, it can be recommended that lucerne be established there. The most appropriate cultivar of the two tested for these saline conditions is SuperCuf because there are limited differences between the physio-morphological parameters when irrigated with the different saline water treatments, but this will also depend on the way the lucerne will be used. Under less severe saline environments, the use of coated seed will result in higher leaf areas, which will enable seedlings to recover from possible damage. The germination and emergence percentage of coated seed was often higher than the non-coated seed treatments, which is an additional advantage over the improved handling of the seed. Additional information which would be valuable to this recommendation is to identify is the coating is equally effective when the dominant salt is not NaCl.

According to this data, the sowing density of coated and non-coated lucerne can safely be decreased with 20% if the growing conditions are suitable for lucerne production. If it is expected that there will be a higher seedling mortality rate, a higher sowing density can be justified. Information that would add great value to the sowing density trial would be to monitor the density of live seedlings over time as the stand is establishing. It would also be useful to determine whether there are significant differences in the size of the crowns and the number of tillers per plant, as this would give an indication which mechanism of adaptation was responsible for the similarities of the sowing density stands.

Some genetic information regarding physio-morphological characteristics and tolerance to salinity would be of great interest. This will help to determine whether cultivars in general act differently to the seed coating or whether cultivars tolerant of extreme soil conditions such as salinity, will act similar to the seed coating.

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