A COMPARISON OF SELECTED ENHANCED (COATED) AND NON-ENHANCED GRASS SEED TYPES FOR RE-SEEDING OF DISTURBED AREAS

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Disclaimer

The trials contained in this publication were designed to meet certain criteria as set by the sponsor and the results were therefore purpose driven. Certain experiments were carried out in controlled environment and for this reason the interpretation of the results cannot take into consideration the numerous factors, which may occur out in the field on commercial farming level. Those include climate (moisture and temperature), soil management, soil type, specific genetic predisposition and many others.

Advance Seed Company

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And GOD said,
Let the earth bring forth grass,
the herb yielding seed,
and the fruit tree yielding fruit after his kind,
whose seed in itself,
upon the earth: and it was so.

- Genesis 1:11 -

Opgedra aan Pappa & Mamma...

ABSTRACT

A comparison of selected enhanced (coated) and non-enhanced grass seed types for re-seeding of disturbed areas.

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Restoration and rehabilitation activities are presently considered to be a major priority in environmental management, whether the activity implies the restoration of neglected cultivated pastures or degraded rangelands due to overgrazing and climatic impacts, or the rehabilitation of the mining and industrial areas. However, the goals are not easily achieved, mainly due to the high input costs, including that of re-seeding activities. Re-seeding success is influenced by the quality and effectiveness of the used seed regarding germination and establishment under natural field conditions. If techniques can be developed to enhance the effectiveness of germination and establishment percentage of the seed in restoration and rehabilitation sites, a better cover, density and biomass yield can be expected, which will improve the rehabilitation process.

It is known that commercially available grass seed has a better germination percentage and establishment percentage in comparison with seed locally harvested, which may include many impurities such as sticks and stones. The availability of the locally harvested seed types, especially of certain ecotypes adapted to specific environments, can be poor. Advance Seed Company (Krugersdorp, South Africa) has taken commercially available grass seed to the next level by enhancing (coating) the seed with a multitude of different treatments to ensure better handling of the seed in re-seeding applications. These treatments also have advantages such as a higher seed to soil contact, growth stimulants included in the treatment, higher seed purity and the protection of the seed against predation by ants and other insects and against harsh chemicals in the soil, which might have an influence on the germination percentage of the seed and the establishment of seedlings.

The objective of this study was to investigate whether or not certain enhanced grass seed types of selected grass species will have a better germination and establishment percentage, fresh and dry above- (leaves) and below–ground (root) biomass yield (glasshouse trials) and dry above-ground biomass yields (natural fields trials) in

comparison with non-enhanced types. The predation of enhanced and non-enhanced seeds by ants and other insects, as well as the development of the vascular tissue in the transitional region of the seedlings was also investigated.

The grasses assessed included enhanced and non-enhanced seed types of *Chloris gayana* (Rhodes grass), *Cynodon dactylon* (Couch grass), *Digitaria eriantha* (Common finger grass) and *Eragrostis curvula* (Weeping love grass). In the case of *E. curvula,* four seed types, including the non-enhanced seed type were tested. These included non-enhanced seed, seed treated with "plain coat", enhancement with "organic insecticide on the base of the coat" (i.e. insecticide between the enhancement and the seed) and enhancement with "organic insecticide on the base of the coat and as an overspray" (i.e. insecticide between the enhancement and the seed, as well as spraying the insecticide over the coated seed). The above mentioned species are commonly used in grass seed mixtures for rehabilitation and restoration purposes. Seeds were supplied by Advance Seed Company. The seed enhancement treatments as well as the non-enhanced seed types were tested under various conditions. The chemical composition of the enhancement treatment used in the coating process is only known by the seed technicians at Advance Seed Company.

All the seed supplied by the seed merchant had a purity of >95%. With the application of dormancy breaking in the germination tests the non-enhanced seed types of *Chloris gayana* had the higher germination percentage of the seed type or the same species. Other differences included the germination percentage being significantly higher for the enhanced seed type of *Cynodon dactylon* than the non-enhanced seed type. Lower germination percentages were noted in the comparison of the *E. curvula* seed types, were the non-enhanced seed type had a higher germination percentage in comparison with the enhanced seed types. In the germination tests without dormancy breaking being applied, these results differ. With regard to the establishment percentages, similar statistical differences were noted in both the Coco Peat Moss medium and the Hygromix growth medium.

In the above- and below-ground biomass production trials in the glass house the only significant difference were noted in the biomass production of *D. eriantha* plants. In the case of the dry above- and below-ground biomass yield the plants of the non-enhanced seed types of *D. eriantha* yielded a significantly higher biomass in comparison with the

plants harvested from the enhanced seed type of the same species. With regard to the natural field trials a few significant differences were noted.

The results indicated that the enhanced seed types of *Chloris gayana* and *Cynodon dactylon*, the non-enhanced seed type of *D. eriantha* as well as the non-enhanced and "organic insecticide on base and as overspray" enhancement of *E. curvula* can be used in re-seeding restoration and rehabilitation practices. *Eragrostis curvula* enhanced with "plain coat" is not recommended to be used for re-seeding in disturbed areas.

Keywords: biomass; coated seed; disturbed areas; enhanced seed; re-seeding

OPSOMMING

'n Vergelyking tussen geselekteerde behandelde (omhulde) and onbehandelde grassaadtipes vir die herverstiging van gras in versteurde gebiede.

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Die belangrikheid van die restourasie en rehabilitasie van versteurde gebiede word deesdae as 'n prioriteit in omgewingsbestuur geag, of dit nou die restourasie van verwaarloosde aangeplante weiding, gedegradeerde landelike weivelde a.g.v oorbeweiding en klimaatsverandering, of die rehabilitasie van myne en industriële gebiede beteken. Hierdie doelwitte is egter moeilik bereikbaar aangsien insetkostes, insluitend die koste van die hervestiging van grasse, geweldig hoog is. Die suksesvolle hervestiging van grasse word beïnvloed deur die kwaliteit en die effektiwiteit van die saad ten opsigte van ontkieming en vestiging onder natuuurlike omstandighede. Indien tegnieke ontwikkel kan word wat die ontkiemings- en vestigingsyfer van die gebruikte saad verbeter, kan 'n beter bedekking, digtheid en biomassaproduksie verwag word wat die rehabilitasieproses 'n hupstoot kan gee.

Dit word algemeen aanvaar dat komersieël beskikbare grassade 'n beter ontkiemings- en vestigingsyfers in vergelyking met plaaslik geoeste saad het. Die saad wat plaaslik geoes is kan moontlik baie onsuiwerhede soos stokkies, kaf en klippies bevat. Saad wat plaaslik geoes word, veral van sekere ekotipes wat by die omgewing aangepas is, kan baie skaars wees. Advance Seed Company (Krugersdorp, Suid-Afrika) het met 'n verbeteringstegniek vorendag gekom, deur 'n behandeling (omhulsel) van verskeie stowwe om die saad te plaas wat beter hantering van die saad in hervestigingsprosesse verseker. Ander voordele van die behandelde (omhulde) saad is dat die saad-tot-grond kontak hoër is, groeistimulante kan in die omhulsel geplaas word, hoër saadsuiwerheid word verseker en die omhulsel beskerm die saad teen saadpredasie en teen sterk chemikalieë in die grond wat moontlik 'n negatiewe invloed op die ontkieming en vestigingstempo van die saad kan hê.

Die doel van die studie was om die behandelde (omhulde) saad, te vergelyk met die onbehandelde saad t.o.v. ontkiemings- en vestigingsyfers (laboratorium), vars en droë bogrondse (blare) en ondergrondse (wortels) biomassabepalings (glashuis) en droë

bogrondse biomassa-opbrengs (veldproewe). Die saadpredasie van behandelde en onbehandelde saad, asook die ontwikkeling van die vaatweefsel in die oorgangsarea van die saailinge is ook ondersoek.

Die grasse wat in die proewe getoets is het behandelde (omhulde) en onbehandelde saad van *Chloris gayana* (Rhodes-gras), *Cynodon dactylon* (Kweekgras), *Digitaria eriantha* (Gewone-vingergras) en *Eragrostis curvula* (Oulandsgras) ingesluit. In die geval van *E. curvula* is vier behandelings, insluitende die onbehandelde saad, getoets. Hierdie behandelings sluit onbehandelde saad, 'n "gewone behandeling", 'n behandeling met "organiese insekdoder op die basis van die behandeling" (d.w.s. insekdoder tussen die saad en die behandeling) en 'n behandeling met "organiese insekdoder op die basis en oorgesproei" (d.w.s. insekdoder tussen die behandeling en die saad, asook insekdoder oor die behandeling gespuit) in. Die grasspesies word in die algemeen in saadmengsels in restourasie- en rehabilitasiewerk gebruik en is verskaf deur Advance Seed Company. In die studie is die saadbehandelings en onbehandelde saad tipes getoets onder verskeie omstandighede. Die chemiese samestelling van die behandelings is onbekend aan die publiek.

Al die saad verskaf deur die saadmaatskappy het 'n suiwerheid bo 95%. Wanneer na die kiemingspersentasies gekyk word, word uit die resultate afgelei dat die onbehandelde saad van *Chloris gayana* beter presteer het as die behandelde saad. Ander verskille ten opsigte van die kiemingspersentasies sluit *Cynodon dactylon* in. In hierdie geval het die behandelde saad van die betrokke spesie , hoër kiemingspersentasie in vergelyking met die onbehandelde saad vna dieselfde spesie. In die geval van *E. curvula* het die behandelde saad 'n swakker kiemingspersentasie as die onbehandelde saad. Hierdie resultate verskil egter in die ontkiemingstoetse waar dormansiebreking nie gebruik is nie.

In die resultate van die verstiging van die saailinge in die glashuisomgewing, is soortgelyke resultate in beide die gebruikte mediums waargeneem. In die bo- en ondergrondse vars en droë biomassa meetings is die enigste statisties vergelykbare resultate in die *D. eriantha* spesie waargeneem. Verskeie statisitese verskille was in die resultate van die veldopnames onderskei.

Die resultate weerspieël dat die behandelde saadtipes van *Chloris gayana* en *Cynodon dactylon*, die onbehandelde saad tipe van *D. eriantha,* asook die onbehandelde saad en saad behandel met "organiese insekdoder op die basis en oorgesproei" van *E. curvula* gebruik kan word in die hervestiging van grasspesies in die toepassing van restourasie- en rehabilitasieprosesse. Die saad van *E. curvula* wat behandeld is met "gewone behandeling" word nie aanbeveel vir gebruik in saadhervestigingsprojekte in versteurde gebiede nie.

Sleutelwoorde: behandelde saad; biomassa; hervestiging; omhulde saad; versteurde gebiede.

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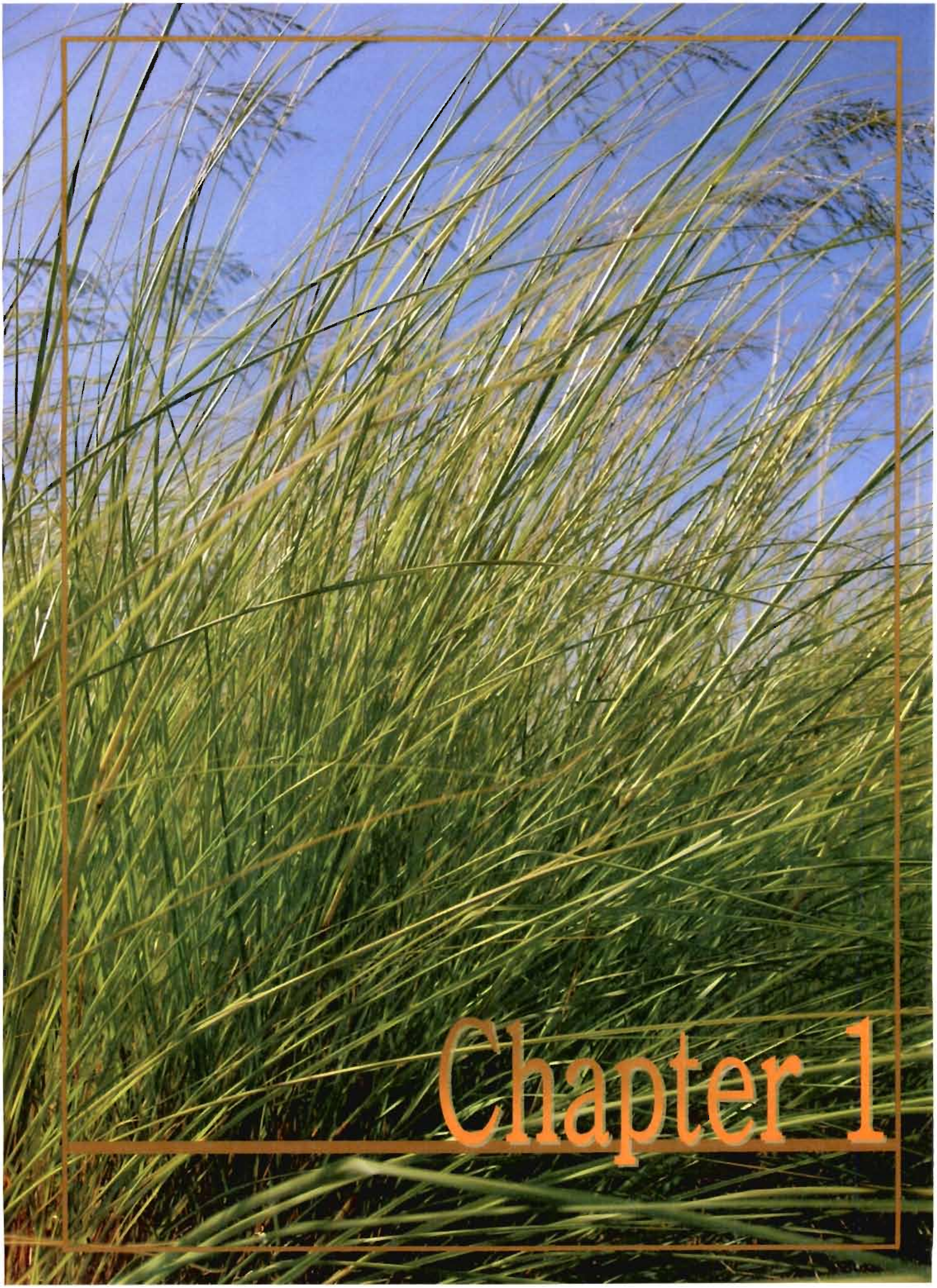


Chapter 1

CHAPTER 1

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

As technology progresses for the good in some instances, it can have negative influences on ecosystem structures and progresses, such as in the case of mining, industrialisation and agricultural activities (Bradshaw, 1997; Schmitzberger *et al.*, 2005). This is an international problem and the environment may need intervention by the main source of disturbance, namely humans (Parker & Pickett, 2000; McIver & Starr, 2001; Van den Berg, 2002; SER, 2004; www.ser.org). The problem of degradation can be reversed by progressing technologies for the restoration and rehabilitation of degraded lands (Urbanska *et al.*, 2000). According to Harris *et al.* (1996), degradation is a combination of events, causing land to become unfit for a variety of uses, i.e. as natural ecosystems, as a consequence of natural or unnatural processes. Many definitions and main goals exist for the discipline of restoration ecology, i.e. "...to provide a scientifically sound basis for the reconstruction and function of damaged or destroyed ecosystems, and produce self-supporting systems which are, at least to some degree, resilient to subsequent damage" (Urbanska *et al.*, 2000). Bradshaw (2000a) and the Society of Ecological Restoration (1995) include the terms renewal and maintenance - also referred to as "aftercare", in their definition of ecological restoration, and define it as "...the process of renewing and maintaining ecosystem health." In ecological restoration, the product is alive and capable of further development as a result of growth and successional processes (Bradshaw, 2000a; Chambers, 2000; Majer, 2000; Parker & Pickett, 2000). However, the end goals of restoration are adjustable to the circumstances to which the ecosystem was subjected, cultural and political practices as well as to the availability of resources needed in the restoration process of the particular ecosystems (Chambers, 2000; Clark, 2000; Edwards & Abivardi, 2000; SER, 2004; www.ser.org). Bradshaw (2000a) mentioned that four concepts are commonly used in restoration ecology: restoration, rehabilitation, remediation and reclamation. These concepts are described in Table 1. Damaged or degraded ecosystems, habitats, water and soil quality, communities and species are several of the topics where restoration can be applied (Bradshaw, 2000a). While disturbance, stress and degradation are characterised as the problems, restoration, reclamation – in some cases considered as the first stage of restoration - and rehabilitation are seen as the solutions (Harris *et al.* 1996).

Table 1.1: Definitions of common concepts used in restoration ecology.

Concept	Definition
Restoration	*"repair or re-establishment of a natural community by reinstating as many as possible of the species and processes that evolved together in response to the physical environment and to one another over thousands of years or more."* (Packard, 1997),
Ecological Restoration	*"the act of restoring to a former sate or position...or to an unimpaired or perfect condition"*, and *"...is the process of assisting the recovery of an ecosystem that has been degraded, damaged, or destroyed."* (SER, 1995; www.ser.org)
Rehabilitation	*"the action of restoring a thing to a previous condition or status."* (OED (1971) as taken from Bradshaw (2000a), and *"...applied to areas which formerly had no growth at all, but with careful fertilization and landscaping works may be used to grow a limited number of species."* (Harris *et al.*, 1996).
Remediation	*"the act of remedying."* (OED (1971) as taken from Bradshaw (2000a)
Reclamation	*"the making of land fit for cultivation."* (OED (1971) as taken from Bradshaw (2000a), and *"...is the process by which previously unusable land is returned to a state whereby some use may be made of it."* (Harris *et al.*, 1996)

In many instances, the main goal of restoration are to assist in the re-establishment of species and functional characteristics of previously existing ecosystems (Ehrenfeld, 2000; Block *et al.*, 2001). However, it is easier said than done, because of environmental changes occurring over the long-term. The ecosystem will then be restored to a particular reference condition, with desirable functional characteristics (Urbanska *et al.*, 2000).

In degraded ecosystems, the vegetation is the most noticeable loss, in comparison to the loss of animals and soil (Bradshaw, 2000c). The loss of vegetation can either be replaced by natural processes, such as secondary succession (Bradshaw, 2000c) or by human involvement. The restoration applied by humans can be seen as artificial restoration and, depending on the aim of the restoration, the technologies to be applied can either be active or passive (for example in van der Merwe, 1997; Morgan, 1997; van der Merwe & Kellner, 1999; McIver & Starr, 2001). Active restoration uses various techniques for example ripping, ploughing and re-seeding, whereas passive restoration does not interfere with the natural process of rest in order for restoration to take place.

1.1.1 The importance of seed in restoration practices

Plant populations are directly influenced by the caryopsis - which are according to ISTA (2006) a "naked grass-fruit in which the testa is united with the pericarp", but for all practical purposes will be referred to as seeds in the dissertation, and the ability of the seed to develop in a reproductive plant, regarding the replacement of dead individuals and the population increase in local and new areas (Hulme, 1998; Urbanska, 2000). Seed is thus an important component in active restoration and rehabilitation (Snyman, 2003) for the re-introduction of seed by re-seeding methods increase the frequency, density and establishment of species significantly (Warren *et al.*, 2002). Certain seeds are better adapted, depending on the environmental factors of the region where re-seeding restoration applications were carried out. Van den Berg and Kellner (2005) reported that, when species are over-sown, i.e. using higher than recommended seeding ratios, the highest frequencies were observed for *Digitaria eriantha* and *Chloris gayana*. These plots were situated in the Middelburg area, Eastern Cape, South Africa and the site was characterized by saline patches (Van den Berg & Kellner, 2005). The germination tests carried out on *E. curvula* indicated that, although the germination test values were high (63% normal seed – intact seedling, and 28.5% fresh seed – seed which failed to germinate in the germination period, but have the potential to germinate and develop into a normal seedling), the establishment in the restoration plots were poor (Anon., 2006; Van den Berg & Kellner, 2005). According to Van den Berg and Kellner (2005), these findings were also observed in restoration trials carried out by Snyman (2003).

The selection of the appropriate grass seed for the re-vegetation of disturbed areas, which includes the active restoration of degraded rangelands, as well as the rehabilitation of mining areas, depends on a multitude of factors (Van den Berg, 2002; Van den Berg & Kellner, 2005). Degraded rangelands are characterised by "the reduction or loss of biodiversity and productivity", as well as a decrease in the height and cover, palatability and yield of grasses (Van den Berg & Kellner, 2005; Van den Berg & Zeng 2006). These distinctive characteristics may be overcome by introducing seeds as well as seedlings to the degraded areas, which may have a positive influence on the vegetation cover and the abundance of plant species diversity (Warren *et al.*, 2002; Visser *et al.*, 2004). One of the most limiting factors in active restoration and rehabilitation activities which involve re-seeding processes, is the germination and establishment of grasses in especially harsh environments (Bradshaw, 2000b; Van den Berg, 2002). The latter is characterised by erratic rainfall, variable temperatures, increased soil salinity, soil crusting, pathogens within the soil and herbivory (Tongway & Ludwig, 1996; Bradshaw, 2000c; Magnússon, 2000; Van den Berg & Zeng, 2006). These harsh environments could cause negative sporadic natural vegetation changes (Hoffman *et al.*, 1990; De Wet, 2001; Van den Berg, 2002; Van den Berg & Kellner, 2005; Van den Berg & Zeng, 2006).

Other factors, for example soil type, seed quality, the application and seeding ratio, and whether the seed can be easily applied by the communities and land users in areas that have to be cultivated or are being affected by land degradation, are taken in consideration when restoration applications are planned (De Wet, 2001; Van den Berg, 2002). Degraded rangelands, as well as areas where desertification is common, are consequences of climatic conditions and are a problem encountered by rangeland managers (farmers) (Van den Berg & Kellner 2005). Restoration to a sustainable state is impossible to achieve due to human involvement and is slow because of unpredictable rainfall and changes in the environmental conditions that occurred due to degradation and disturbances (Snyman, 1999, 2003). The goal of restoration of degraded rangelands should be to restore the disturbed areas to an acceptable state or optimum state.

The selection of species to be re-introduced in such areas to increase the biomass and vegetation cover in a short time frame is generally one of the problems in restoration and rehabilitation efforts (Van den Berg, 2002). In South Africa, rangelands are an important land use as it occupies over 70% of the surface area (Snyman, 1998a). It is therefore very important to manage the vegetation accordingly, especially for sustainable animal production (Snyman, 2003). The most economically suitable restoration practices are in the interest of the rangeland manager because numerous risks are involved in the practices, there are no specific guidelines to follow and constant monitoring of the succession of the vegetation after restoration must be implemented (Van der Merwe, 1997; Van der Merwe & Kellner, 1999; Snyman, 2003).

1.1.2 Selecting seed for restoration

Restoration is very expensive because of the cost of seed, equipment, technologies, inputs by experienced environmental managers as well as the time scale for the implementation of restoration and must be done effectively to obtain good and reliable results (Snyman, 1999; Edwards & Abivardi, 2000). The seed that is purchased from seed companies must therefore be thoroughly tested to determine the recommended purity percentage and germination percentage according to the Plant Improvement Act (No. 53 of 1976) (Mayer & Poljakoff-Mayber, 1989; Apfelbaum *et al.*, 1997), before any recommendations can be made to the land user. Van den Berg and Kellner (2005) reported that locally collected seed should be used cautiously in active restoration practices, although the conservation of the genetic material is important. The seed can be impure and the germination percentages very low (Edwards *et al.*, 2000). Martínez-Ruiz *et al.* (2007) mentioned that the seed companies must seek to use indigenous ecotype seed to conserve genetic material on mined areas as well as other degraded lands (Apfelbaum *et al.*, 1997).

Van den Berg and Kellner (2005) also observed that seed supplied by a registered seed merchant had a higher percentage of purity and higher germination percentages in comparison to seed harvested form local seed ecotypes. It is therefore recommended to use seed from a seed company. Before seed from a seed lot may be made available for purchase, the seed lot must be declared in terms of the Plant Improvement Act (PIA) and be in compliance with the provisions relating to the seed as well as the seed samples (PIA No. 53 of 1976 – Table 4 and Table 6 – Appendix A). The requirements to be met for the seed to be in compliance with the PIA, with regards to physical purity and germination, are stated in Table 4, while the prohibited weed seeds are listed in Table 6 (see Literature review).

The harvested seed of local ecotypes may have native genetic material, but sometimes it is necessary to introduce new genetic material of the same native species. The genetic material of the native species may not be competitive enough to survive certain harsh environmental conditions and therefore does not have very high germination percentages and may not be able to aid in the recovery of the natural restored or rehabilitated state of the degraded rangelands (SER, 2004). Another factor may be that the native species might germinate, but the survival and establishment percentages can be very low (Banerjee *et al.*, 2006). Seed have certain mechanisms to ensure dispersal and survival of the species. These dispersal mechanisms may have a negative influence with regards to planting the seed in a cultivated system, as the seeds are blown away by the wind if it has not been subjected to post harvest purifications, which are fortunately carried out by seed companies (Taylor *et al.*, 1998; Banerjee *et al.*, 2006). Banerjee *et al.* (2006) noted in their experiments, focusing on native plant regeneration with regards to irrigation, preparation and amendments on the establishment of seedlings, that native species are not protected against possible saline soils in the specific area, as well as the chemical and microbial influences from the surrounding soil environment. The enhancement of the seed – i.e. the main focus point of this study, may act as a barrier which protects the seed against harmful chemicals in the soil environment.

The enhancement of seeds is defined by Taylor *et al.* (1998) as treatment improving germination or seedling growth, or the facilitation of delivery of seeds and required materials at the time of sowing, after seed is harvested from the natural environment and before sowing the seed. Enhancement covers three aspects, including pre-sowing hydration treatments (priming), coating technologies and seed conditioning (Taylor *et al.*, 1998). In this study, the enhancement technique focused on the coating technologies (of the applicable seed types of the selected grass species), including pelleting, which means "the deposition of a layer of inert materials that may obscure the original shape and size of the seed, resulting in a substantial weight increase and improved plantability" (Taylor *et al.*, 1998). With film coating, as explained by Taylor *et al.* (1998), the shape and the general size of the raw seed are retained, while a minimal weight gain is achieved. The pelleting method is used on the seed types of the selected grass species tested in this study. Both these coating methods may contain pesticides, polymers, dyes or biologicals. This study used seeds coated with AgriCOTE^{GT} by

Advance Seed Company[1] (refer to as ASC form here on – see 6.2 Companies and Personal Communications) (Advance Seed, 2006).

Seed predation by rodents and ants (Russell *et al.*, 1967; Andersen & Ashton, 1985; Bond & Breytenbach, 1985; Kelt *et al.*, 2004), as well as the availability of seed from seed companies, play an important role in the success of re-seeding technologies when restoring degraded rangelands and rehabilitating mining areas. Bradshaw (1997) noted that the damage caused by mining activities to the environment are crucial with the emphasis on the re-establishment and re-introduction of vegetation as well as the remediation of the soil component for the successful achievement of rehabilitation goals.

Advance Seed Company is a South African based agricultural farming, processing and trading organization, established in 1948 which has been coating seed for many years (Advance Seed, 2006). The seed coating is applied with a new automated and computer-controlled coating technique at the plant in Krugersdorp. This is the only seed coating plant on the African continent which coats grasses and other crops to fit the customers needs (Advance Seed, 2006). Many land users purchase coated (enhanced) seed, as they believe that this seed has a higher germination percentages and the plants establish better. Advance Seed Company has a privately owned, Government approved seed testing laboratory, operating according to the ISTA rules, located on the premises, ensuring that only high quality seed reaches the market. These seeds are also used in the rehabilitation of mine tailings and the stabilisation of steep hills (Advance Seed, s.a.). Since very few scientific experiments have been carried to test the germination and establishment capacity of the enhanced grass seed under natural conditions, this project was launched by ASC.

1.1.3 Objectives

The general objectives of the project are therefore to evaluate and compare enhanced (coated) and non-enhanced (non-coated) seed, provided by ASC for use in re-seeding practices, with regards to the performance of the seed under various experimental conditions. The seed types of the selected grass species normally used in re-seeding practices were provided to the North-West University[2] (refer to as NWU henceforward – see 6.2 Companies and Personal Communications) to carry out certain experiments.

The specific objectives included:

1. To compare the germination and establishment percentages of the seed types of selected enhanced and non-enhanced grass species.
2. To compare the above- and below-ground biomass yield of plants grown from enhanced versus non-enhanced seed types of selected grass species, in two different mediums.
3. To evaluate the predation by ants and small insects on the enhanced and non-enhanced seed types of the selected grass species.
4. To compare differences in the structure of the vascular tissue in the transition root region of seedlings grown from enhanced versus non-enhanced seed types of *Digitaria eriantha*.

The seed-enhancement technologies were therefore investigated regarding the increase of the germination potential as well as the establishment, growth percentages and biomass yield of certain seed types of grass species for restoration and rehabilitation activities.

The project was carried out in collaboration with ASC and is a continuation of already existing projects concerning the testing of seed types of selected grass species for restoration applications.

1.1.4 Hypothesis

Environmental factors can not always be altered to meet the needs of the experiment in natural conditions and will vary from place to place. It is however important to also test a wide array of controlled conditions (such as the laboratory and glass house) to eventually come to a mutual conclusion regarding the reactions and behaviour of biological components (in this case commercial available seed) in nature for better decision making.

When enhanced and non-enhanced seed types of selected grass species are compared, it is expected that the seed of the enhanced grass species will have a better seed germination and establishment percentages, as well as higher above- and below-ground biomass yields. The roots of the enhanced seed of the selected grass species will develop better and the structure of the vascular tissue will be better adapted for water conduction. The seed predation of the enhanced seed will also be lower.

1.2 Literature Review

1.2.1 Project overview

This project consisted of three components. The first included laboratory experiments, involving purity analysis and germination tests, as well as anatomical studies of root development. The second component entailed glasshouse trails where the above- and below-ground biomass of fresh and dry plant material were determined and compared. The third component, carried out in the natural field, involved vegetation surveys on an established site, comparing density, frequency and biomass yield of the different seed types of grass species, as well as the investigation of seed predation by ants and small insects.

1.2.2 Seed enhancement (coating)

According to ASC's website, the AgriCOTE^{GT} enhanced seeds have several advantages (Advance Seed, 2006). The seeds are coated with an enhanced layer which increases the seed to soil contact. The coating can absorb water, which can lead to better emergence of the seedlings in poor soil seed bank conditions. AgriCOTE^{GT} also contains growth stimulants, nitrogen, phosphorous, potassium, molybdenum, calcium and other nutrients ensuring better growth of the plant (Figure 1.1). The coated (enhanced) seeds are better anchored in the seed bank and small, light seeds that are not easily sown are enhanced with the coating to ensure easier handling when using a planter or when sowing by hand. Seed can also vary in size and shape with chaff, resulting in the difficulty of sowing the seed (Apfelbaum *et al.*, 1997; Taylor *et al.*, 1998). The enhanced layer also protects the seed from the harmful effect of fertilizers as well as from predation by rodents, birds and insects, especially if the coating layer includes insecticides (Advance Seed, s.a.). There are, however, some disadvantages concerning the enhancement of seeds. These include that it increases the weight of seed resulting in less seed per kilogram and it requires a higher seeding rate, both resulting in an increase of the seed costs per hectare.

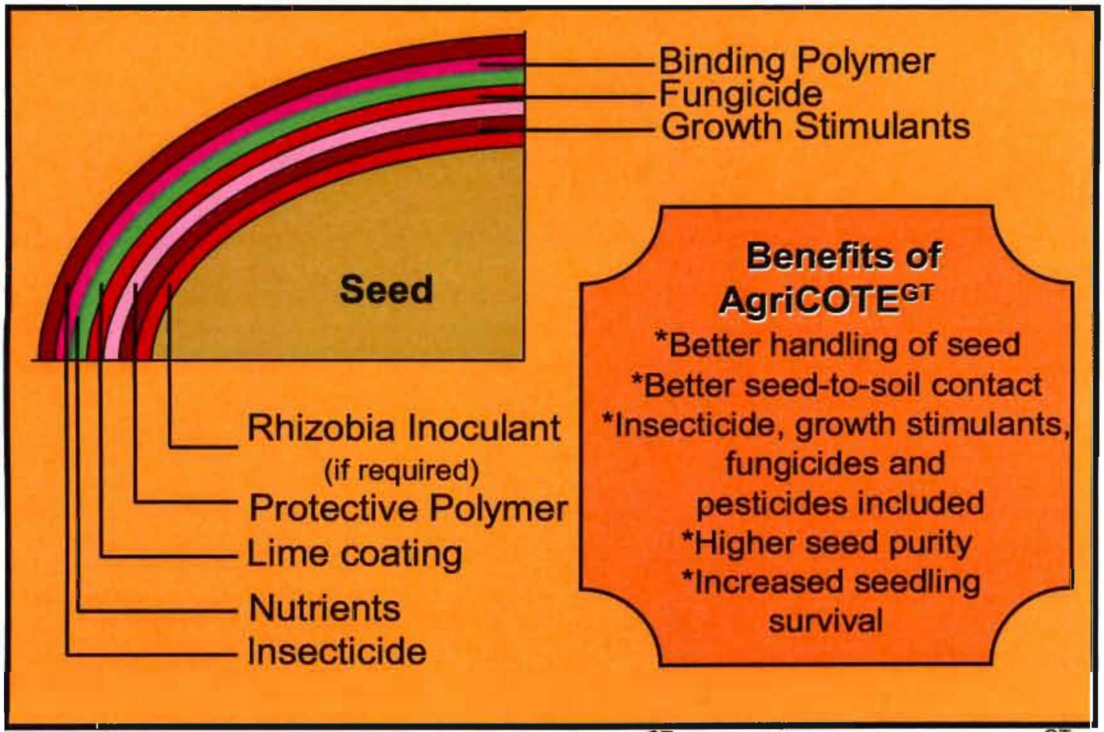


Figure 1.1: Diagram indicating the AgriCOTE^{GT} coating protection (AgriCOTE^{GT}, s.a).

1.2.3 Seed testing and the Plant Improvement Act (PIA) (No. 53 of 1976) (Appendix A)

The seed to be sold are subjected to post harvest purification and the seed are harvested at the appropriate time to ensure viability (Van den Berg & Kellner, 2005). The germination rate of seed is of utmost importance and germination is defined as the process during which imbibition of water by the seed takes place and a protrusion of any part of the embryo from the seed coat appears (Mayer & Poljakoff-Mayber, 1989). Purity and germination tests carried out according to the International Seed Testing Association's (ISTA) Rules ensure the consumer of high quality seed regarding the purity and germination percentages and that these requirements comply to the Plant Improvement Act (No. 53 of 1976) (Mayer & Poljakoff-Mayber, 1989). The relevant law is The Plant Improvement Act (PIA) (No. 53 of 1976), which states the following:

"To provide for the registration of premises from which the sale of certain plants or the cleansing, packing and sale of certain propagating material may be undertaken; to prescribe the conditions subject to which such plants or propagating material may be sold for the purposes of cultivation; to provide for the recognition of certain varieties of plants; for a system of certification of plants and propagating material with the object of maintaining the quality of certain plants and propagating material, and ensuring the usefulness of the products thereof for agricultural and industrial purposes; and for the control of the import and export of certain plants and propagating material; and to provide for incidental matters."

In Table 4 of the PIA (No. 53 of 1976), the purity analysis is subjected to Columns 3, 4 and 5, stating the maximum other matter, other seed and weed seeds that may be less or equally present in the seed lot. If, however, these maximum numbers are exceeded, the seed lot can be both re-cleaned and re-tested, depending on the number of weed seeds found, or the decision can be made not to sell the seed.

With regards to the germination tests, only Column 6 in Table 4 is applicable. In Column 6, the percentage found may be more or equal to but not less than the required germination percentage stated. The normal seedling component is used to determine the germination percentage of the seed lot. Normal seedlings refer to the seedlings that germinated from the seed without any abnormalities, such as stunted growth, underdeveloped roots and shoots.

With regards to the PIA (No. 53 of 1976), the seed used in the study was tested for purity and germination by ASC. However, the seed was tested in this study to compare the different enhancements and to compare the laboratory germination results to the results obtained in the glasshouse.

1.2.4 Biomass

The growth of seedlings in restoration activities can be monitored using various important parameters, including the biomass yield of the vegetation. Both the above-ground (shoots) and below-ground (roots) parts of the plant are important in the growth and regeneration of vegetation because of the physiological processes taking place in these parts (Kanninen *et al.*, 1982). Numerous biomass production experiments have been carried out in stock rating and ecological vegetation studies (Silverton, 1980; Deshmukh, 1986; Gross *et al.*, 1991; Dodd *et al.*, 1994; Bonser & Reader, 1995; Tilman *et al.*, 2001). The biomass production is an important factor regarding the food source for animals. Biomass produced within a growing season is an indication of the primary productivity of the plant and biomass will increase with the number of healthy individuals (Tilman *et al.*, 2001; Chen *et al.*, 2007). Grazing animals prefer the palatable above-ground parts of the plant and are very dependant on these parts for their survival throughout the year, while granivores, rodents and insects utilize the roots and the seeds as part of their dietary requirements (Kabi & Bareeba, 2007). The evaluation of biomass production is thus inevitable in long term monitoring of the productivity of plants (Oomes, 1992).

The most suitable conditions are also important to maximize optimum biomass yield and various experiments have been carried out to determine the optimum conditions and minimum input needed for sustainable biomass yield. According to an experiment involving shoot and root biomass and their reaction to certain types of gradients, using the four grass species *Themeda triandra*, *Aristida junciformis* (both short to medium height species) and *Hyparrhenia hirta* and *Eragrostis curvula* (both taller species), carried out by Ghebrehiwot *et al.* (2006), significant effects were observed on shoot biomass when the grasses were subjected to different degrees of shading, quantities of nutrients, amounts of water and cutting. Shoot biomass was the highest in the treatments where the species were unshaded, nutrients and water were abundant and no cutting took place. In the treatments where the nutrients were low (similar to the above- and below-ground biomass experiment carried out in this study) the shoot/root ratio of the tallest species was near to 1:1. According to Ghebrehiwot *et al.* (2006), this was an indication that the same amount of biomass was produced above- and below the ground. Thus, both shoot and root production was negatively influenced by a shortage in water and nutrients. Ghebrehiwot *et al.* (2006) also remarked that the relative influences of nutrients and water are vague regarding the organisation of the grassland community.

Themeda triandra roots possessed a greater biomass allocation in comparison with the shoots, clearly illustrating the important role the roots play in the maintenance of the grown plant throughout its life span. Ghebrehiwot *et al.* (2006) also support the ecological theory, which states that when a species produces the highest biomass with regards to a particular treatment (high-nutrient or low-nutrient) when planted in a monoculture, that species will be the best competitor in the appropriate treatment. With regards to this study, we are of the opinion that when the enhanced and non-enhanced seed types of selected grass species are compared, the seed type of the selected grass species which yields the highest biomass in the natural field will also yield the highest biomass when the soil is ameliorated with fertilizers. Fertilizers in the soil, e.g. phosphorous, can "burn" the seed and cause damage. The coating can protect the seed against this damage.

Furthermore, many re-vegetation efforts used in various types of active restoration practices include tillage and mulching (Banerjee *et al.*, 2006). Both these practices are very labour intensive and costly. The pre-treatment of soil is also an option, but Banerjee *et al.* (2006) reported in their study on abandoned desert farmlands that treatments, e.g. mulching, imprinting, chiselling and fertilization, used in their study had no effect on the germination of the seeds or the canopy cover (irrigated or not). Brooks (2003) reported that the pre-treatment of soils may enhance the germination of weed seeds as they utilize nutrients better. Sowing enhanced seeds may provide the rangeland manager with less hassle when re-introducing vegetation to a degraded area by means of re-seeding, for the coated seeds are easier to plant and are not blown away by the wind (Advance Seed, s.a.). Banerjee *et al.* (2006) also mentioned the competition of weed seeds that can be abundant on formally agricultural lands where degradation took place. This must also be taken into consideration when re-seeding of such areas is planned. The germination and establishment of the re-introduced seed must out-compete the weed species to ensure optimal cover results. Irrigation may favour the weed species as well, but it may increase the germination of the introduced species initially (Banerjee *et al.*, 2006).

1.2.5 Root development and the vascular system

After the seed has germinated it is important for the young seedling to establish and develop a good root system. Efficient root development is crucial to the success of the seedling. In this study, the development of the transition region of the enhanced and non-enhanced seed was compared. The transition region is the region of the plant axis where the contrasting level structures of the vascular systems of the shoot and root are joined together and the applicable development of this region is crucial to a young seedling, determining the normal development of the shoot and root (Esau, 1965; Fahn, 1990). The root systems originate as an adventitious root, after which lateral roots produce secondary lateral roots (Robinson *et al.*, 2003). Roots supply the plant with much needed water and minerals, absorbed from the surrounding soil, particularly nitrogen (N) and phosphorus (P) (Neary *et al.* 1999; Atkinson, 2000; Tyree, 2003, Pietola and Alakukku, 2005). Root development therefore has an impact on the development of the rest of the plant. For the roots to penetrate the soil successfully, the root locally deforms the soil or causes friction. This may lead to the destruction of the root tip (Robinson *et al.*, 2003). The friction between the roots and the soil is reduced by high turgor pressure in the zone behind the apical meristem, as well as the secretion of lubricants by the root cap (Bengough, 2003). According to Robinson *et al.* (2003), radial symmetry is important in the anatomy of the roots. Roots provide anchorage to the plant and connect it with available minerals and water in the soil. In contrast with popular belief, "the deepest roots occur in tropical savannas", making roots a very important structure for the absorption of water and minerals in deep water tables (Robinson *et al.*, 2003).

In general, plant roots face biological, chemical and physical obstructions which influence their ability to grow and to utilize resources in soil (Robinson *et al.*, 2003). These obstructions can be overcome or be minimized by inherent root features such as how well they penetrate the pores in the soil, branching patterns of the roots as well as their ability to utilize water and solute supplies (Robinson *et al.*, 2003). Morphological and ecological properties of roots include the topology and size of the roots, the capacity to anchor the systems as well as the association with microbes (Robinson *et al.*, 2003). The topology of a root includes the branching or architectural pattern of the root in the soil (Taub & Goldberg, 1996).

Roots do not have their own independent carbon supply and therefore rely on the shoots to fulfil their carbon needs (Robinson *et al.*, 2003). To view the progress and development of roots, computerized programs (Asseng *et al.*, 2000) or methods causing minimal disturbance are implemented in root biology studies (Robinson *et al.*, 2003). However, computerised programs were not used in this study. The method used in this study for the root development of seed types of selected grass species causes only minimal disturbance to the root.

According to Snyman and du Preez (2005), organic matter in soil can increase as a result of the root mass in the soil not being degraded and the roots thus steadily contribute to the carbon (C) source in the soil. Great root biomass is thus an important factor in the sustainable development of a system not entirely affected by degradation. Roots are also a benefit to the soil and contribute to pore spaces which in turn given rise to "better infiltration capacity". When a plant can ensure abundant above- and below-ground cover and root development and the establishment and distribution of the root within the soil can be assured, effective use of water can be expected. This will indirectly lead to rangelands being utilized in an appropriate manner, which is important in South Africa as rainfall is one of the limiting factors in the environment (Snyman, 1998b; Snyman & du Preez, 2005). Grazing management systems are not directly affected by root development (Snyman, 2005), but roots can give the rangeland manager an idea of the growth patterns, water utilization and production which may lead to better animal production (Neary *et al.* 1999).

According to Snyman (2005), roots are often under the influence of the surrounding environment. The concentrations of solutes in the soil influence the development of the roots (Robinson *et al.*, 2003). To evaluate the full extent of the seed enhancement, no additional soil amelioration was applied to the medium.

In this comparison of root development, the enhancement of the seed, supplied by ASC, may give the developing seedling an advantage to overcome possible nutrient deficiencies in the soil. When the development of the root is not normal and functional, the plant's tolerance to drought as well as the absorption function of the root may decrease (Snyman, 2005). A better root system may ensure that water is optimally utilized and that water is used in the biomass production of the above-ground parts. For the survival of a seedling, the distribution as well as the establishment of the roots must be good (Snyman, 2005). Snyman (2005) emphasizes the importance of the inclusion of roots in rangeland dynamic studies as the roots are fundamental in the development of the seedling.

Root development is closely connected to the differentiation of the vascular system, thus comparative anatomical studies were performed on the roots of enhanced and non-enhanced seedlings (Russell, 1977; Atkinson, 2000). The apical meristem is the area where the entire root system develops in grasses. According to Russell (1977), vacuolation occurs about 0.5 cm from the apex and the apical meristem differentiates into the vascular stele and the cortex. The xylem, which is associated with the transportation of solutes and water, and the phloem, which assists in the movement of sugars from the shoot to the root, are encased by the pericycle and form the vascular stele (Evert, 2006). The stele is surrounded by the endodermis, the inner layer of the cortex in the root. In this study, the transitional region (xylem and phloem) of enhanced seed was compared to the transition region (xylem and phloem) of the non-enhanced seed.

The pathway of water movement from the root to the shoot is through the xylem tissue that consists of dead conducting tissue i.e. vessel elements and/or tracheids. Live xylem parenchyma cells surround these elements and control the flux of solutes into and out of the conducting elements (Robinson *et al.*, 2003). Grasses are monocotyledons and therefore secondary growth does not occur in these plants (Russell, 1977). Water loss through stomatal evaporation is a triggering mechanism for water to move through the xylem from the root to the shoot (Gunning and Steer, 1996). Another component in the vascular tissue of plants is the phloem that translocates sugars from the leaves to the roots. Phloem consists of sieve elements and companion cells. The vascular tissue thus connects the above-ground shoot and the below-ground root, which are interdependent on each other for the plant to survive (Russell, 1977; Robinson *et al.*, 2003).

The extension and elongation of the roots are influenced by nutrient supply, amongst other factors already mentioned. The morphology of the plant, as well as the size of the plant may differ due to the quantity of absorbed nutrients (Russell, 1977). The nutrients made available by seed enhancement may increase the seed's chances of developing into a seedling and ultimately into a mature plant. The concentration of nutrients surrounding the seed, in the case of the enhanced seed, makes the nutrients readily available and accessible to the root and this may lead to better root development. Nutrients may not be uniformly available under natural conditions in the soil area were roots tend to elongate. In agricultural practices, nutrient deficiencies are corrected with the addition of fertilizers (Russell, 1977). The enhancement on the seed used in this project already contains nutrients and beneficial chemical additions to ensure better establishment of the seedling, which means that fertilizer additions can be minimized in the early stages after re-seeding. Additional fertilizers and soil fertility - whether in cultivated rangelands, natural field or mining areas – can, however, not be neglected. By the time plants have reached maturity, the enhancements may have dissolved and no longer benefit the plant. To study the total effect of the enhancements, seeds were intentionally germinated in an environment deficient of nutrients.

As mentioned, roots and shoots are interdependent. Shoots supply the roots with carbon and the roots supply the shoots with minerals and water. For this exchange to be successful, the root and shoot must be effectively connected by a "long-distance mass-transportation system" (Tyree, 2003). Wide vessels transport water and minerals more effectively than narrow vessels (Robinson *et al.*, 2003). Elongation of roots occurs at the zone of elongation behind the apical meristem. The root then enlarges radially through the activity of the vascular cambium (Robinson *et al.*, 2003). Root growth occurs very rapidly. Young fibrous roots can increase their dry mass by 25% per day. The interactive root and shoot activities are regulated by specific mechanisms (Farrar and Jones, 2003). These mechanisms need to function optimally for roots and shoots to develop to their full potential. The distribution of nutrients and water may be problematic because the flow of the nutrients and water are depended on the diameter of the vessel (Robinson *et al.*, 2003). The diameter of the vessel expands as growth is taking place (Robinson *et al.*, 2003).

Various morphological and anatomical changes accompany root growth and these may influence root activity (de Neergaard *et al.*, 2000). Root activity is defined by Atkinson (2000) as "the temporal changes in the amount of a soil resource or evidence of a material being moved in to the above-ground part of the plant". Root activity also includes hormone production, storage and mobilization.

Roots are not easily removed from the soil and attempts to remove below-ground root biomass may easily cause damage to individual roots (Hutchings and John, 2003). Atkinson (2000) reported that root growth, i.e. elongation, is of importance in the development of the root. Although there are many shortcomings in root measurements, root growth is usually measured by observing the change in root length directly. Soil may interfere with direct observations, unless soil particles are *carefully* removed.

Alternatively, as in this study, the plant may be grown in a medium from which the roots can easily be removed (de Neergaard *et al.*, 2000).

Root development and distribution may reflect climate and soil patterns and may be influenced by these patterns (Robinson, *et al.*, 2003). The uptake of nutrients can be altered by an increased root length. Root length of roots from enhanced and non-enhanced seeds is also compared (Robinson *et al.*, 2003). The hypothesis is that the roots of the enhanced seed will have an advantage during the initial stages of development as the adventitious roots have minerals already available. Pecháèková *et al.* (1999) remarked that roots do not respond in the same manner in different soil types and horizons. Thus, roots of seed sown in different soil types will develop differently, depending on the environmental factors.

Both "root growth rate" as well as "root branching rate" may improve when more nutrients are available in the soil (Robinson *et al.*, 2003). Studies by Hutchings and John (2003) confirmed that roots have a positive morphological response to the availability of nutrients.

When comparing grass root structures, it is evident that two root systems are present in the commencing stages of development, i.e. the seminal root - functioning until the second type of root, which are the adventitious roots, originating from the crown node at the coleoptile base, develop. The adventitious roots are the functional roots of the plant. At germination, the protecting sheath of the primary root (the coleorhiza), and the coleoptile expand, after which the primary root and the mesocotyl expand in length (Oregon State University, 2000).

The transition region from the root to the shoot is considered to be very important, because this is the connection between the root and the shoot, where the transportation of much needed water and minerals takes place. According to Lauenroth and Gill (2003), a seedling's life cycle commences with a primary root and the shift of the primary root to adventitious roots will determine the "long-term survival" of the seedling. This crucial stage may be a factor in the development of the vascular system to be monitored.

The anatomy of roots is highly variable and major differences occur between species as well as between individuals of the same species grown in different environments (Tyree, 2003). Root anatomy may be a factor contributing to, in the first instance, radial and in the second instance, axial movement of water, though variable, for water, according to Tyree (2003), "moves radially from the root surface to the stele and then axially from the stele to the shoot". With regard to this comment by Tyree (2003), implying that root anatomy can play a role in water movement and the importance of water as an abiotic component in the successful development of the grass plant to reach maturity, our focus was set on the stele, containing the vascular system (xylem) responsible for the movement of water from the roots to the rest of the plant. The stele also contains the phloem responsible for translocating sugars to the roots. Material may also be stained with different staining agents to enhance the contrast of elements being studied (de Neergaard *et al.* 2000).

According to Mc Donald *et al.* (2002), Goller (1977) conducted the largest anatomy survey on adventitious roots of grasses. Special attention was given to the diameter of the stele as well as the cortical cell arrangement. Niklas (1985) remarked that the conductivity in xylem strands - which are larger in diameter, will be higher compared to smaller strands, in the comparison of the tracheid diameters and the proportion of xylary elements to xylem parenchyma. Further remarks by Niklas (1985) suggested that tracheid diameter is not the only conspicuous factor determining the xylem strand conductivity. The number of the tracheids must also be taken into account. Plants with larger xylem strands as well as tracheids with a greater mean diameter may be better able to grow towards resources and have the chance to exploit them to their full potential (Niklas, 1985).

The root vascular system development and the individual root development are important. Therefore the focus of this study was whether or not the enhanced seed will show differences in the morphological and anatomical development of the root. Another aspect that was incorporated in the study was the question of whether or not the enhanced seed does indeed have an advantage to better develop and establish in comparison with non-enhanced seed. The hypothesis is that vascular tissue differentiation and root development in enhanced seeds will be superior tot that of non-enhanced seeds.

Digitaria eriantha (see Chapter 2) was chosen to be evaluated in this regard because it is such a common grass species used in ecological restoration as well as in mining practices. Cultivation only requires a low capital input to establish the species and will yield maximum outputs, such as palatability and great biomass production (Donaldson, 2001; van Oudtshoorn, 2005). *Digitaria eriantha* is also an indicator of decreaser species situated in good veld (van Oudtshoorn, 2004).

1.2.6 Seed predation

Seed predation is one of the greatest problems of re-seeding activities, whether on pasture grass seeds or even seeds from Proteaceae or woody species such as *Acacia erioloba* (Bond & Breytenbach, 1985; Barnes, 2001). Ants are known to be one of the most active predators. They re-locate seeds to deep beneath the soil surface where conditions are not suitable for seeds to germinate and seedlings to develop (Andersen & Ashton, 1985; Russell *et al*., 1967).

The genera mainly known as harvester ants are *Meranoplus*, *Monomorium*, *Chelander* and *Pheidole* (Russell *et al.*, 1967). Results obtained from experiments by Bond and Breytenbach (1985) suggest that the dispersal of seeds by ants may have a direct effect on the contents of seed banks. The seed predation by rodents and birds is influenced by ants, as the ants usually discover seed faster than the other predators. Seed predation may assist in seed dispersal and seed predators are important regarding plant demography as well as the influences of selection pressures on the characteristics of seeds (Hulme, 1998). Seeds are rich in nutrients in comparison with other plant parts, making them an ideal food resource (Hulme, 1998). Seed predation by ants is, however, influenced by the weather as ant activity peaks in warmer weather conditions (Bond & Breytenbach, 1985).

To determine the predation by ants is difficult, for the removal of the seeds as set out in baits (or traps) could be different from the seed removal taking place in nature and many variables, i.e. the weather, can influence the process (Andersen & Ashton, 1985). The activity of ants is site and species specific, and may vary from region to region, as well as from season to season (Andersen & Ashton, 1985; Kelt *et al.*, 2004). Andersen & Ashton (1985) and Predavec (1997) have shown that, in Australia, ants are regarded as the main seed predators with similar seed removal levels than in South America. According to studies carried out by Kelt *et al.* (2004), seed predation in South America is dominated by rodents and birds, with the predation by ants being less important as dominance differs from site to site. Levels of seed removal are much higher in Africa and the northern hemisphere (Kelt *et al.*, 2004).

Naturally, seeds have the ability to "escape" seed predation by being dispersed when ant activity is normally low or by being dispersed at intervals. This is different in re-seeding practices when restoring degraded rangelands and the rehabilitation of mining areas, as seeds are mostly sown in bulk, with a high seeding rate to maximize the germinating chances of the seed. This makes the seed vulnerable to predation, which emphasizes that seeds need to be protected from ants. The enhancement on the seeds may act as a detergent against seed predation, as seeds are then unrecognizable, according to the size and mass of the seed. Insecticides and pesticides that are used as a coating can act as a repellent to the ants and other predators. This hypothesis - that enhanced seed treated with insecticides are less prone to ant seed predation - was also tested in a preliminary project.

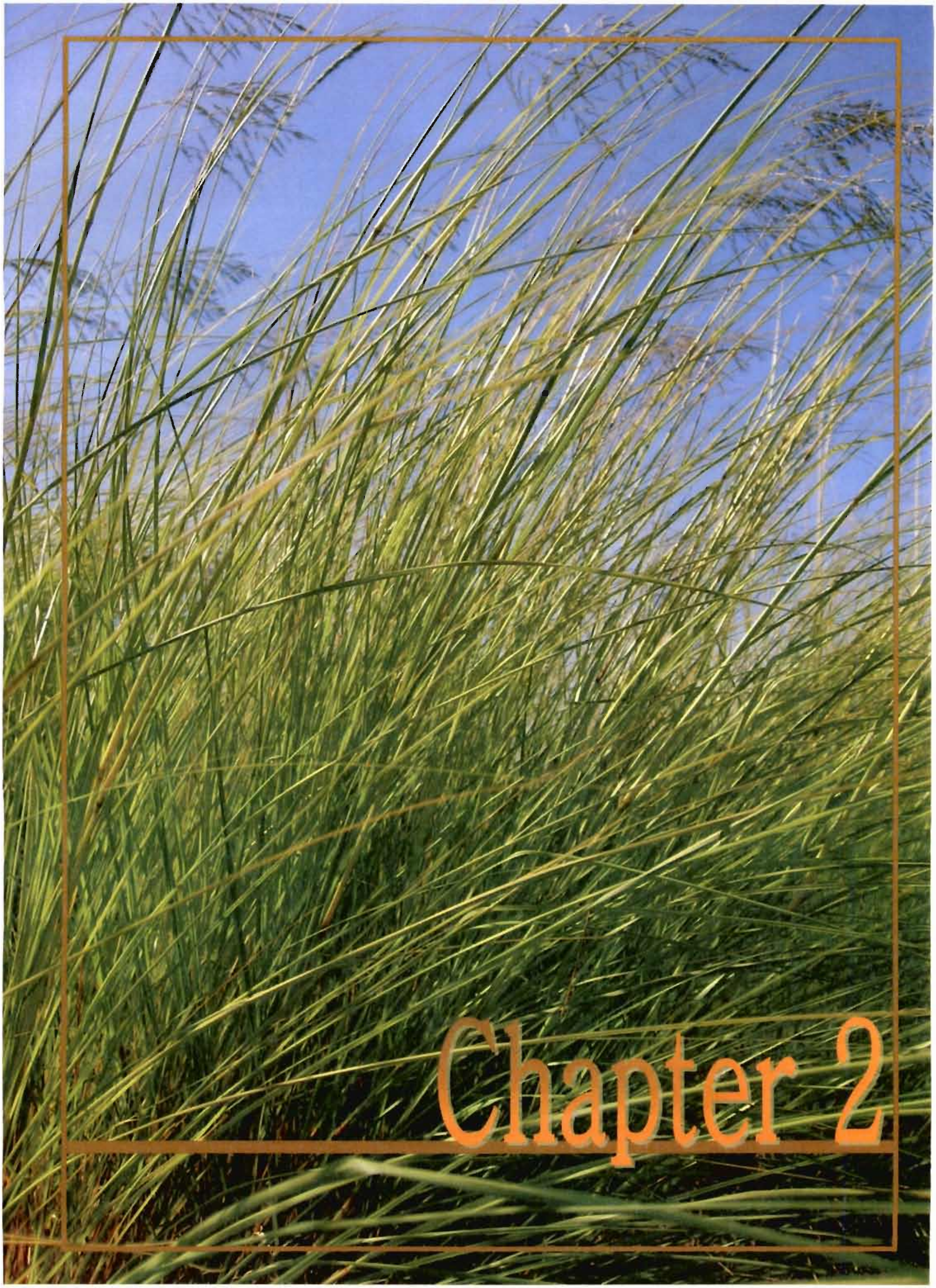
1.2.7 Previous projects

As mentioned, this study is a continuation of already existing projects regarding the testing of grass seed types for restoration applications. These include studies carried out by other post-graduate students of the NWU, e.g. De Wet (2001), where coated (enhanced) and non-enhanced seed types of selected grass species were monitored in the natural field where rip plough cultivation was applied in different areas. A soil seed bank analysis was carried out. In all the study areas, the non-enhanced seed showed a higher seedling survival rate, with the exception of *Chloris gayana* in one of the study areas. The selected species in the different study areas reacted differently to the rip plough cultivation technique. Overall, after five growth seasons, the species composition in all the study areas improved after the particular restoration applications (De Wet, 2001).

Van den Berg (2002) studied different restoration technologies, with regards to purity and germination tested, over-sown re-seeding and re-establishment trials as well as soil seed bank analysis were carried out. In the results of this project it is mentioned that almost all the species established in the natural field trials, except for *Pteronia membranacea* and *Eriocephalus ericoides.* A combination of brushpacking, over-sowing, ripping and organic material had a positive effect on the establishment of the species that were sown in. With regards to the purity analysis and the germination percentage of the seed, the seed obtained from a seed company had a higher purity in comparison to local harvested seed and the coated (enhanced) seed had a low germination percetnage. The soil seed bank studies indicated that, when degradation increased at a site, the density of the emerging seedlings decreased (Van den Berg, 2002).

Another study, similar to the study carried out by Van den Berg (2002), is the study by Boateng (2002), where the different restoration techniques used by Van den Berg (2002), with the addition of lime application, were tested in two degraded areas. The rip plough cultivation was the most successful in the stimulation of the germination and establishment of most of the species (Boateng, 2002).

Buys (2002) studied the active restoration application when organic blocks and supplement feed blocks were introduced to active restoration intervention. These blocks contained seed mixtures and the organic block was placed under natural and controlled conditions where the seeds could germinate and establish. The supplement feed block was fed to cattle and buffalo and the dung of these animals were collected and placed in the greenhouse to evaluate the germination of these seeds after passing through the rumen of the animals. The germination potential was also evaluated in the laboratory and the results vary from species to species. The results regarding the organic and supplement block under controlled conditions were more successful in comparison with the natural field trials (Buys, 2002).



Chapter 2

CHAPTER 2

MATERIAL AND METHODS

2.1 General

In the three types of experiments (laboratory trials, glasshouse trials and natural field trials, Chapter 1) of the project, four different sub-tropical grass species, supplied by ASC, South Africa, were used. The species were *Chloris gayana*, *Cynodon dactylon*, *Digitaria eriantha* and *Eragrostis curvula*. Non-enhanced and enhanced (coated with AgriCOTE^{GT}, Figure 2.1) seed types were tested for all the selected species, with the exception of the *E. curvula* seed types, for which the tests also included seeds enhanced with an "organic insecticide on the base of the coat" and an "organic insecticide on the base of the coat and as overspray" treatment. A short description of the grass species tested, including the batch number of each, is given below.

2.2. The selected grass species used in study

2.2.1 Description and uses

(a) *Chloris gayana* Kunth (Gibbs Russell *et al.*, 1990)
(Batch number: 9072 (B0009) (non-enhanced, Figure 2.1 A. ii.) and E9072 (B0009) (enhanced, Figure 2.1 A.i.)

According to Animal Feed Resources Information Systems (sine anno), *Chloris gayana* (Rhodes grass) is a perennial, tuft-forming grass with a fine stem that can grow up to 1.5 m high. It establishes mostly on fertile loam soils and flowers from November to May (Van Oudtshoorn, 2004; Gibbs Russell *et al.*, 1990). It is mainly used for grazing and making hay. Pastures with newly sown *Chloris gayana* should not to be overgrazed initially, but may be grazed moderately when properly established. The seeds are sown in conjunction with seed mixtures and cannot recover from extensive grazing (Donaldson, 2001). *Chloris gayana* is not often seen in the natural environment, due to its high palatability (van Oudtshoorn, 2004; Animal Feed Resources Information System, sine anno; Donaldson, 2001). In general, *Chloris gayana* is utilised as natural and cultivated pastures (Van Oudtshoorn, 2004). The grass establishes easily and grows for 3–5 years (Donaldson, 2001). *Chloris gayana* needs a minimum annual rainfall of 625 mm and shows a preference for warmer regions. Areas with summer rainfall of 600-1200 mm are acceptable for growth (Donaldson, 2001; Van Oudtshoorn, 2004). The grass is drought resistant, but prefers moist soils that are very well drained. All *Chloris* spp are sensitive to acidic soils, with a preference for soils with a pH of 6.0 – 8.0. They are also frost sensitive. A characteristic property of *Chloris gayana* is that it has a superior salt tolerance (Donaldson, 2001). For optimal production, *Chloris* spp should be burned, slashed and fertilized to ensure the palatability and quality of the grass (Donaldson, 2001). Besides the fact that *Chloris gayana* is an excellent grass to be used in planted pastures, it is also an ideal grass to stabilise disturbed areas, such as mining sites and road verges (Van Oudtshoorn, 2004). When re-seeding this chaffy seed in rows, 6-8 kg/ha seed is generally needed (Donaldson, 2001, Dickson *et al.*, 2004; ISTA, 2006). According to the ISTA (2006), chaffy seeds, in contrast to non-chaffy seeds, have a structure which adheres to each other or other objects. This can cause other seeds to become trapped and the seed cannot be easily cleaned, mixed or sampled. Seed samples are regarded as being chaffy when chaffy structures make up one third or more of the total. *Chloris gayana* is indigenous to Africa, India and China (Van Oudtshoorn, 2004; Gibbs Russell *et al.*, 1990).

(b) ***Cynodon dactylon*** **(L.) Pers.** (Gibbs Russell *et al.*, 1990)
(Batch number: 8949 (B0012) (non-enhanced, Figure 2.1 B. ii) and E8949 (B0012) (enhanced, Figure 2.1 B. i.)

Cynodon dactylon (Coach grass) is very palatable, adaptable to different conditions and able to grow in every type of soil. *Cynodon dactylon* flowers from September to May and the seed is classified as chaffy (Gibbs Russell *et al.*, 1990; ISTA, 2006). This grass is found in moderate climates all over the world and requires a rainfall of 550-650 mm/a to ensure productivity (Donaldson, 2001; Van Oudtshoorn, 2004). *Cynodon dactylon* is a perennial grass species with rhizomes and stolons which form a thick mat, enabling the grass to be a great soil stabiliser in eroded places, but often to become a weed in ameliorated areas (van Oudtshoorn, 2004). *Cynodon dactylon* is regarded as a pioneer grass which farmers sow in a mixture to establish pasture grasses. It is also used in gardens with fertile, loamy soils. *Cynodon dactylon* is an indicator of overgrazed lands, commonly seen in the Grassland, Savanna, Nama-Karoo and Fynbos biomes (Mudau, 2006; Gibbs Russell *et al.*, 1990). Most of the *Cynodon* spp varieties are tolerant to acid, alkali and salty soils and are planted as a summer pasture (Donaldson, 2001). Hot temperatures are favourable for luscious growth. The herbage quality and the carrying capacity are high, while the forage value is lower. Due to its stoloniferous growth form, the grass has a high vegetative reproduction ability and can easily form dominant stands in the environment. When *Cynodon dactylon* - is established with seed, instead of through vegetative methods, between 6-10 kg/ha of the seed must be sown in cultivated pastures (Donaldson, 2001).

(c) *Digitaria eriantha* Steud. (Gibbs Russell *et al.*, 1990)
(Batch number: 9038 (non-enhanced, Figure 2.1 C. ii.) and E9038 (enhanced, Figure 2.1 C. i.)

Digitaria eriantha (Smuts finger grass) is a palatable, perennial tuft-grass (Donaldson, 2001; van Oudtshoorn, 2004; Gibbs Russell *et al.*, 1990). It sometimes has long hairy stolons and branched stalks growing to a height of 1 200 mm. Florets are formed between January and April (van Oudtshoorn, 2004; Gibbs Russell *et al.*, 1990). The grass is found in the dry regions of Southern Africa, mostly in sandy soils, but can adapt to many habitats and soil types, including light sandy to loam soils, which are well-drained with low acidity. It can also be seen along wetlands and undisturbed areas (Van Oudtshoorn, 2004; Dickson *et al.*, 2004). *Digitaria eriantha*, used in seed mixtures, requires a minimum annual rainfall of 500mm and does not easily establish during the dry seasons in soils with high clay contents (Donaldson, 2001; Dickson *et al.*, 2004). *Digitaria eriantha* is a good summer grazing grass as it has a high forage value. Establishment of the grass seed, which is regarded as a chaffy seed (ISTA, 2006), is often difficult, as the seed is very fine and small with varying quality, but when used in a grass seed mixture it can establish successfully within the first three growth seasons (Donaldson, 2001). *Digitaria eriantha* is indigenous to southern Africa, but is used in planted pastures all over the world (Van Oudtshoorn, 2004).

(d) *Eragrostis curvula* (Schrad.) Nees (Gibbs Russell *et al.*, 1990)
(Batch number: 9098 (non-enhanced, Figure 2.1 D. iv) and E9098 (enhanced, Figure 2.1 D. i.; ii.; iii.)

Eragrostis curvula (Weeping love grass) – a non-chaffy seed (ISTA, 2006) - has a cosmopolitan distribution as it occurs in many regions in Africa and around the world. Florets form from August to June (Gibbs Russell *et al.*, 1990). It is a wiry perennial grass that grows in high rainfall areas in sandy, acidic or loamy soils. This grass species is often seen where land is overgrazed. Due to its easy establishment and growth it can be found in a variety of vegetation types and is very palatable. *Eragrostis curvula* also aids in the control of erosion (Skerman *et al.*, 1996). Weeping love grass forms dense tufts and prefers areas with a minimum annual rainfall of 525 mm, and may occur in areas with a rainfall higher than 650 mm/a (Donaldson, 2001; Dickson, *et al.*, 2004). The grass is intolerant to water logging and alkaline soils (Donaldson, 2001). *Eragrostis curvula* has proven to be a successful rotational crop in the cooler regions of Southern Africa, as well as for conservation on old lands and road verges because it establishes easily. This grass is also used in planted pastures and is often important in nematode niches (Donaldson, 2001; van Oudtshoorn, 2004). When planted in rows, 1.5 - 2.5 kg/ha or 2 – 4 kg/ha is used and it is often recommended to be sown in conjunction with *Eragrostis teff* (Donaldson, 2001; Dickson *et al.*, 2004). *Eragrostis curvula* originally occurred in the southern and eastern parts of Africa, but is currently also utilised in other tropical and subtropical countries (Van Oudtshoorn, 2004).

2.3 Germination percentages of seed types of the selected grass species

2.3.1 Seed testing

According to the *Training Manual for Seed Analysis* (Anon., 2006), the testing of seeds for purity and germination has been developed to reduce the risk of sowing seed that are of poor quality. Thus the goal of seed testing is to determine the quality as well as the viability, of the seed to be sown or planted. The quality involves that more pure seed than inert matter, other crops and weeds is present in the seed lot. The viability refers to whether the seed has the potential to germinate. It is, however, not possible to predict the behaviour of these biological products, with regards to the germination and establishment, correctly and the analysis of the seeds is dependant on the experience of the seed technician regarding the sampling, purity, germination and moisture content of the seed lot being tested (Anon., 2006). According to Anon. (2006), the specific objectives of seed purity testing include, "the determination of the percentage composition by weight of the sample being tested and by inference the composition of the seed lot, and to determine the identity of the various species of seeds, including inert particles constituting the sample."

The seed purity tests were carried out in the laboratory at the NWU, according to International Seed Testing Association Rules (ISTA, 2006). The seeds were provided by ASC.

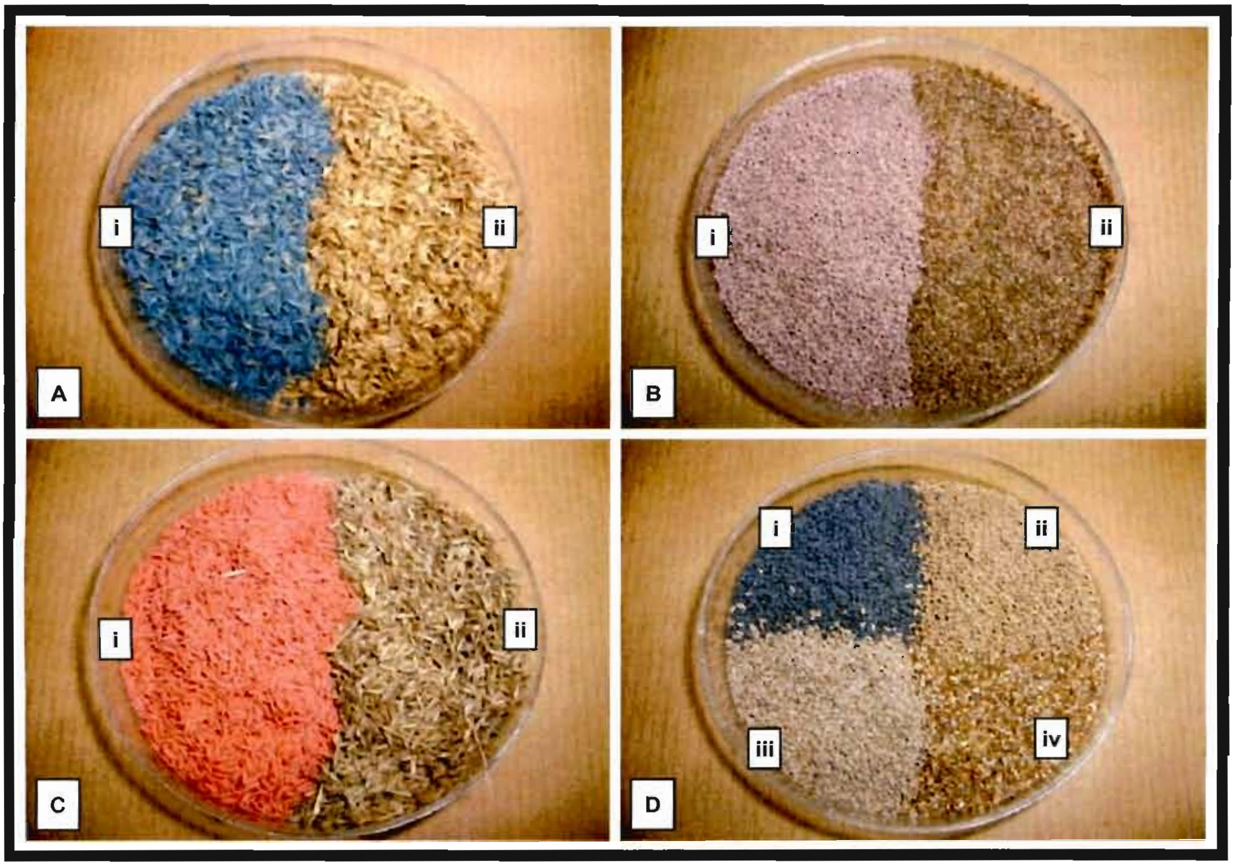


Figure 2.1: The seed types of the selected grass species used in the project:

A. *Chloris gayana*: **i.** Enhanced and **ii.** Non-enhanced.

B. *Cynodon dactylon*: **i.** Enhanced and **ii.** Non-enhanced.

C. *D. eriantha*: **i.** Enhanced and **ii.** Non-enhanced.

D. *E. curvula*: **i.** "Organic insecticide on base of coat", **ii.** "Organic insecticide on base of coat and as overspray", **iii.** "Plain coat" and **iv.** Non-enhanced.

2.3.2 Seed purity

For the seed purity tests, the seed sample must be representative of the whole seed lot. The seed analyst must therefore divide the sample received and record it accordingly, so that it is representative of the lot. The submitted sample was opened to ensure that it contains the correct seed to be tested. Due to the fact that the project involved three components (laboratory tests, glasshouse trials and natural field trials), only pure seed was used in all the trials. The mass of the submitted sample received was higher than the requirement of the ISTA Rules. The submitted samples were divided into the working sample according to the ISTA laboratory dividing techniques to comply with the prescribe working sample mass as stated in Column 4 of Table 2A of the ISTA Rules (Anon., 2006).

The estimated mass of the sample must at least contain approximately 2500 seed units (Anon., 2006). This was used to determine the working sample mass of the enhanced seeds (ISTA, 2006). Seeds are spread in a pan and scooped randomly from at least five (5) places in the pan. From the five (5) randomly selected places, eight (8) replicates of a 100 pure seeds are counted. Each replicate is weighed in grams, according to the four figure rule. The variance, standard deviation and coefficient of variation are calculated (Chapter 5). If the coefficient of variation does not exceed 6.0 for chaffy seeds, or 4.0 for other seeds, the results can be calculated. If the coefficient of variation exceeds the relevant limit, a further eight (8) replicates are counted and weighed. The standard deviation is subsequently calculated for the sixteen replicates and any replicates which deviate from the mean by more than twice the calculated standard deviation are discarded. (Anon., 2006). The working samples' mass must be recorded and any gain or loss of mass must be compared to the sum of the components after the analysis was completed. A re-test of the sample is needed when there is a discrepancy of more than 5% of the initial mass (Anon., 2006; Appendix B (i) & (ii)).

The dividing method used in this project, as required by the ISTA Rules (Table 2.7.2.A.4), is the hand halving method (Figure 2.2.A), which is mostly restricted to chaffy seeds (Anon., 2006).

The working sample was spread onto a clean surface, mixed with a flat-edged spatula and then divided by half. The halves were divided again, leaving four (4) portions. The four (4) portions were halved giving eight (8) portions (Figure 2.2 A), which were arranged in two rows of four (4) portions. Four alternating portions were combined and the remaining four portions removed. The sample was repeatedly halved and portions were removed until the required working sample mass was obtained (Anon., 2006). The seed purity separation was done on the working sample for each of the grass seed samples, which included the determination of pure seed (used in the germination tests), inert matter and other crops in the specific grass seed sample. Thereafter, each particle was examined (Anon., 2006) and recorded on the seed purity chart (Appendix C (i) & (ii)). Visible impurities dominating the sample were recorded on the seed purity chart. According to Anon. (2006), for a unit to be considered a as pure seed, the structure of the unit must at least contain one ripened ovule (hard to distinguish), with or without the necessary parts, such as achenes, schizocarps/mericarps, nutlets and caryopses, florets and spikelets. The seed viability was not taken into consideration when the seed purity analysis was done (Anon., 2006).

After the above mentioned components were determined and recorded on the purity chart (Anon., 2006; Appendix C (ii)), impurities, such as identified "other crops", were separated from the pure seed component and weighed in grams to the same decimal place as the working sample (Anon., 2006). The components were weighed and the mass recorded on the purity chart (Appendix C (i) & (ii)). The pure seed component was needed for the germination tests.

2.3.3 Seed germination

According to Anon. (2006), the object of the germination tests "is to determine the maximum germination capacity of the seed, i.e. seed lot, which in turn can be used to compare the quality of the different seed lots and also to estimate the field planting value". The testing for germination took place under controlled laboratory conditions, where the external factors were represented by standardised conditions, as prescribed for the seed types of the selected species in the ISTA Rules (ISTA, 2006). The germination of seed included the emergence, as well as the development of the seedlings, to the point where the important structures (leaves and roots) are visible. This is an indication that the seedling is able to develop further under favourable conditions in the natural field (Anon., 2006).

Grass seeds require moisture, oxygen, suitable temperatures and light to germinate. Certain species require higher temperatures and moisture for optimum germination, as well as a suitable period of germination. These requirements have been determined by the Germination Committee of the International Seed Testing Association (ISTA) (Mayer & Poljakoff, 1989). The periods of germination were determined per species. Some of the species may have the ability to be dormant until the seeds are subjected to moisture for a certain period of time (Tainton, 1999; Anon., 2006). The medium (substrate) on which the germination test took place is also specified by the ISTA Rules. Before any seed can be distributed by seed companies, the seed purity and germination percentage must be in compliance with Table 4 (Provisions Relating to Seed Samples) of the Plant Improvement Act no. 53 of 1976 (Appendix A).

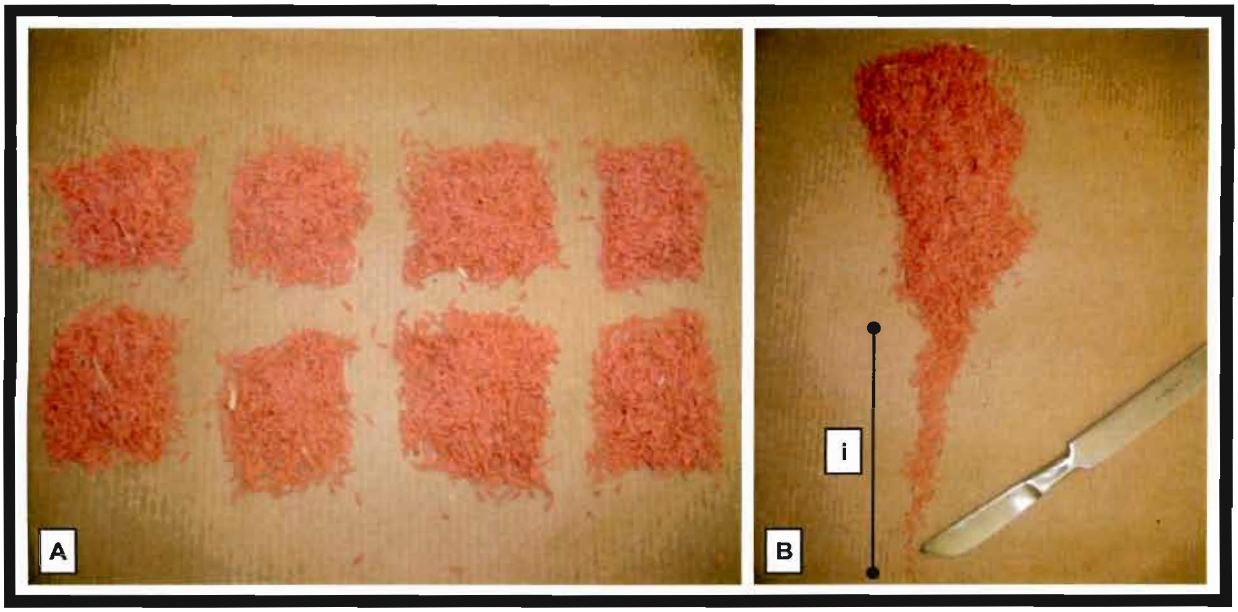


Figure 2.2: **A.** The eight (8) portions obtained in the ISTA Rules hand halving method as described in the text. (Seed in picture: enhanced *D. eriantha*).
B. The random counting of the pure seed component to obtain 4 x 100 replicates used in the germination tests: i. refers to the tail (explained in the text). (Seed in picture: enhanced *D. eriantha*).

2.3.4 Germination tests

Seed purity and germination tests took place in the laboratory and growth chambers of the NWU.

According to Anon. (2006), the seed used in the germination tests must be obtained from the well mixed pure seed component. In the germination component, 4 x 100 seeds were counted at random with a seed spatula as with the seed purity (Figure 2.2.B). A line of seed was carefully spread out ending in a fine line, referred to as the "tail" (Figure 2.2.B) and the seeds were counted in the strict order of their position on the line until 4 x 100 seeds were obtained (Anon., 2006).

The germination containers (plastic containers of 10 x 10 cm) were sterilised with 95% ethanol in order to prevent contamination with fungi or other micro-organisms. The ethanol was left to evaporate, after which the substrate and seeds, using the top paper method (Anon., 2006) was placed in the containers (Figure 2.3).

The top paper procedures for the germination tests in the growth chambers were carried out as follows (Anon., 2006):

The pure seed (as determined in the purity testing – Appendix C (i) & (ii)) was used and four (4) replicates of one hundred seeds counted. The replicates were spaced out in plastic containers, lined with cellulose paper and 125 mm Ö Anchor circles (Figure 2.3). The paper was wetted with potassium nitrate (KNO_3) for dormancy breaking (germination test one), or water when dormancy breaking was not required (germination test two) (Anon., 2006). The cellulose paper and 125 mm Ö Anchor circles provided a medium for the seeds to germinate. Various dormancy breaking methods can be used. In this project, two sets of germination tests were carried out.

The first set of germination tests included an integration of the (ISTA, 2006) dormancy breaking method with pre-chilling of the seeds at 5°C for five (5) days, according to ISTA (2006). After the pre-chill period, the containers were incubated in the NWU growth chambers at 25°C and evaluated as prescribed. Evaluation dates were calculated from the day that the containers were taken out of the 5°C pre-chilling and placed in the growth chambers (Anon., 2006) and not from the day the samples were pre-chilled.

The germination evaluation took place at different intervals for the selected grass seed samples. All the dates were the same, whether or not dormancy breaking was used. The first evaluation for the *Chloris gayana* (non-enhanced and enhanced grass seed types of the selected species) took place seven (7) days after incubation. The final evaluation was carried out after fourteen (14) days (ISTA, 2006).

Figure 2.3: The container with the "planted" seeds used in the Top-paper germination tests method.

For the *Cynodon dactylon* (non-enhanced and enhanced grass seed types) the first evaluation was carried out seven (7) days after the first day of incubation, and a second evaluation took place after fourteen (14) days of incubation. The final evaluation was carried out twenty-one (21) days after incubation (ISTA, 2006). The first evaluation for the *D. eriantha* (non-enhanced and enhanced grass seed types) was done four (4) days after incubation and the final evaluation after ten (10) days (ISTA, 2006). For the *E. curvula* (non-enhanced seed and seed enhanced with "plain coat", "organic insecticide on the base of the coat" and the type enhanced with "organic insecticide on the base and as overspray" grass seeds types) the first evaluation was done after six (6) days and the final evaluation after ten (10) days (ISTA, 2006). In the second set of germination tests, dormancy breaking was not applied and the evaluation dates were calculated from the day the samples were placed in the growth chambers.

During the first evaluations, normal seedlings, as well as seeds with secondary fungus infection, i.e. dead seed, were removed from the containers. The un-germinated seeds were left until the next evaluation date. During the final evaluation, abnormalities, dead seeds and fresh seeds that did not germinate were recorded on a germination chart (Appendix D).

2.4 Establishment percentage of the seed types of the selected grass species

2.4.1 Glasshouse trials with different growth mediums

After the germination tests, germination and establishment tests were carried out in the glasshouse at NWU at a day temperature of 25°C and a night temperature of 20°C. For each seed type of the selected grass species, one hundred seeds were sown in a tray and the growth medium was kept wet with tap water (Figure 2.4.A and 2.4.B). Four (4) replicates, adding up to a total of 400 seeds were tested per seed type of the selected grass species. The establishment tests were carried out in two separate growth mediums, i.e. Hygromix and Coco Peat Moss growth mediums (for the composition of the mediums, refer to Chapter 5). The germination and establishment of the seeds were monitored weekly for seven (7) weeks in both the mediums.

The reason for the use of the two separate growth mediums is that Hygromix is a growth medium with added nutrients in comparison with Coco Peat Moss, which does not have as much nutrient present in the mix as with Hygromix. Hygromix ensures fast and effective growth of the plant and was used to optimise the seedlings chances to produce biomass, used in the biomass determinations under controlled conditions, while the Coco Peat Moss was used as a germination medium, for no added nutrients are present in the medium to support the plant to yield a biomass.

2.4.2 Biomass production monitoring

After seven (7) weeks, the fresh and dry above- (leaves) and below- (root) ground biomass was determined for the seedlings planted in the Hygromix growth medium. The seedlings for the biomass calculations were randomly selected, uprooted and the growth medium was washed off with water, after which the parts were left to air dry (Figure 2.4.C and 2.4.D). The fresh above- (leaves) and below- (root) ground parts were separated and weighed, using a two decimal balance scale (0.00 g). Thereafter, the above- and below-ground parts were dried for 24 hours at 60°C. The dry above- (leaves) and below- (root) ground biomass was then determined. These procedures for the biomass monitoring took place at the end of each month for four (4) consecutive months (April 2006 – July 2006). Only the biomass production after the four month period of growth is discussed in the results, and not the biomass production rate over the four months. The fresh and dry above- and below-ground biomass was used to compare the fresh and dry growth yield of the seedlings after four months.

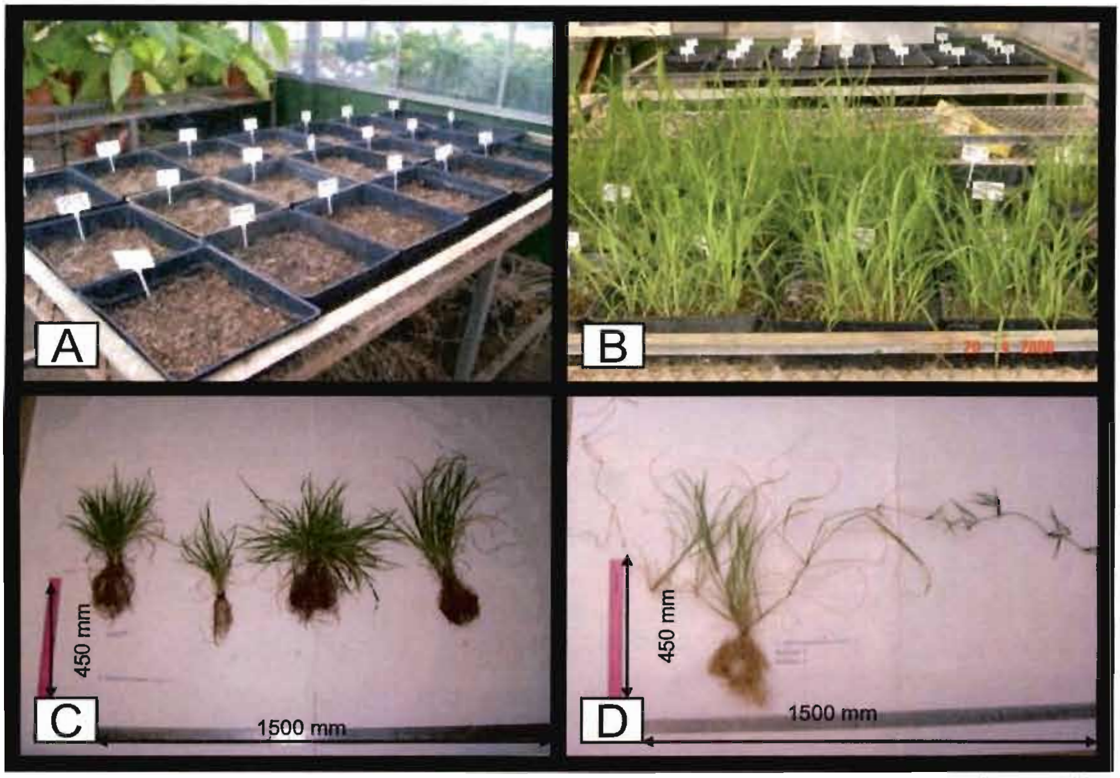


Figure 2.4:

A. The trays in which seeds were planted in Hygromix growth medium and kept moist.

B. The seedlings in the Hygromix growth medium after a growth period of seven (7) weeks.

C. *Digitaria eriantha* non-enhanced grass seed type as an example of the uprooted and washed seedlings used in the biomass monitoring.

D. *Chloris gayana* non-enhanced grass seed type as an example of the uprooted and washed seedlings used in the biomass monitoring.

To exclude the competition of light and water between the seedlings in the trays, seedlings in the Hygromix growth medium were planted in separate bags (in a growth medium supplied by the NWU Botanical Garden consisting of one part saw dust mixed with one part compost) in May 2006. Three (3) seedlings per tray (4 trays per seed type of the selected grass species) were used for the biomass monitoring, resulting in twelve (12) seedlings per seed type for the first three months (April 2006 – June 2006). Because of limiting seedlings after the fourth month of growth, for July 2006, only one seedling per tray was used in the biomass monitoring, resulting in four (4) seedlings per seed type of the selected grass species. The average for the fresh above- and fresh below-ground biomass of each seed type of the selected grass species, as well as the average dried above- and dried below-ground biomass of each seed type of the selected grass species was calculated and recorded.

The results were statistically analysed and plotted on graphs (SigmaPlot 8.0) to indicate the average biomass of the seed types of the selected grass species after the four month period, as well as the statistical significance using the computerised program, Statistica to calculate the t-tests and p-values (Steyn *et al.*, 1998).

2.5 Natural field trials

2.5.1 Study site description

The experimental site is situated at the experimental farm of the North-West Department of Agriculture, Conservation and Environment[3] (DACE), Potchefstroom – see 6.2 Companies and Personal Communications - (26° 44'30.60" S and 27°04'04.47" E, Figure 2.5). The land was cultivated seven (7) years prior to the establishment of this experimental site, for the production of cash crops.

The experimental site is located in the center of the Gm 11 Rand Highveld Grassland vegetation type (Mucina & Rutherford, 2006) and the Grassland Biome (Rutherford & Westfall, 1994) in the North-West Province of South Africa (Figure 2.5). According to Acocks (1988), this site is situated in the VI. Pure Grassveld Type No. 48. *Cymbopogon-Themeda*-veld. This veld type is also characteristic of the Gauteng, Free State and Mpumalanga Provinces, where the altitude ranges between 1300-1635 m, up to 1760 m in specific places. According to Rutherford & Westfall (1994), the Grassland Biome's altitude ranges from 300 m on the coastal plateaus to 2850 m above sea level on the Drakensberg. The landscape is characterised by a flat to rolling, and in some instances mountainous, topography.

2.5.1.1 Vegetation and Landscape features of the experimental site

Sloping plains and series of rocky ridges with sparse woodlands (savannoid) are features of this landscape. Within the woodlands, species such as *Protea caffra*, subsp. *caffra*, *P. welwitschii*, *Acacia caffra* and *Celtis africana* occur. This is a species-rich environment, with sour grasslands in alteration with shrubland, which is characterised by low and sour slopes with rocky outcrops. Other grass species common in these areas and widely recorded on the plains include species of *Themeda*, *Eragrostis*, *Heteropogon* and *Elionurus*. Another common feature of this vegetation type is the diversity of herbs belonging to the Family Asteraceae, as well as shrubs with the dominant genus *Rhus* (especially *R. magalismontana*) (Mucina & Rutherford, 2006).

The hemicryptophytes of the Poaceae are one of the characteristics of the Grassland Biome, with a moisture dependent canopy cover which will decline in areas of lower rainfall (Nicholson, 1988). The structure of the canopy is influenced by grazing. The Grassland Biome is mainly divided into the "sweet" and "sour" grass veld types (Rutherford & Westfall, 1994). The "sweet" grasses, are more palatable and occur within areas with an annual rainfall of below 625 mm/a. These grasses have lower fibre content. In winter, the nutrient level remains high. The "sour" grasses dominate in areas with an annual rainfall of above 625 mm. These grasses have higher fibre content and loose their nutrient status in winter. Another characteristic of the Grassland Biome is that the grass plants can be managed by grazing, fire and mowing (Rutherford & Westfall, 1994).

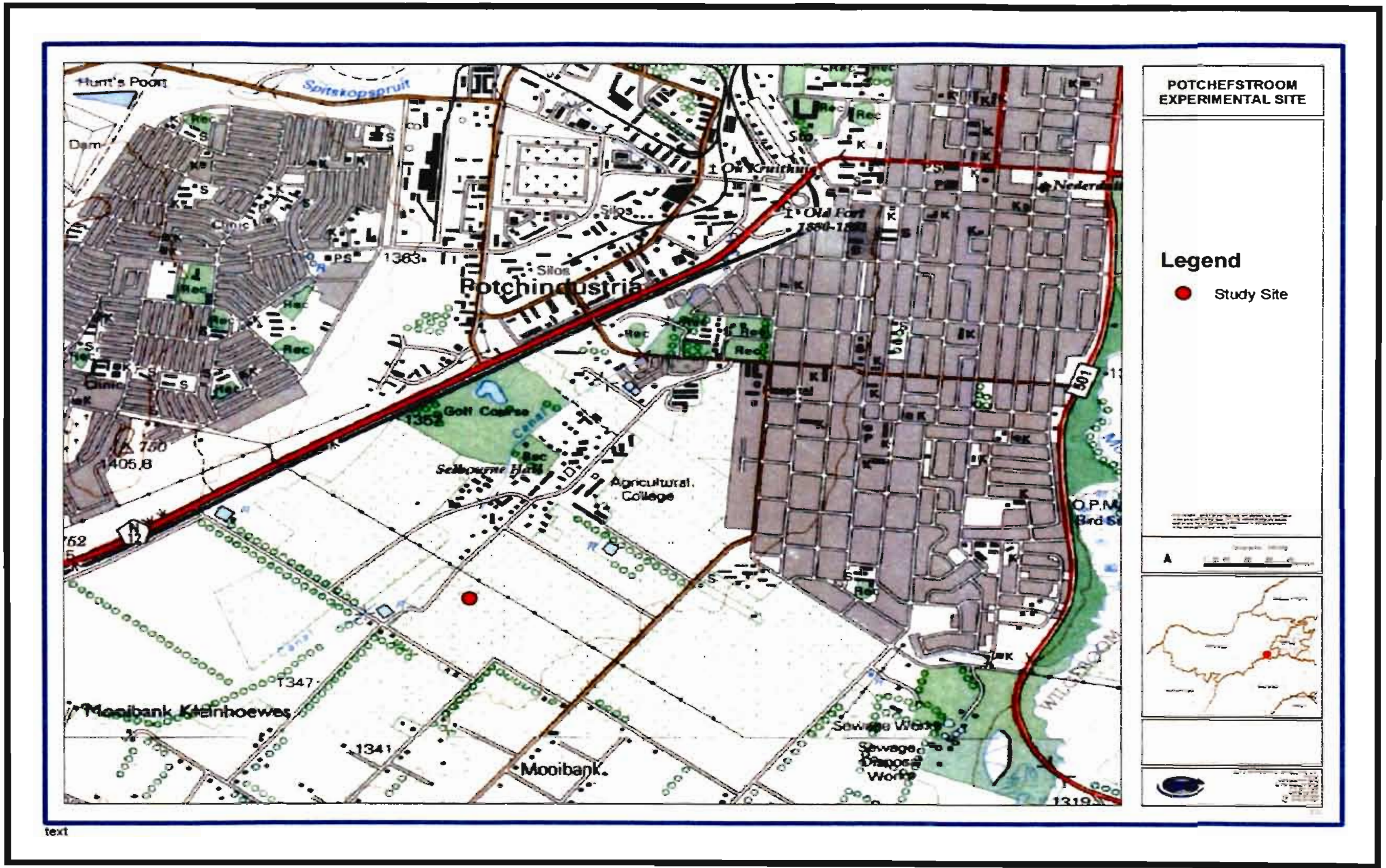


Figure 2.5: The natural field trial experimental site at DACE, Potchefstroom. Map supplied by GISCOE[4] – see 6.2 Companies and Personal Communications. (See red dot).

According to Rutherford & Westfall (1994), the above-ground plant production may vary between 1 000 $kg.ha^{-1}$ per annum in the drier regions, to 6 000 $kg.ha^{-1}.a^{-1}$ in the regions where temperature, as well as rainfall, is higher. The plant production will determine the occurrence of fire, which normally occurs naturally in the wetter regions.

2.5.1.2 General description of the vegetation of this veld type

According to Rutherford & Westfall (1994) *Themeda triandra* Forssk. is the most important species in the Grassland Biome. C_4 grasses tend to dominate the biome, with exception of the high Drakensberg regions, where, according to Vogel *et al.* (1978) and Rutherford & Westfall (1994), the C_3 type grasses are important. The rarer plants in this biome are more dominant in the wetter areas. Mucina & Rutherford (2006) classify the Rand Highveld Grassland vegetation type to have the following important taxa, which include graminods for example, *Ctenium concinnum*, *Cynodon dactylon*, *Digitaria monodactyla*, *Diheteropogon amplectens*, *Eragrostis chloromelas*, *Heteropogon contortus* and *Loudetia simplex* (d); succulent herbs such as *Aloe greatheadii* var. *davyana*; low shrubs, for example *Anthospermum rigidum* subsp. *pumilum*, *Indigofera comosa*, *Rhus magalismontana* and *Stoebe plumose*. Succulent shrubs include *Lopholaena coriifolia* and geoxylic suffrutex include *Elephantorrhiza elephantina*. Biogeographically important taxa include (all Northern sourveld endemics) geophytic herbs, such as *Agapanthus inapertus* subsp. *pendulus* and *Eucomis vandermerwei*; the succulent herb, *Huernia insigniflora* and the low shrub, *Melhania randii*. Endemic taxa include herbs such as *Melanospermum rudolfii* and *Polygala spicata*. *Anacampseros subnuda* subsp. *lubersii*, and *Frithia humilis* are part of the succulent herbs. Succulent shrubs include *Crassula arborescens* subsp. *undulatifolia* and *Delosperma purpureum,* and small trees, such as *Encephalartos lanatus* and *E. middelburgensis* are known in this veld type.

2.5.1.3 General description of the geology and soil

According to Mucina and Rutherford (2006), the study site of the natural field trials form part of the Gm 11 Rand Highveld Grassland vegetation type. The geology of this region consists of the quartzite ridges of the Witwaterand Supergroup and the Pretoria Group as well as the Selons River Formation of the Rooiberg Group (from the Transvaal Group). The natural field trial site form part of the Ba, Bc, Bb and Ib land types.

The Grassland Biome's lithosphere area consists of the red-yellow-grey latosol plinthic catena soil group. Following this soil is a combination of black and red clays and solonetzic soils, freely drained latosols and black clay soils, the latter being limited to the Grassland Biome. Undifferentiated rock and litosols, lime-poor, weakly developed soils on rock, and undifferentiated swamps and alluvial plains are other soil groups included in this biome (Rutherford & Westfall, 1994). More leached soils are characteristic of the moist grasslands, which have a higher rainfall and are more dystrophic than the general eutrophic soils of the dry grassland. The Grasslands are maintained by fire and the removal of woody species by animals (Rutherford & Westfall, 1994). Veld mismanagement may lead to soil erosion occurring on the steeper slopes and erodable solonetzic, duplex soils in the wetter regions.

Soil sampling took place in 2007 as background data, and were analysed by Eco Analytica[5] – see 6.2 Companies and Personal Communications. A composite sample of each replicate plot was taken at a 300 mm depth with a soil auger (Figure 2.9). The pH (KCl), phosphate (P) (Bray 1) as well as potassium (K), calcium (Ca) and magnesium (Mg) (ammonium acetate) contents of the soil was determined.

2.5.1.4 General description of the climate

The experimental site is situated in an average warm-temperate region with seasonal summer-rainfall (October – April) and very dry winters. According to Mucina and Rutherford (2006), the long-term mean annual precipitation (MAP) is 654 mm, ranging between 570 mm and 730 mm, with the measured precipitation at the study site for the period January 1990 – September 2007 being 604 mm (Figure 2.7). The MAP for the region where the natural field trials study site is located is a bit lower and the coefficient of variation of MAP is 28%. Frost occurs more in the west, ranging from 30 – 40 days (Mucina & Rutherford, 2006). Rainfall and minimum and maximum temperature data were provided by the *Institute for Soil, Climate and Water*[6] (ISCW) – see 6.2 Companies and Personal Communications - from the *Potchefstroom Oil Seeds and AGR; NIGG*. Stations.

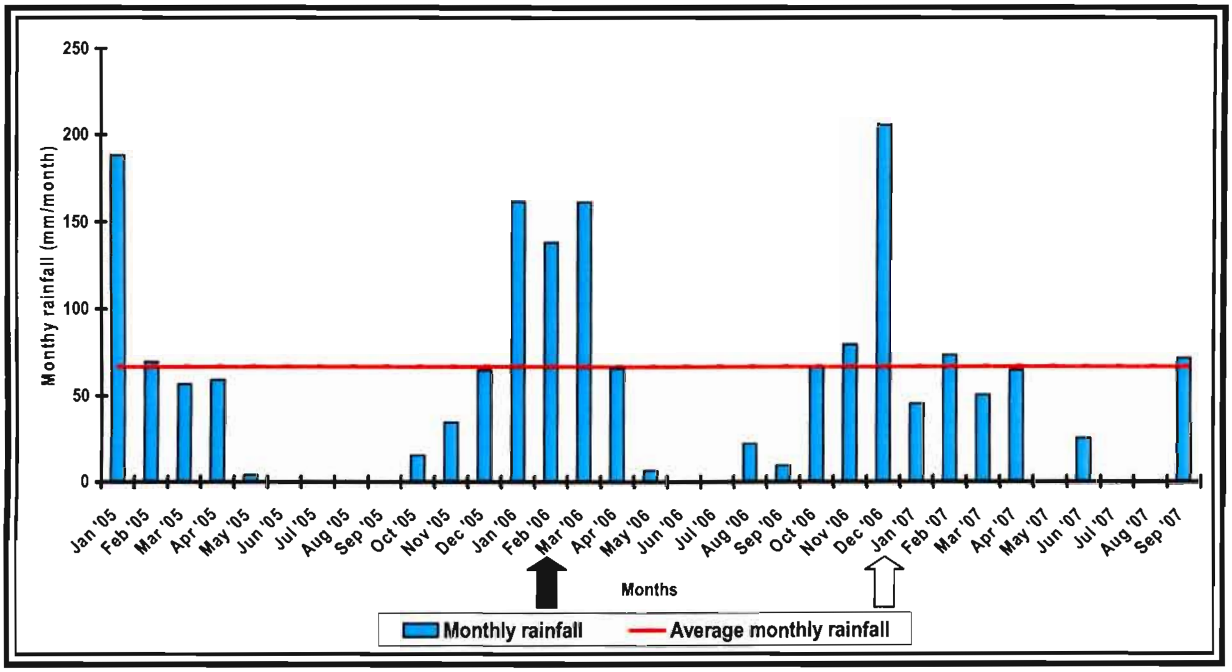


Figure 2.6: The monthly and average rainfall as from January 2005, throughout the growing period (March 2006 – January 2007) on the Potchefstroom natural field trial experimental site recorded by the ISCW. Black arrow – planting period and white arrow - period during which the ecological surveys were carried out.

The temperature and rainfall data, as well as planting and monitoring times are given in Figures 2.6 – 2.8. During the planting period, the average monthly rainfall was 161.5 mm (Figure 2.6) and the minimum and maximum temperatures were 13.7°C and 27.1°C (Figure 2.8), respectively. The average rainfall of the six months (September 2005 – February 2006) preceding planting was 69.1 mm. The average rainfall during the period when the ecological surveys were carried out was 45.1 mm (Figure 2.6) and the minimum and maximum temperatures 16°C and 30.8°C (Figure 2.8), respectively. Planting took place on 16 and 17 March 2006 and ecological monitoring on 23 January – 5 February 2007.

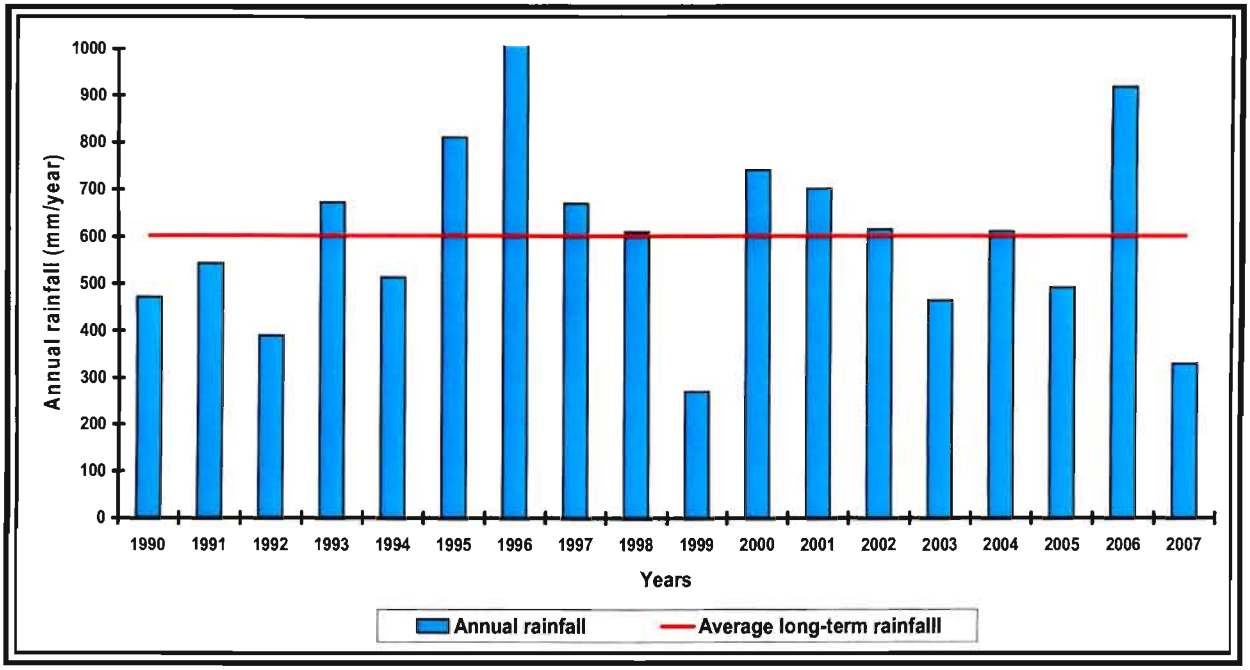


Figure 2.7: The average annual rainfall from 1990 - 2007, at the natural field trial experimental site in Potchefstroom, as recorded by the ISCW.

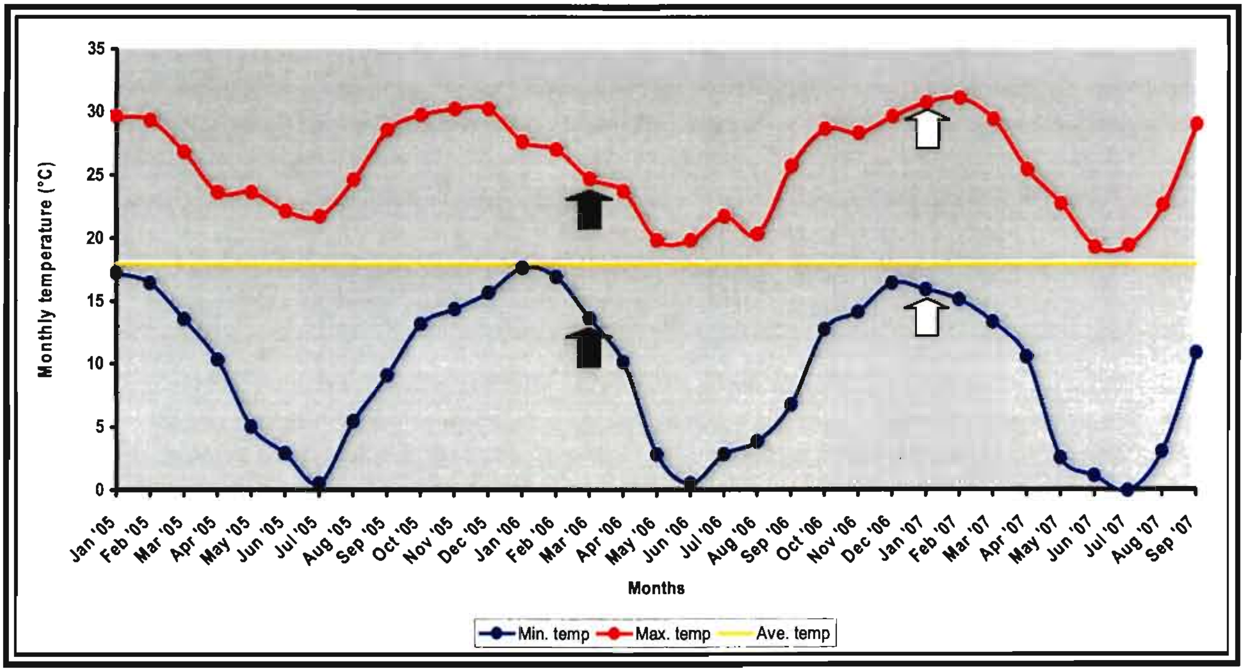


Figure 2.8: The minimum and maximum temperatures as from January 2005, throughout the growing period (March 2006 – January 2007) at the natural field trial experimental site in Potchefstroom, as recorded by the ISCW. Black arrow – planting period, white arrow - period during which the ecological surveys were carried out.

2.6 Experimental design of study site

Three (3) replicate plots for each of the seed types of the selected grass species were planted at the experimental site mentioned in section 2.5.1, in a split-plot design (Figure 2.9 A). The land on which the plots were laid out was ploughed and rolled before the selected grass seeds were sown (Figure 2.10 A, B, C). The experimental site was based on a randomised experimental design (Figure 2.9 A). Each plot was divided into 10 sub-plots with a size of 100 m² (Figure 2.9 A & B). In each sub-plot, seven (7) rows were sown by hand one meter apart and 6 m long (Figure 2.9 B & 2.10 D).

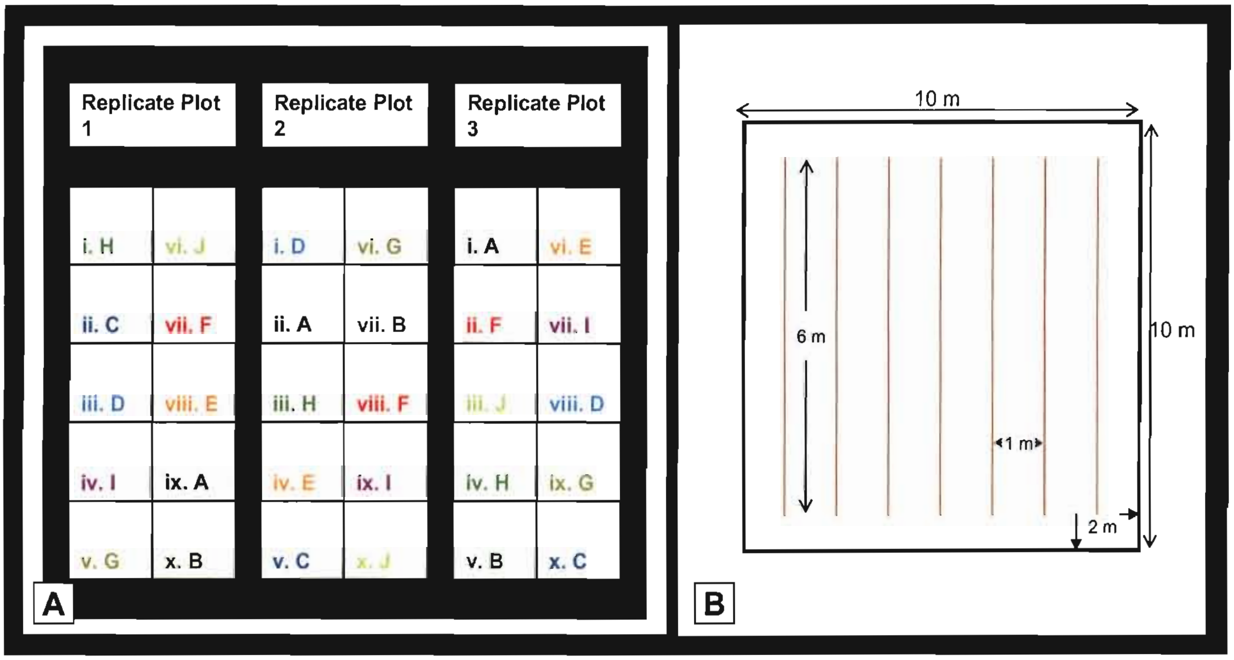


Figure 2.9: **A.** The randomised experimental design of the natural field trials study site. Number of sub-plots (i – x) with seed types (A – J, Table 2.1).
B. Sub-plot size and lay-out of rows. The 7 rows are 6 m long and 1 m apart, with an edge of 2 m surrounding each plot.

In each row, 4.2 g of seeds were sown for each of the *D. eriantha* (non-enhanced and enhanced grass seed types), *Chloris gayana* (non-enhanced and enhanced grass seed types) and *E. curvula* (non-enhanced and enhanced grass seed types), respectively.

For the *Cynodon dactylon* (non-enhanced and enhanced grass seed types), 6 grams of seeds were sown per row. The type of seed, batch number, mass per 100 seeds, number of seeds per row, as well as the number of seeds sown per plot are given in Table 2.1. The site was watered (25 mm/week) by means of a dragline sprinkle system.

Problematic weeds were chemically controlled with MCPA 400, and also mechanically removed (Figure 2.10 F). Vegetation monitoring was carried out after a growing period of nine months (Figure 2.10 E).

Figure 2.10: **A.**, **B.** and **C.** The cultivation and preparation of the natural field before sowing the seed types of the selected grass species. **D.** Sowing the seeds in rows by hand. **E.** Grass wards after a nine month growing period (January 2007). **F.** Mechanical control of the weeds (June 2007). **G.** Plots were cut in August 2007. **H.** One month after the plots were cut (September 2007).

Table 2.1: Seed types of the selected grass species, batch number, mass (g) of 100 seeds as well as number of seeds planted at the natural field trial study site. A - J are the seed types sown, correlating with Figure 2.9.

Ref. no.	Seed type of grass species	Batch number	Mass (g) of 100 seeds	Number of seeds per row	Number of seeds per plot
(C)	*Chloris gayana* [Non-Enhanced]	9072 (B0009)	0.0568	22 388	156 7196
(D)	*Chloris gayana* [Enhanced]	E 9072 (B0009)	0.1330	4 511	31 577
(F)	*Cynodon dactylon* Variety Blackjack [Non-enhanced]	8949 (B0012)	0.0271	15 498	108 486
(E)	*Cynodon dactylon* Variety Blackjack [Enhanced]	8949 (B0012)	0.032	13 125	91 875
(A)	*Digitaria eriantha* [Non-enhanced]	9038	0.0288	15 000	105 000
(I)	*Digitaria eriantha* [Enhanced]	E 9038	0.1064	3 959	27 713
(J)	*Eragrostis curvula* [Non-enhanced]	9098	0.0334	12 575	88 025
(B)	*Eragrostis curvula* [Plain enhanced]	9098	0.0647	3 246	22 722
(G)	*Eragrostis curvula* [Enhanced with organic insecticide on base of coat]	9098	0.0647	6 492	45 444
(H)	*Eragrostis curvula* [Enhanced with organic insecticide on base of coat and as overspray]	9098	0.0647	6 492	45 444

2.7 Vegetation surveys

The vegetation surveys were carried out nine to ten months after establishment of the site (Figure 2.10 E). Vegetation surveys were carried out in order to sample and compare the density, frequency and dry matter production of the selected enhanced and non-enhanced seed types of the grass species subjected to natural environmental conditions. The following vegetation sampling methods were used:

2.7.1 Density

The density was determined by randomly placing 1 m x 1 m quadrats in each sub-plot and counting the number of individuals of the seed types of the grass species as well as estimating the occurrence of weeds present in the quadrat (Kent & Coker, 2003).

2.7.2 Frequency

The descending point method (Kent & Coker, 2003) was used to determine the frequency of the grasses planted. Kent and Coker (2003) described frequency as being the probability of the occurrence of a species in a particular survey area.

In each 6 m long row, a tape measure was positioned over the row and the species at every 0.5 m in a radius of 30 cm nearest to the points were recorded. Other species, such as weed species and bare patches were also noted. Direct hits on the living base of the tufts were also recorded as a basal hit, representing the basal cover of the plot. To eliminate the edge effect, the first and last point in the beginning and end of each row was discarded. The nearest species was identified to be either the planted species or other species, because seed not present in the seed bank may have been imported to the study site by animals, insects or wind.

2.7.3 Dry matter production

The biomass production of the seed types of the selected grass species planted was recorded by harvesting the grass by clipping the above-ground material at 5 cm above the ground with a sheep shear (Pieper, 1988). Each species was placed in a brown paper bag and dried at 60°C for 24 hours. After the drying period, the average dry matter (DM) was determined and noted as grams per sub-plot. Thereafter, the grams per species were calculated and converted to kg/ha (kilograms per hectare – see conversion 2.1). The plots were further cut at 20 cm above-ground in August 2007 (Figure 2.10 G), to calculate the productivity of the new growth in the next season and future studies (Figure 2.10).

The conversion of the grams per square meter to kilograms per hectare were calculated as follow:

<u>Conversion</u>

$$\text{Dry matter production (kg/ha)} = \text{dry matter production (g)} \times \frac{10\,000\ \text{m}^2}{1\,000\text{g}}$$

For example, the conversion of the g/m^2 *Chloris gayana* (enhanced) to kilograms per hectare:

$$\text{Dry matter production (kg/ha)} = \text{dry matter production (g)} \times \frac{10\,000\ \text{m}^2}{1\,000\ \text{g}}$$

$$\text{Dry matter production (kg/ha)} = 226.07\ \text{g/m}^2 \times \frac{10\,000\ \text{m}^2}{1\,000\ \text{g}}$$

$$\text{Dry matter production (kg/ha)} = 2260.7\ \text{kg/ha} \sim \underline{\mathbf{2261\ kg/ha}}$$

2.8 Root development and vascular tissue evaluation

Root development studies on *Cynodon dactylon* and *Festuca arundinacea* affected by cutting interval and growth regulators were previously carried out in the USA by Beyrouty *et al*. (1990), who monitored root development and growth in natural field conditions with cameras recording the growth through plexiglass tubes. In their observations of *Cynodon dactylon* using the plexiglass, a two-stage root establishment pattern was observed. The first stage was characterised by minimal root development and stolon proliferation followed by the production of total root length after two seasonal productions. In the case of *F. arundinacea,* the total root length peaked one and a half years after establishment and overall the trends in root growth differed from the patterns of shoot growth. Other root development experiments included that of Jurena and Archer (2005), who investigated whether or not above-ground growth would be greater in the absence of a barrier and whether shallow root species would be less affected by these barriers in comparison with species with a deeper root system (Jurena and Archer, 2005). The grasses were grown in clear flexible cylinders with standard potting soil to mimic natural field conditions. In their experiment, artificial barriers were placed in the pathway of root growth and the root growth could be monitored through the flexible cylinders. The barriers had no affect on either the shallow or deep rooted species' above-ground or root biomass.

The initial objectives stated that the root development (morphological) will be compared in this study. These morphological development evaluation experiments were carried out, by using a transparent agar medium, Gelrite as a germination and growth medium. After the essential sterilisation process of the seed before planted on the Gelrite medium, the seed did not germinate on the medium and fungal infection occurred. No seedlings were therefore available for the evaluation of root development. Although the seed germinated and established successful in the perlite medium, the medium made it difficult to uproot the seedlings without damaging the roots. This was a second option to evaluate the root development, which also was not successful. The seedlings grown in the perlite medium were however used in the anatomical experiments. The anatomical comparison of the xylem and phloem as the major conducting tissue were evaluated, because the transition region from the stem to the roots was not damaged.

The seed used in the anatomical study was entirely different to the type used previously. The reason for this was that the coating was altered and different coatings were applied on the *D. eriantha* by Advance Seed Company. *Digitaria eriantha* are also a species which are widely used in South Africa, present in the mixtures of seed used in the restoration and rehabilitation practices of mining areas, degraded rangelands and cultivated pastures (van Oudtshoorn, 2004). For the experiments, non-enhanced seed as well as seed coated in multiple enhancements of *D. eriantha* was used. Five (5) different enhancement methods form a different seedlot than the *D. eriantha* used in the other experiments in the study was used (Table 2.2). The seed enhancements included seed treated with a normal treatment (K), seed treated with a dormancy breaking coating (L), seed treated with a phosphate enriched coating (M), seed treated with a treatment that included extra growth stimulant (N) and seed treated with an organic fungicide (O). The main aim of these treatments (coatings) is to enable the seed to have a higher germination and establishment percentage than the untreated seed (P) (Table 2.2). The seed used in the anatomical study was also supplied by ASC. The composition for the coatings (treatments) was unknown as the company has exclusive rights to the composition of the coatings and can only be disclosed by the seed company.

Table 2.2: Seed types of *D. eriantha* used in the root development and vascular tissue evaluation.

Ref. no.	Description of enhancement treatment of *D. eriantha*
(K)	Normal treatment
(L)	Dormancy breaking treatment
(M)	Phosphate enriched
(N)	Extra organic growth stimulant
(O)	Organic fungicide
(P)	Non-enhanced

The following procedures were followed in these trials:

The seeds were individually germinated as in germination tests (2.3.2 Seed germination). Seeds were checked every day and the seeds that started to germinate, i.e. when the root or shoot emergence, were transferred to pure perlite growth medium (available from Ocean Agriculture[7] – see 6.2 Companies and Personal Communications) in seed trays (Figure 2.11). In order to note the age of each transferred seed, the rows were recorded on the seed trays. The trays were kept wet with distilled water for consistency. Initially more water was given and the amount of water was gradually reduced in order not to cause fatality in the seedlings.

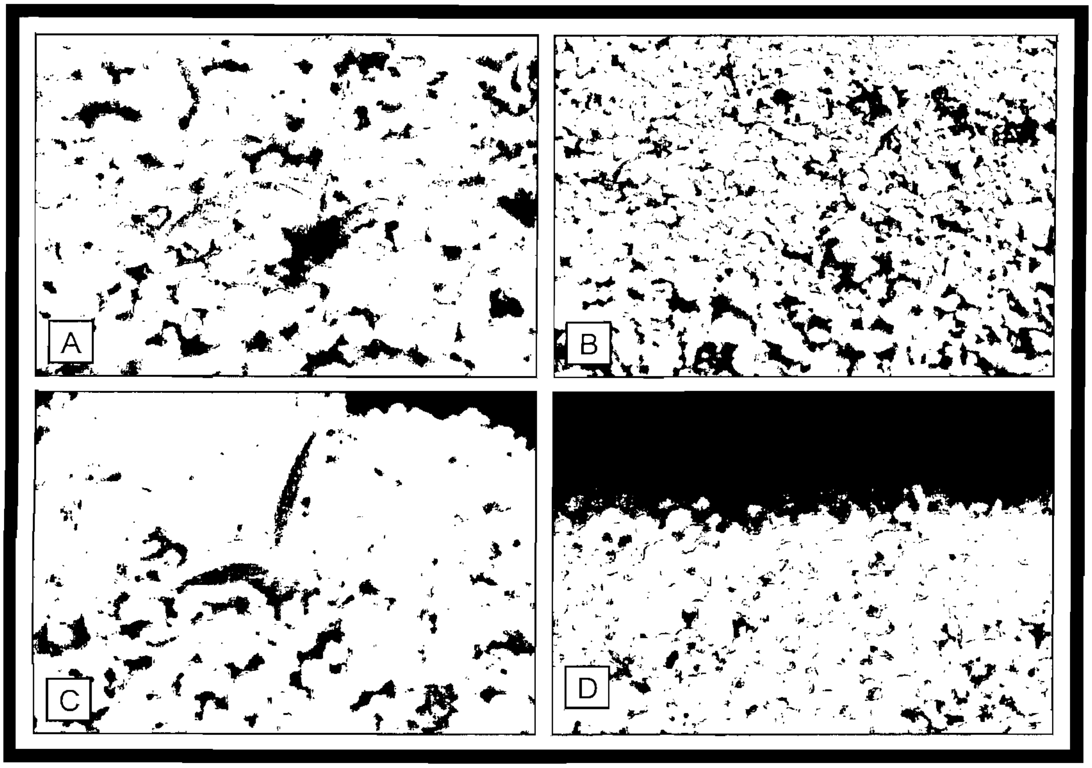


Figure 2.11: **A.** and **B.** Seedlings of *D. eriantha* (enhanced with organic fungicide) in the perlite medium.
C. and **D.** Seedlings of *D. eriantha* (enhanced with extra organic growth stimulant) in the perlite medium.

After a growing period of 10 – 16 days, the seedlings were harvested and preserved in 4% aqueous paraformaldehyde at pH 7.2.

After the seedlings were fixed in the paraformaldehyde for a week, four seedlings of each enhancement treatment, as well as of the non-enhanced seed type, of which the adventitious roots have started to develop, were identified. The roots were removed and the seedlings were cut at approximately 2 mm above the seed at the transition region.

The seedling samples were embedded in LR (London Resin) white resin and subsequently cut with a Reichert-Jung ultra cut microtome at 0.8 μm. The specimens were stained with 0.05% toluidine blue and 0.05% neofuchsin in water and permanent slides were mounted. Light micrographs were recorded with Nikon DXM1200F digital camera mounted on a Nikon E800 microscope and captured in the EclipseNet program, where the measurements were also taken. The transition regions of the selected grasses were compared.

2.9 Seed predation

Although the monitoring of seed predation may vary between trials depending on the aim (Andersen & Ashton, 1985; Barnes, 2001; Bond & Breytenbach, 1985), the type of experiment used in this study was discussed with a few entomologists and scientists who have experience with these types of trials.

First, a pilot study was carried out to test various aspects to be refined in the final seed predation experiment. In each of the sub-plots four petri dishes were placed level with the soil and two dishes were filled with a thin layer of soil from the plots, at a random spot with-in the sub-plot (Figure 2.12). In the first petri dish 30 seeds of the seed types of the selected grass species to be tested were placed in a petri dish with a layer of soil. In the second petri dish 30 seed from the opposing seed type of the selected grass species - enhanced or non-enhanced, depending on which treatment was planted in that particular sub-plot – treatment. The two petri dishes without the soil layer were also filled with either 30 non-enhanced and 30 enhanced seed type. The two petri dishes were covered by either a tin cover (referred to as closed) or mesh cover (referred to as "open") - manufactured from expanded metal by the Instrument Making Department[8] NWU - see 6.2 Companies and Personal Communications - (Figure 2.13) – to exclude predation by other organisms, such as birds and rodents. Small insects can also act as predators on seed used in cultivation. This experiment was carried out for the seed types of the selected grass species mentioned in Table 2.2 and in sub-plots indicated in Figure 2.14. At each replicated plot, two petri dishes of the same seed were placed, giving a total of 6 repetitions for the entire experimental site for each trial, i.e. "soil and open" (SO), "no soil and open" (NSO), "soil and closed" (SC) and "no soil and closed" (NSC). The objective of the seed predation trials was to compare whether or not the non-enhanced seed are more susceptible to seed predation by ants and other small insects compared to the enhanced seed types. The trials were placed in the field and left for a period of 48 hours, after which the petri dishes were retrieved and the predation noted.

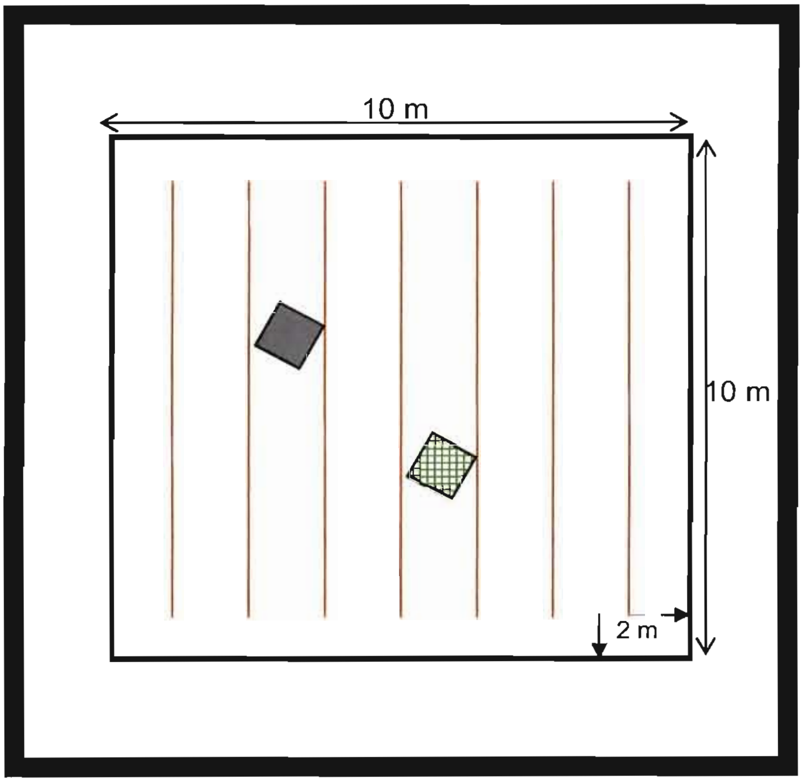


Figure 2.12: Seed predation trial lay-out of the pilot study in the sub-plot. The green square represents the mesh hood ("open") and the grey square is an indication of the tin hood ("closed").

Table 2.3: Seed types of the selected grass species used in the seed predation trials.

Ref. no.	Seed types of the selected grass species
(C)	*Chloris gayana* [Enhanced]
(D)	*Chloris gayana* [Non-enhanced]
(I)	*Digitaria eriantha* [Enhanced]
(A)	*Digitaria eriantha* [Non-enhanced]
(G)	*Eragrostis curvula* [Enhanced with organic insecticide on base of coat]
(J)	*Eragrostis curvula* [Non-enhanced]
(E)	*Cynodon dactylon* Variety Blackjack [Enhanced]
(F)	*Cynodon dactylon* Variety Blackjack [Non-enhanced]

Replicate Plot 1		Replicate Plot 2		Replicate Plot 3	
i. H	vi. J *	i. D *	vi. G *	i. A *	vi. E *
ii. C *	vii. F *	ii. A *	vii. B	ii. F*	vii. I *
iii. D *	viii. E *	iii. H	viii. F *	iii. J *	viii. D *
iv. I *	ix. A	iv. E *	ix. I *	iv. H	ix. G *
v. G *	x. B	v. C *	x. J *	v. B	x. C *

Figure 2.13: Sub-plots in which the seed predation experiments took place (marked *).

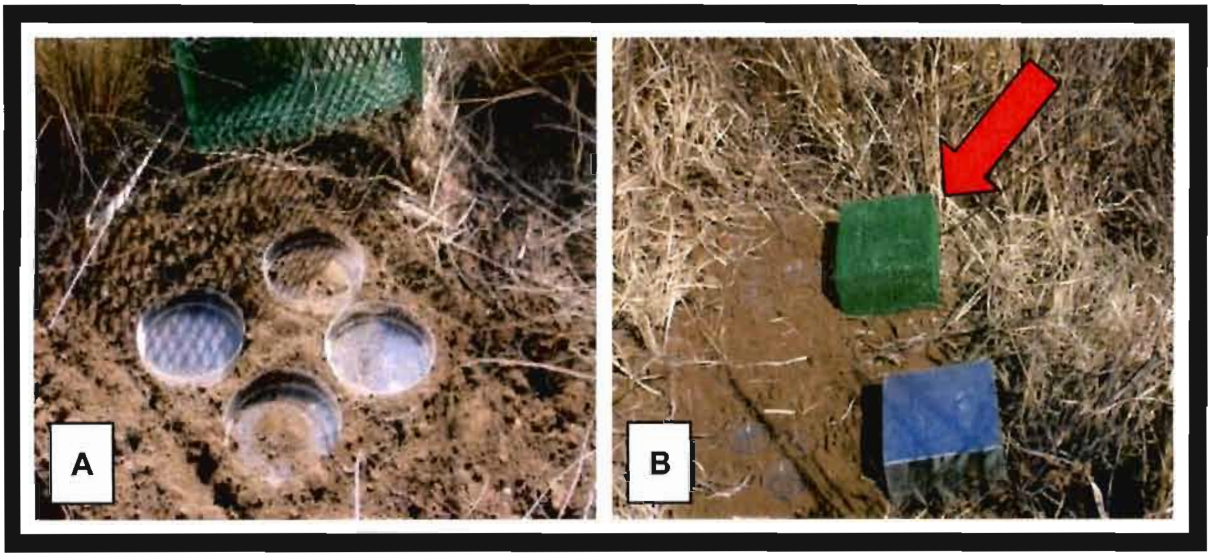


Figure 2.14: **A.** The petri dishes and mesh hood ("open") used in the seed predation trials.
B. The wire hood ("open" - red arrow), as well as the tin hood ("closed") used in the trials.

2.10 Statistical analysis

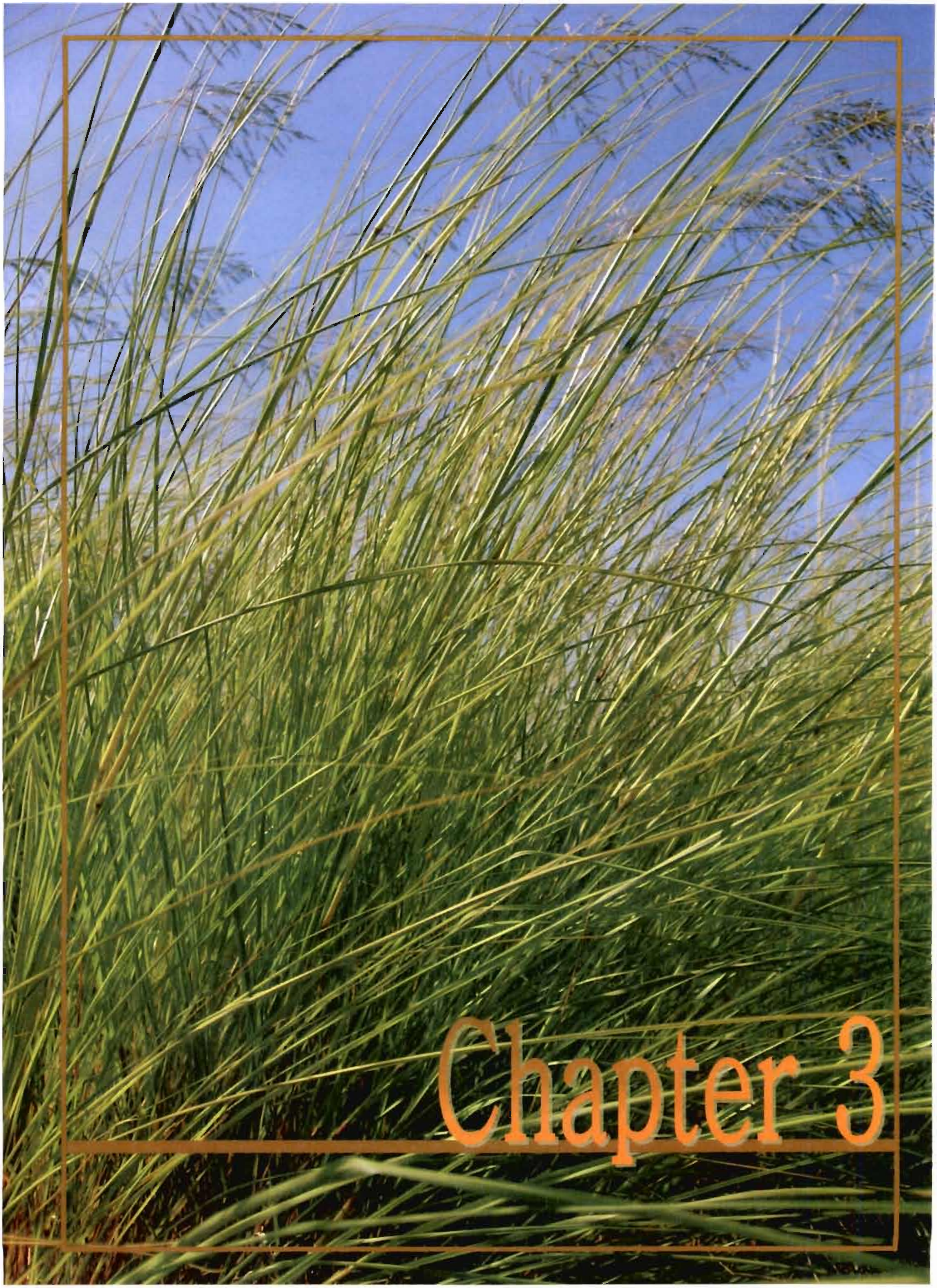
As mentioned the purity and germination tests was carried out according to the ISTA Rules (ISTA, 2006). The results are described in Chapter 3. A One Way ANOVA followed by Specific Contrast in Statistica was statistically carried out on the all the data, which include the laboratory, glasshouse and natural field trials - and will be discussed accordingly (Steyn *et al.*, 1998). The statistical analysis of the data obtained in the experiments are comparisons of the non-enhanced and enhanced seed types of each species with each other and not interspecies, for example, comparing the non-enhanced and enhanced seed types of *Chloris gayana,* and hence the same for the other species. In the case of the *E. curvula* all the enhancements were compared to the non-enhanced seed type separately. The results are given in graphs, and the bars on the graphs are an indication of the standard error.

No statistical data analysis was carried out on the seed predation data as well as on the measurements in the anatomical study.

The site (natural field trials) data were analysed by using the Principal Component Analysis (PCA) in the Multivariate data analysis CANOCO package (Ter Braak, 1988).

The PCA is an indirect gradient analysis, which is evident in this method. Eigen-values and the eigen-vectors – an indication of the factor by which the frequency value of each species is multiplied to determine the value of the plot on the PCA-axis – were calculated (Kent & Coker, 2003).

The Eigen-value represents the relative contribution of each component to the explanation of the total variation in the data as well as the highest possible degree of correlation of all the species/variables with the principal axis (Kent & Coker, 2003). The second ordination axis Eigen-value is much lower than the Eigen-value of the first ordination axis. In PCA, the axis is extracted in descending order of importance, in terms of the contribution to the total variation in the data set (Kent & Coker, 2003).



Chapter 3

CHAPTER 3
RESULTS AND DISCUSSION

3.1 General

As stated in the general objectives, this project focused on the evaluation and comparison of the seed types of the selected grass species of the same species to be used in re-seeding practices with each other with regards to the performance of the seed under various experimental conditions. The seed types of the selected grass species were: enhanced and non-enhanced seed of *Chloris gayana, Cynodon dactylon, D. eriantha* and several enhancements of *E. curvula* - non-enhanced, "plain coat", enhancement with "organic insecticide on base of coat" and enhancement with "organic insecticide on base of coat and as overspray".

These enhanced and non-enhanced seed types were compared under controlled conditions with regards to the germination and establishment percentages of the species. Seed purity analysis formed part of the germination test and the pure seed obtained from the purity tests were used in the germination tests, as stated in the ISTA Rules (ISTA, 2006). The pure seed component was also used in the glasshouse trials. The seeds germinated and established under controlled conditions and the above and below ground biomass of the seed types of the selected grass species were compared.

The dry matter (DM) biomass production was determined under natural conditions in the cultivated fields. Together with these determinations, ecological surveys to determine frequency, density and basal hits were carried out to give an indication of how the enhanced or non-enhanced seed types of the selected grass species performed in comparison with each other. Pilot seed predations trials, with special attention to the seed predation caused by ants and insects, were performed.

The activity of ants depended on temperatures and seasons. Because of unforeseen weather conditions in the trial period, the ant activity was low. The seed predation results can therefore only be seen as pilot study with preliminary results and need to be investigated further. The preliminary trials were, however, an indication of which method would be more specific and applicable in the final trials to evaluate seed predation by ants (see Chapter 4).

The experiment for root development was abandoned due to a fungal infection on the un-germinated seed as a result of the sterilisation process which were reduced to a minimum to not have an influence on the subject of the enhanced (coating) seed, which could be chemically remove by the ethanol used in the sterilisation process.

Certain seed-enhancement technologies were therefore investigated regarding the increase of the germination potential as well as the establishment, growth percentages and biomass yield of certain grass seed types for restoration and rehabilitation activities. The results are discussed below.

3.2 Germination percentage of selected grasses

3.2.1 Seed purity

The seed purity analysis yields the pure seed component of the seed types of the selected grass species as the percentage purity (ISTA, 2006). After the seed purity analysis was carried out on the seed types of the selected grass species, the overall results (average higher than 95%) indicated that all the seed types had a high percentage of pure seed, which supports the statements made in previous studies regarding the purity of seed supplied by the seed merchant, ASC (Table 3.1) (De Wet, 2001; Buys, 2002; Van den Berg, 2002). The major impurities in the enhanced seed types were chipped coatings and those in the non-enhanced seed types were straw debris and chaff (see Seed Analysis Report, Appendix E (i) & E (ii)). The enhanced seed type of *Chloris gayana* had a higher purity (97.6%) in comparison with the non-enhanced type (96.1%). In contrast, the non-enhanced seed types of both *Cynodon dactylon* (100.0%) and *D. eriantha* (98.3%) had a higher purity than the enhanced types with purities of 99.4% and 97.4%, respectively. Seed purity of a 100.0% was noted in the all the seed types of *E. curvula,* except in the "plain coat" seed type, which yielded a 98.8% purity. These results are in compliance with Table 4, namely Provisions Relating to Seed Samples, of the PIA no. 53 of 1976, which stipulates the maximum contents of inert matter, other seed and declared weed seeds that may be present in the specific seed lot (Appendix A). If the values given in Table 4 (PIA) are exceeded, the seed lot is regarded as impure.

Table 3.1: Seed purity of enhanced and non-enhanced seed types of the selected grass species (%),★ indicating the seed types with the higher seed purity.

Species	Purity (%)
Chloris gayana (non-enhanced) (*C. gay* N)	96.1
Chloris gayana (enhanced) (*C. gay* E)	★97.6
Cynodon dactylon (non-enhanced) (*C. dac* N)	★100.0
Cynodon dactylon (enhanced) (*C. dac* E)	99.4
Digitaria eriantha (non-enhanced) (*D. eri* N)	★98.3
Digitaria eriantha (enhanced) (*D. eri* E)	97.4
Eragrostis curvula (non-enhanced) (*E. cur* N)	★100.0
Eragrostis curvula (plain coat) (*E. cur* P)	98.8
Eragrostis curvula (enhanced with “organic insecticide on base of coat”) (*E. cur* B)	★100.0
Eragrostis curvula (enhanced with “organic insecticide on base of coat and as overspray”) (*E. cur* O)	★100.0

3.2.2 Seed germination

The seed germination test results are presented in Figure 3.1, as well as in the Seed Analysis Report (Appendix D & E (i) & E (ii)). The germination trials entailed two sets of germination tests. In the first germination test, dormancy breaking treatments were used (ISTA, 2006). The dormancy breaking treatments included the substituting of distilled water with potassium nitrate (KNO_3), where after the seed was pre-chilled at 5°C for five (5) days. The germination tests commenced after placing the seed in the growth chambers. In the second set of germination tests, no dormancy breaking treatments were applied (Figure 3.1).

Dormancy breaking

In the germination tests were dormancy breaking treatments were applied, the following results were obtained (Figure 3.1). The non-enhanced seed types of *Chloris gayana* (28%) had a significantly higher germination percentage ($p<0.001$) than the enhanced seed type of *Chloris gayana* (12%). A significant difference was also noted in the case of the *Cynodon dactylon* seed type, were the germination percentage of the enhanced seed type (17%) was higher in comparison with the non-enhanced seed type (7%) (p=0.006). The enhanced seed type with “organic insecticide on base” and the “plain coat” types showed germination percentages of 47% and 7%, respectively. These germination percentages were both significantly lower than the germination percentage of the non-enhanced seed type (p=0.029, p<0.001) (Appendix F (a)).

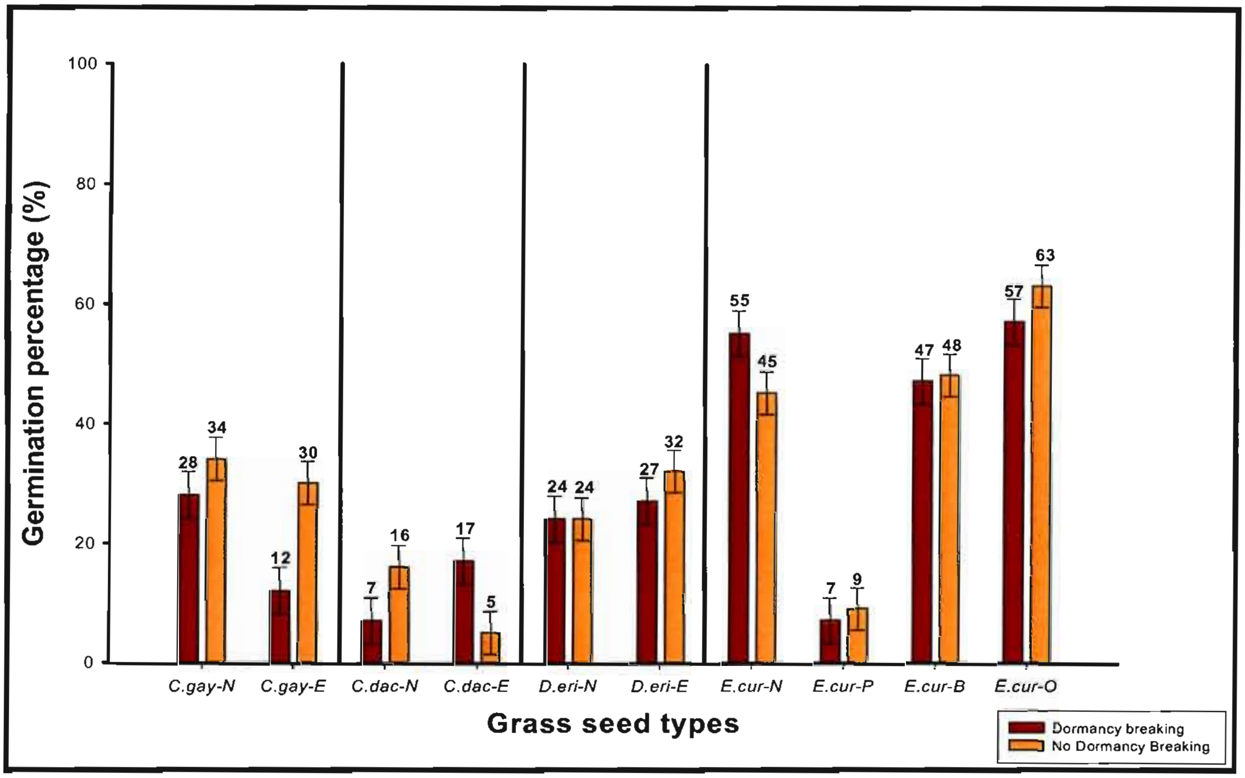


Figure 3.1: Germination results (capacity - %) with (KNO_3) and pre-chilled at 5°C for 5 days and without dormancy breaking for grass seed types of the selected grass species (Abbreviations – Table 3.1).

The germination percentages for *D. eriantha* enhanced and non-enhanced seed types, however did not differ significantly. This was the same in the case of the enhanced *E. curvula* seed type with “organic insecticide on base of coat and as overspray” were the germination percentage of 57% did not differ significantly from the germination percentage of the non-enhanced seed type of the same species.

Germination without dormancy breaking

In the germination trials were the dormancy breaking methods was not used, not all the species showed significant differences in the germination percentages when comparing the non-enhanced seed types of the selected species. A significant difference were however noted between the enhanced and non-enhanced seed types of *Cynodon dactylon,* where the enhanced seed type had a significant lower germination percentage (5%) in comparison with the non-enhanced seed type (16%) ($p=0.011$) (Figure 3.1). In the germination test regarding the *E. curvula* seed types the different enhancements, the seed type enhanced with "organic insecticide on base of coat and as overspray", had a significantly higher germination percentage of 63% in comparison with the non-enhanced seed type (45%) ($p<0.001$), while seeds with an enhancement with "organic insecticide on base" had a germination percentage of 48%, but did not differ statistically from the non-enhanced seed type. The non-enhanced type yielded a germination percentage of 45%, which were significantly higher than the germination percentage of the "plain coat" seed type being only 9% ($p<0.001$) (Appendix F (b)).

The *D. eriantha* enhanced seed type had a germination percentage of 32%, however this was not significantly different from the germination percentage of the non-enhanced seed type (24%).

ASC also carried out some germination trials at their own laboratories (Table 3.2). However, no statistical analysis was carried out on this data, as the aim of the germination tests carried out by the seed company was to verify whether the seed lot was in compliance with the PIA (No. 53 of 1976) (Appendix A – Table 4). The results of the tests carried out at the laboratory of the NWU in this study were compared to the results from ASC.

The data were obtained from Louise Kotze[9] (see 6.2 - Companies and Personal Communications) at ASC and showed that all the non-enhanced seed types for the species tested had higher germination percentages in comparison with the enhanced seed types (Figure 3.2 and Table 3.2). The germination tests carried out by ASC were not subjected to any dormancy breaking methods (Figure 3.2 and Table 3.2).

When comparing the results of the germination test without the use of dormancy breaking methods, obtained by NWU and ASC no corresponding results were evident. The non-enhanced seed type of *Chloris gayana* tested by NWU had a germination percentage of 34%, while the seed tested by ASC's germination percentage was 32%. These germination percentages are both higher in comparison with both the germination percentages of the enhanced seed types of *C. gayana* tested by NWU (30%) and ASC (27%). The non-enhanced seed type of *D. eriantha* tested by the NWU had a germination percentage of 22% in comparison with the percentages obtained from ASC, which was 33%. The enhanced seed type of *D. eriantha* tested by NWU had a germination percentage of 32%, which is much higher in comparison to the germination percentages from ASC (23%).

In the case of the germination tests carried out on the *Cynodon dactylon* and *E. curvula* seed types, a great difference was noted when comparing the results of the NWU and ASC. The germination tests carried out by NWU had a germination percentage of 15% for the non-enhanced seed type of *Cynodon dactylon* while the germination percentage for the enhanced seed type of *Cynodon dactylon* was only 5%. The latter is in contrast with the 70% germination percentage of the enhanced seed type of both *Cynodon dactylon* at a 75% germination percentage and the non-enhanced seed type of *Cynodon dactylon*, also supplied by ASC.

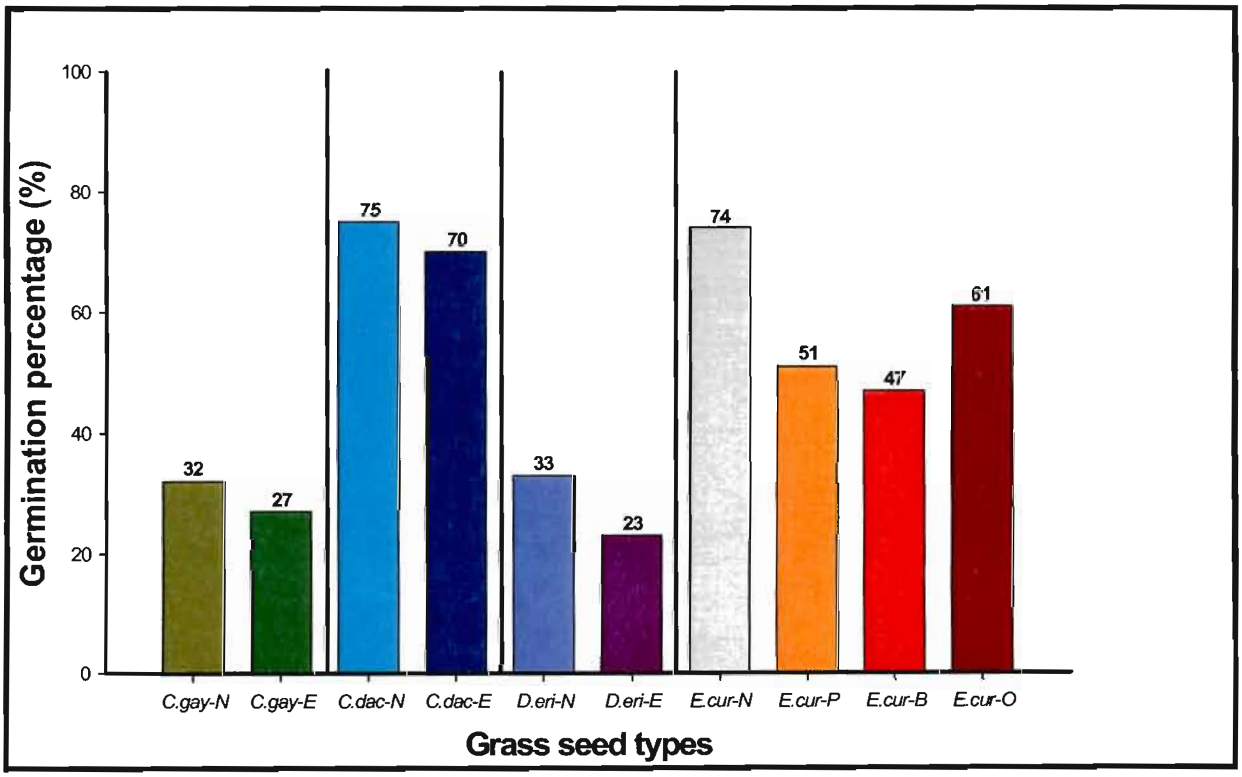


Figure 3.2: Germination results (%) for seed types of the selected grass species as carried out at the laboratories of ASC. (Abbreviations – Table 3.1).

The germination percentage of the non-enhanced seed type of *E. curvula* obtained at NWU was 45%, which is a much lower germination percentage obtained from ASC (74%). The *E. curvula* seed type enhanced with a "plain coating" only had a 9% germination percentage at the NWU, but a germination percentage of 51% in tests carried out by ASC. The germination percentages of *E. curvula* enhanced with "organic insecticide on base of coat", obtained from NWU (48%) and ASC (47%) had similar germination percentages. The *E. curvula* seed type enhanced with "organic insecticide on the base of coat and as overspray" tested by NWU had a germination percentage of 63% and the seed tested by ASC had a germination percentage of 61%.

Although some of the results obtained from ASC and NWU corresponds with each other, the majority of the results do not correlate. This could be as a result of the differences in the testing dates and the possibility of dormancy changing over time. It could also be argued that the storing methods of the seeds tested by ASC and NWU, although from the same batches, differ, for example the seed merchant store the seed in big quantities under a certain temperature, while after the seed was transported and several temperature changes were in countered, the biological viability of the seed could have been altered, hence the differences in the results of ASC and NWU.

According to the data received from ASC, the following seed types of the selected grass species are in compliance with the PIA (No. 53 of 1976) (Appendix A – Table 4): *Chloris gayana* non-enhanced (32%) and enhanced (27%) and *E. curvula* non-enhanced (74%). *Cynodon dactylon* is not listed in the PIA (No. 53 of 1976) and could therefore not be compared. *D. eriantha* non-enhanced (33%) and enhanced (23%), and *E. curvula* "plain coat" (51%), "organic insecticide on base" (47%) and "organic insecticide on base of coat and as overspray" (61%) germination percentages were lower in comparison with the PIA (No. 53 of 1976) (Appendix A: Table 4). In practice, this could have a negative effect on the company selling the seed, for the seed must have a minimum germination capacity before the seed can be commercially sold (ISTA, 2006).

Table 3.2: Germination results (%) obtained from ASC compared to the results obtained for this project as carried out at the NWU laboratories (dormancy breaking and without dormancy breaking) for the seed types of the selected grass species tested, as well as the germination percentage required by the PIA (No. 53 of 1976) (Appendix A: Table 4).

Seed type of the selected grass species	NWU – Dormancy breaking treatment (%)	NWU – No dormancy breaking treatment (%)	ASC (%)	Minimum % Germination PIA (No. 53 of 1976) (Appendix A: Table 4)
Chloris gayana Non-enhanced	28	34	32	20
Chloris gayana Enhanced	12	30	27	20
Cynodon dactylon Non-enhanced	17	15	75	Not listed in PIA (No. 53 of 1976)
Cynodon dactylon Enhanced	7	5	70	Not listed in PIA (No. 53 of 1976)
D. eriantha Non-enhanced	24	22	33	40
D. eriantha Enhanced	27	32	23	20
E. curvula Non-enhanced	55	45	74	70
E. curvula Plain coat	7	9	51	70
E. curvula Enhanced with "organic insecticide on base of coat"	47	48	47	70
E. curvula Enhanced with "organic insecticide on base of coat and as overspray"	57	63	61	70

3.3 Glasshouse trials

3.3.1 Establishment of selected grass seedlings in different growth mediums

The seedling establishment (emergence) was assessed in the glasshouse at NWU. Monitoring of seedlings in both the Hygromix and the Coco Peat Moss growth medium without additional nutrients took place on a weekly basis for a period of seven (7) weeks (Figure 3.3 A).

The average seedling establishment was calculated using the data after the seventh week of the trials. These latter seedlings in the Coco Peat Moss medium only reached a young juvenile stage, after which no significant growth could be noted and no biomass production could be measured.

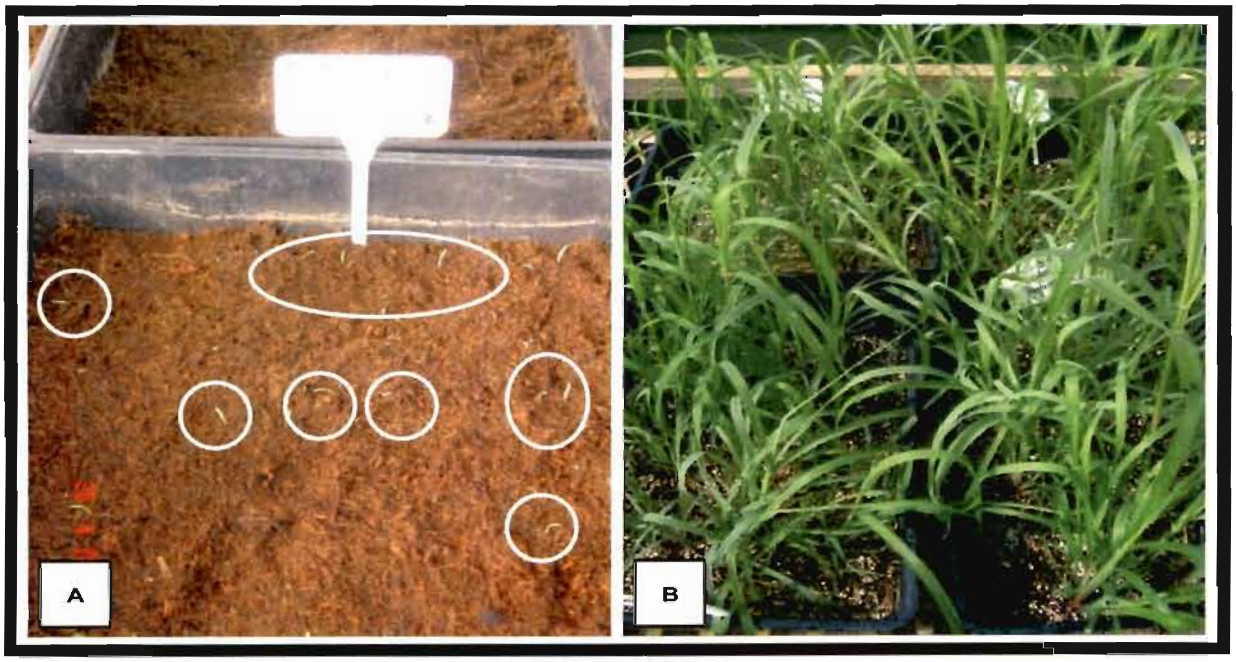


Figure 3.3: **A.** The seedlings (circled in white) of non-enhanced *Chloris gayana* seed type in Coco Peat Moss growth medium 9 days after planting.
B. The seedlings of non-enhanced *Chloris gayana* seed type in Hygromix growth medium approximately one month after the date of planting.

The seedlings that established in the Hygromix growth medium had much better biomass yield and these seedlings were used to determine the biomass of the different species under controlled conditions (Figure 3.3B).

As mentioned, the establishment of the seedlings in the two growth mediums was compared, a higher establishment was noted in the general comparison in the Hygromix growth medium for each of the seed types of the selected grass species, with the exception of both the non-enhanced and enhanced seed types of *Chloris gayana* and *E. curvula* "organic insecticide on base of coat and as overspray". The good growth of the seedlings in the Hygromix growth medium can be attributed to the additional nutrients in the medium; which was absent in the Coco Peat Moss. The Coco Peat Moss, in contrast, required additional amendments to maintain the pH of the growth medium over the growth period but no other nutritional amendments took place except the for correction of the pH .

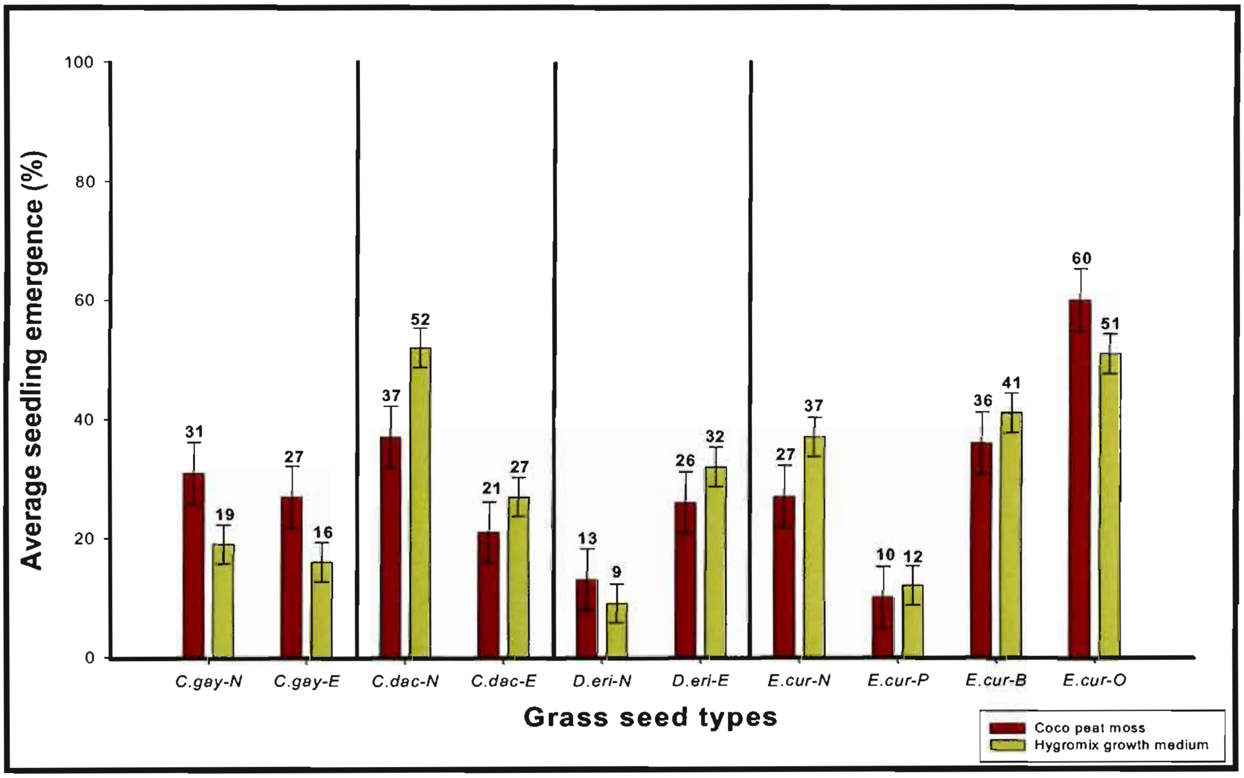


Figure 3.4: Average seedling emergence (%) in Coco Peat Moss and Hygromix growth medium after seven (7) weeks (Abbreviations – Table 3.1).

Seedling establishment percentages in the Coco Peat Moss medium

The non-enhanced seed types of *Cynodon dactylon* had a significantly higher establishment percentages (37%) than the enhanced seed types of these species, with 21% (p=0.005) (Figure 3.4). The *D. eriantha* enhanced seed type had a statistical significant higher establishment percentage of 26% if compared with the non-enhanced seed type (13%) of the same species (p=0.023). The *E. curvula* seed enhanced with "organic insecticide on the base of the coat and as overspray" (60%) showed a significant difference when compared to the non-enhanced seed type (p<0.001). However, the "organic insecticide on base" establishment percentage did not differ significantly from the establishment percentage of the non-enhanced seed type of *E. curvula.* The latter did in fact differ statistically from the "plain coat" seed type, in resulting in significantly higher establishment percentage (p=0.002) (Appendix G (e)).

Seedling establishment percentages in the Hygromix growth medium

Although the non-enhanced seed types of *Chloris gayana* (19%) and *E. curvula* enhanced with "plain coat" had higher establishment percentages in the Hygromix medium than the enhanced seed types with 16% (in the case of the *Chloris gayana* species) and the non-enhanced seed type of *E. curvula*, the differences was however non significant. As the results were in the Coco Peat Moss medium, the non-enhanced seed types of *Cynodon dactylon* and *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" had significant higher establishment percentages in comparison with the enhanced seed type for *Cynodon dactylon* and the "plain coat" seed type for the *E. curvula* (p<0.001 in both instances). The *D. eriantha* enhanced seed type had a significantly higher establishment percentage (32%) in comparison to the non-enhanced seed type (9%) of the same species (p<0.001). As in the Coco Peat Moss medium, the *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" seed type showed a significant higher establishment percentage (51%) than that of the non-enhanced seed type of *E. curvula* (p<0.001) (Appendix G(f)).

Comparison between the two growth mediums

Although the Hygromix and Coco Peat Moss growth mediums varied in composition with regard to the nutrient content, a trend was noted when the establishment of the seedlings of the different seed types were compared (Figure 3.5). When referring to the previous results of both the establishment percentages of the seed types in the Coco Peat Moss medium and the Hygromix growth medium similar statistical significant differences in the results were noted. In both the mediums the non-enhanced seed types of *Cynodon dactylon* and *E. curvula* had a significantly higher establishment percentage in comparison with the enhanced seed type (as in the case of *Cynodon dactylon*) and the "plain coat" seed type were the *E. curvula* species is concerned.

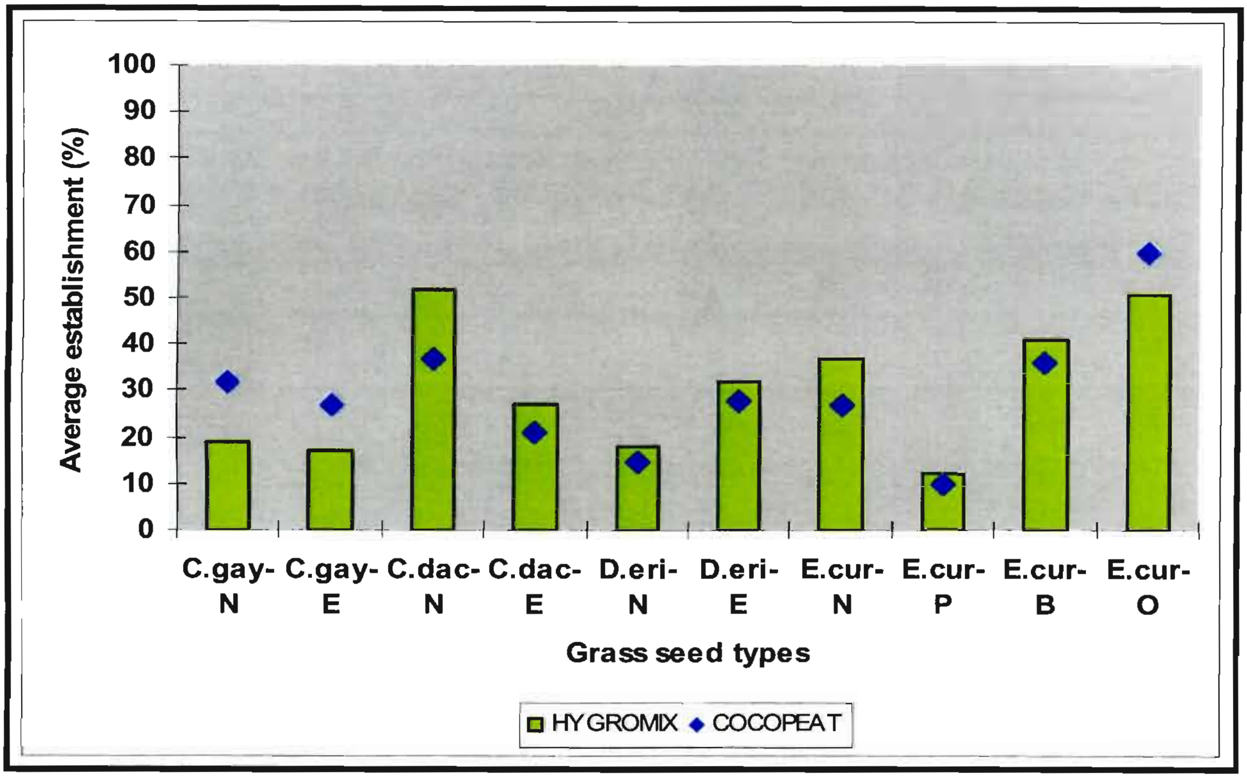


Figure 3.5: A comparison of the seedling establishment (%) of selected grass seed species in the two different growth mediums, namely Hygromix and Coco Peat Moss (Abbreviations – Table 3.1).

The significant difference was repeated in both mediums, were the enhanced seed type of *D. eriantha* and the *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" had higher establishment percentages in comparison with the non-enhanced counterparts. No significant differences were recorded in the comparison of the enhanced and non-enhanced seed types of *Chloris gayana* and the *E. curvula* non-enhanced and enhanced with "organic insecticide on base" seed types.

These results of the establishment percentages correlating in a way of being significantly similar, i.e. that the differences between the establishment percentages of the enhanced and non-enhanced seed types, whether significantly higher or lower, can be indirectly related to the germination capacity of the seed being similar in the different mediums and same conditions. This is however not true for the biomass production experiments, where the seedlings established in the Coco Peat Moss not reaching a seedling state and ultimately not the vegetative state (see 3.3.2 Biomass production).

3.3.2 Biomass production

The seedlings established in the Hygromix growth medium were used to measure the fresh and dry, above- and below-ground biomass production of the seed types of the selected grass species. The fresh biomass of plants is an indication of the condition of a rangeland with regards to esthetical value, and can serve as an attraction to the consumer, which ultimately promoting the dispersal of the caryopsis (or the seed as referred to in the dissertation). The dry biomass is valuable in the sense that it is the material containing the essential nutrients and proteins useful to the livestock (consumer). The dry matter is also used in the calculation of the grazing capacity for the grass species and is important in the management of natural and cultivated areas used as grazing. As for the below ground biomass, the roots help with the compaction and aeration of the soil that is important in sustainable rangelands, cultivated pastures and rehabilitation sites. The fresh above- (FAGBM) and below-ground (FBGBM) biomass production are presented in Figure 3.6 with the dry above- and below-ground biomass production indicated in Figure 3.7. These results were obtained after a four month growth period.

Fresh above- and below-ground biomass

After the four months growing period in the controlled environment the plants from the enhanced seed types of *Chloris gayana* had a higher above-ground biomass (77.12 g) and below-ground biomass (32.97 g) than the plants from the non-enhanced seed types with 64.22 g for the above-ground and 20.83 g for the below-ground biomass production. The FAGBM and FBGBM (25.39 g and 5.12 g, respectively) of the plants of the non-enhanced seed types of *Cynodon dactylon* were higher on average than the fresh above- and below-ground biomass of the plants from the enhanced seed types, with 12.92 g for the above-ground and 3.54 g for the below-ground biomass. The plants of the enhanced seed type of *D. eriantha* only yielded 26.31 g fresh above-ground biomass and 18.56 g fresh below-ground biomass. The fresh above- and below-ground biomass of the plants from the non-enhanced seed type of *D. eriantha* yielded higher biomasses, with 82.78 g and 32.26 g, respectively. The plants from the *E. curvula* non-enhanced seed type (29.36 g) yielded the highest fresh above-ground biomass in comparison with the plants from the enhanced seed types.

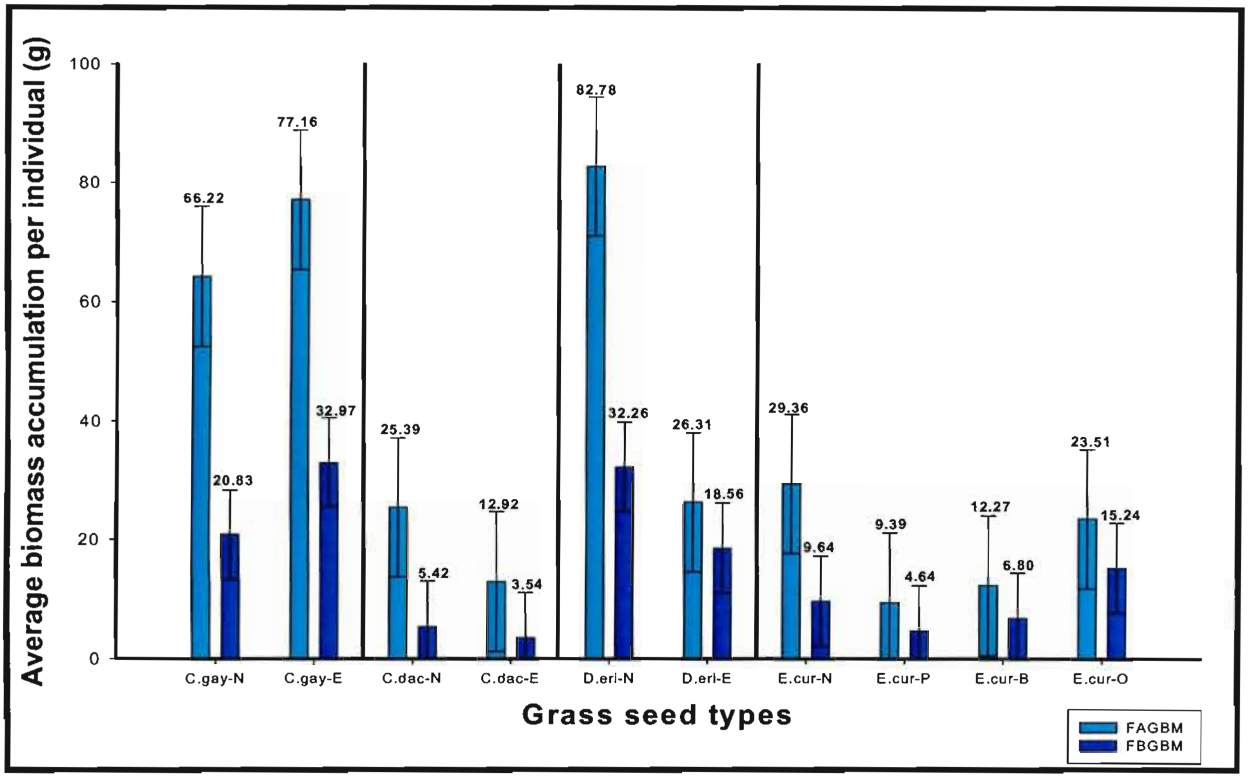


Figure 3.6: The average fresh above-ground (FAGBM) and below-ground (FBGBM) biomass (g) of seed types of the selected grass species after 4 months of growth (Abbreviations in Table 3.1).

The enhancement "organic insecticide on the base of the coat and as overspray" yielded the second highest biomass (23.51 g), followed by the plants from the seed type with "organic insecticide on the base of the coat", yielding 12.27 g, and the "plain coat", yielding the lowest fresh above-ground biomass of 9.39 g. In the case of the fresh below-ground biomass, the plants from the *E. curvula* seed type enhanced with "organic insecticide on the base of the coat and as overspray" (15.24 g) yielded the highest biomass, followed by the plants from the non-enhanced seed type (9.64 g). The plants of *E. curvula* with "organic insecticide on the base of the coat" yielded 6.80 g and the "plain coat" enhancement yielded the lowest biomass, namely 4.64 g. No significant difference was noted at the fresh below-ground biomass production with regards to all the species (Appendix G (a) and G (b)).

Dry above- and below-ground biomass

The average dry above- (DAGBM) and below-ground biomass (DBGBM) production for the seed types of the selected grass species in the glasshouse trials after a growth period of four months are indicated in Figure 3.7.

The average DAGBM and DBGBM (27.55 g and 5.96 g, respectively) for the enhanced seed types of *Chloris gayana* were slightly higher in comparison with that of the non-enhanced *Chloris gayana*, namely 24.37 g and 5.68 g, respectively. The opposite is true for the dry above- and below-ground biomass of *Cynodon dactylon,* where the plants from the non-enhanced seed type yielded a dry above-ground biomass of 7.80 g compared to the 3.93 g of the enhanced seed types. The dry below-ground biomass of the plants of the non-enhanced seed type of *Cynodon dactylon* was 0.99 g, with the enhanced *Cynodon dactylon* plants yielding 0.66 g. The dry above-ground biomass yield of the plants of *D. eriantha* non-enhanced seed type was 27.11 g and that of plants from the enhanced seed type yielded a lower biomass of 15.80 g.

The same trend was noted in the biomass yield of the dry below-ground parts of the plants of the non-enhanced seed type of *D. eriantha*, where the yield was higher (6.11 g) when compared to the enhanced *D. eriantha* (2.86 g).

The plants of the non-enhanced seed type of *E. curvula* yielded the highest dry above-ground biomass (9.92 g). The enhancement with "organic insecticide on the base of the coat and as overspray" yielded the second highest biomass (7.20 g) followed by the enhancement with "organic insecticide on base of coat" (4.71 g). The lowest dry above-ground biomass was the 3.21 g yielded by the plants of *E. curvula* "plain coat" enhancement. The same trend was noted in the case of the dry below-ground biomass of the plants of the *E. curvula* seed types. The non-enhanced plants yielded 1.81 g dry below-ground biomass, followed by the enhancement with "organic insecticide on the base of coat and as overspray (1.49 g). The enhancement with "organic insecticide on base of coat" yielded 1.15 g dry below-ground biomass and again the "plain coat" enhancement yielded the poorest biomass (0.85 g).

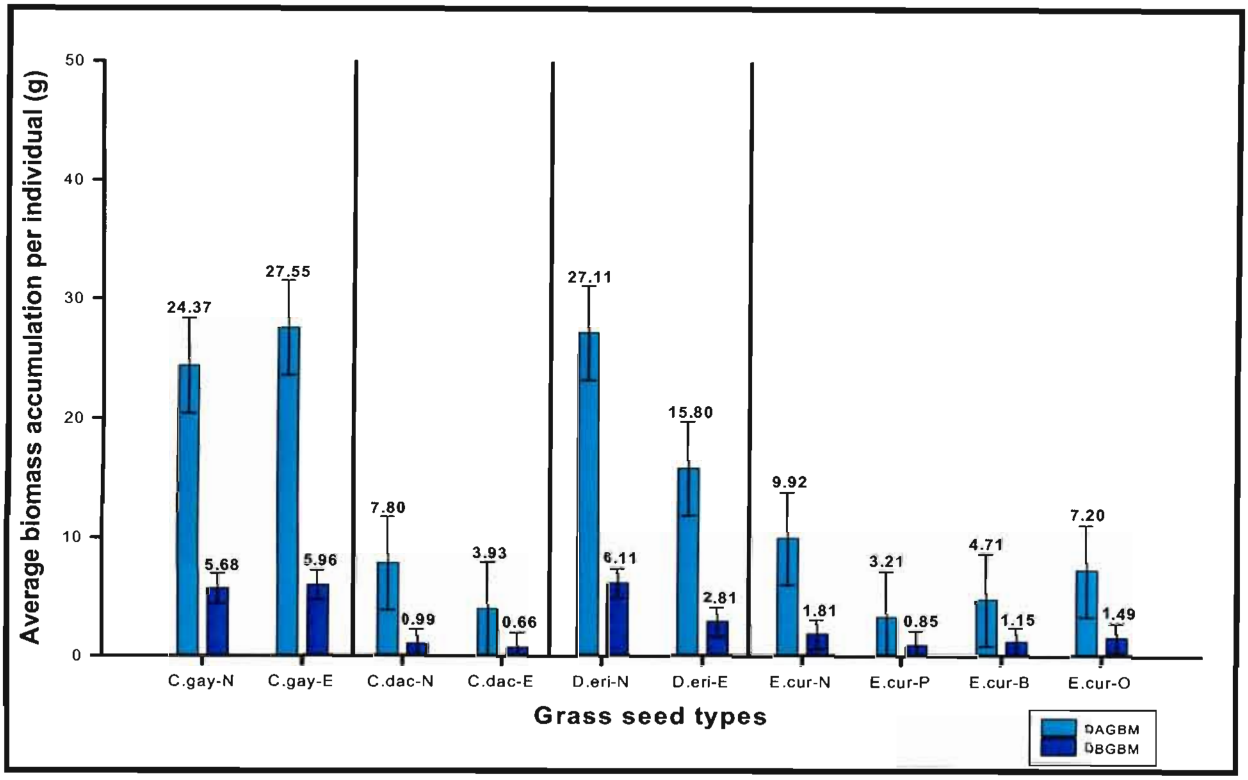


Figure 3.7: The average dry above-ground (DAGBM) and below-ground biomass (DBGBM) (g) of seed types of the selected grass species after 4 months of growth (Abbreviations – Table 3.1).

However, the only significant difference ($p<0.050$) was observed in both the dry above-ground ($p=0.009$) and below-ground ($p=0.016$) biomass of the *D. eriantha* plants. In this case, the plants of the non-enhanced seed types yielded a significantly higher biomass in comparison with the plants harvested from the enhanced *D. eriantha* seed type. In general observation, *C. gayana* was the only species where the plants from the enhanced seed types yielded a higher fresh and dry, above- and below-ground biomass (Appendix G (c) and G(d)).

From the results obtained from the glasshouse trials, similar results was found between the fresh and below-ground biomass of all the seed types, i.e. the fresh above-ground biomass of the enhanced seed type of *Chloris gayana* was higher than that of the non-enhanced seed type of *Chloris gayana* (Figure 3.6). This was also true after the plants were dried for determining the dry belowground biomass.

Drying the plant material is a process to eliminate the water content in the plant, resulting in the dry biomass production (actual proteins and nutrients available for consumption by animals). The above mentioned results indicated that the expected results, i.e. that same trend will exist in the fresh and the dry part of the experiment, is not met. In these seed types, the plants of the *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" had the highest fresh below-ground biomass. However, when the below-ground parts were dried, the roots of the plants of the non-enhanced seed type had the highest dry below-ground biomass. This could be an indication that the water : protein, nutrient-ratio was lower in the non-enhanced *E. curvula* seed type, resulting in the non-enhanced seed type being the better seed type to use, for the useful available proteins and nutrients are more abundant in the non-enhanced seed type of *E. curvula.* Although this could be a reason, further investigation, such as nutrient analysis will bring more clearance to the theory.

3.4 Natural field trials

The natural field surveys were carried out after a 9 month growing period at the experimental farm of the North West DACE, Potchefstroom. The sampling methods are discussed in Chapter 2, Materials and Methods. An example of the datasheet used in the vegetation surveys is included in Appendix H.

3.4.1 Density of the individuals of species

The density of each species was recorded by counting the number of individuals established in three 1 m x 1 m quadrates in each repetition and calculating an average expressed as number of individuals per square metre (Figure 3.8). These averages were converted to number of individuals per hectare (Table 3.3). The average density of the plants from the enhanced seed types of *Chloris gayana* was higher (27 plants/m^2) in comparison with the density of the plants from the non-enhanced seed types (11 plants/m^2). The same was observed for the *Cynodon dactylon* seed types, where the enhanced seed type had a higher density (34 plants/m^2) if compared to the density of the plants from the non-enhanced seed type (8 plants/m^2).

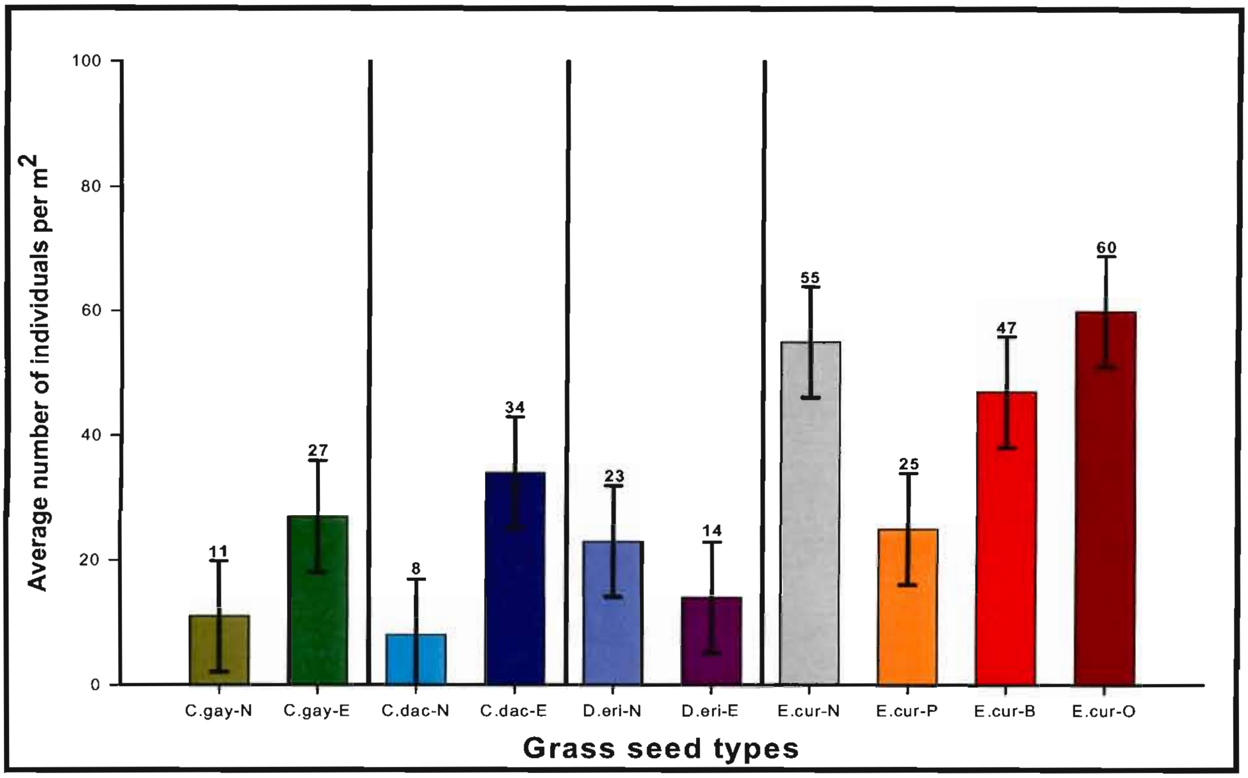


Figure 3.8: Average number of individuals per m² (density) surveyed after 9 months under natural field conditions (See Table 3.1 for abbreviations).

In density surveys carried out in the *D. eriantha* seed types plots, the non-enhanced seed type had a higher density (23 plants/m^2) than the individuals yielded by the enhanced seed type of *D. eriantha* (14 plants/m^2). The *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" had the highest number of individuals per square metre (60 plants/m^2) in comparison with the non-enhanced seed type (55 plants/m^2), enhanced with "organic insecticide on the base of the coat" (47 plants/m^2) and "plain coat" (25 plants/m^2) (Figure 3.8).

For better practical applications, the above plants/m^2 values were converted to give a value reflecting the individuals per hectare (Table 3.3).

The overall density of the plants from the enhanced seed types of all the species had a higher density under the natural field conditions, except for *D. eriantha*, where the density of the plants from the non-enhanced seed type was higher in comparison with the enhanced seed type.

Table 3.3: The average number of individuals per hectare for the seed types of the selected grass species.

Seed type of the selected grass species	Average number of individuals/ha
Chloris gayana (non-enhanced)	110 000
Chloris gayana (enhanced)	270 000
Cynodon dactylon (non-enhanced)	80 000
Cynodon dactylon (enhanced)	340 000
D. eriantha (non-enhanced)	230 000
D. eriantha (enhanced)	140 000
E. curvula (non-enhanced)	550 000
E. curvula (plain coat)	250 000
E. curvula (base and overspray)	470 000
E. curvula (overspray)	600 000

Although the results are given above, the only significant differences, however, were observed between the density of the plants from the *Cynodon dactylon* enhanced seed type ($p=0.007$) and the density of the plants from the non-enhanced seed type of *D. eriantha* ($p=0.003$) (Appendix I (b)).

3.4.2 Frequency

The results of the frequencies of the different grass species which were tested are given in Figure 3.9.

Higher average frequencies were observed for the plants from the enhanced seed types of both *Chloris gayana* (87%) and *Cynodon dactylon* (91%) (Figure 3.9). The difference between the frequencies of the plants from the *Chloris gayana* enhanced and non-enhanced seed types were 17%, while the difference in frequencies between the *Cynodon dactylon* enhanced and non-enhanced seed types was even greater at 28%.

Both the plants from the *D. eriantha* and *E. curvula* non-enhanced seed types had higher frequencies (78% and 94%, respectively) in comparison with the enhanced seed types of *D. eriantha* (68%) and all the enhancement treatments of *E. curvula* (84%), respectively. (Appendix G).

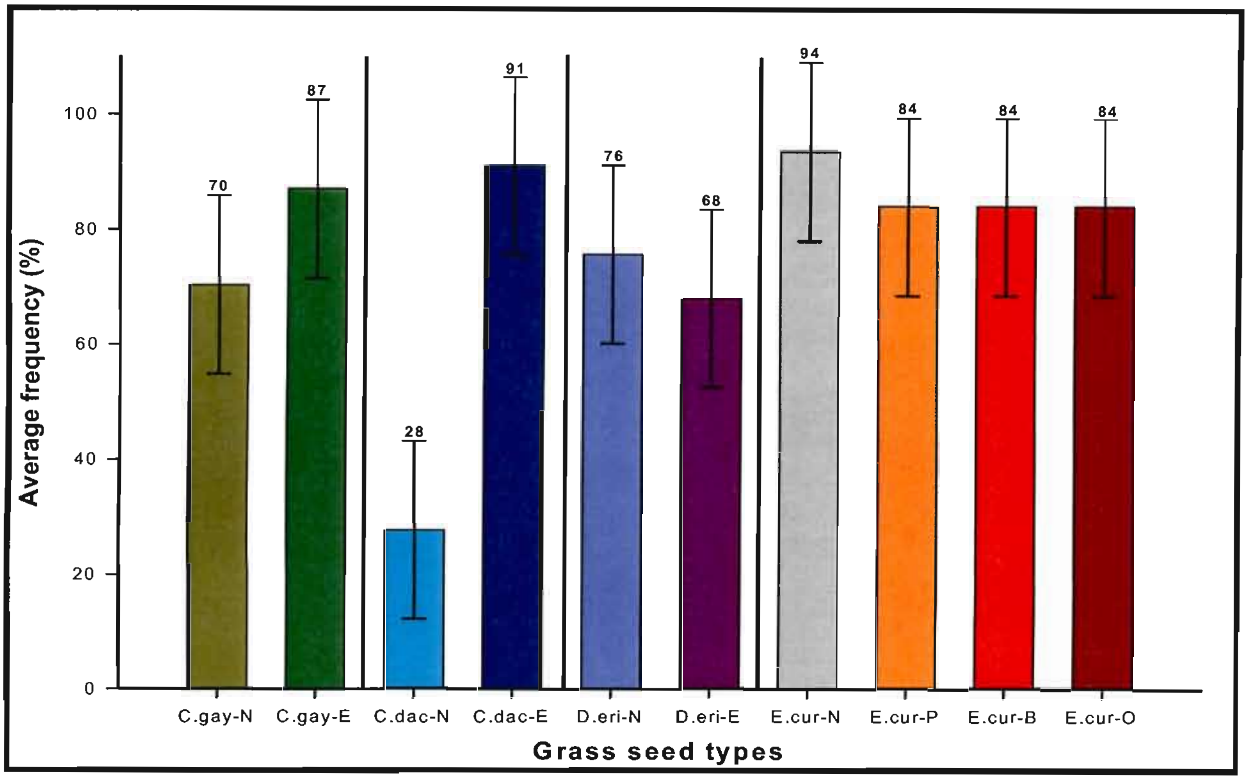


Figure 3.9: Average frequency (%) of the selected grass species surveyed after 9 months under natural field conditions (Abbreviations of species see Table 3.1).

With regards to the frequency surveys, no significant differences were observed between the *E. curvula*, *Chloris gayana* and the *D. eriantha* seed types. The only significant difference between the enhanced and non-enhanced seed types was evident in the results obtained for the *Cynodon dactylon* grass species, where the enhanced seed type had a significantly higher frequency in comparison with the non-enhanced seed type ($p<0.001$) (Appendix I (a)).

When comparing the average density of the plants in the plots with the average frequency, a trend can be seen for all the seed types, except at the *E. curvula* seed types (Figures 3.8 and 3.9). In the density survey, the plants of the *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" had the highest density of all the treatments of this species. In contrast, the plants from the non-enhanced seed type of *E. curvula* had the highest frequency, and all the treated seed had the same average frequency.

3.4.3 Basal cover

Basal hits are an indication of the basal cover of the grass tufts and can be used in biomass (Figure 3.10) and grazing capacity calculations and to determine the Importance Value of the species (Table 3.6). Although only slightly, the basal cover of the non-enhanced seed types of *Chloris gayana* (11%), *D. eriantha* (14%) and *E. curvula* (26%) was higher compared to the enhanced seed types of these species. The enhanced seed types of *Chloris gayana* had the lowest basal cover (9%), followed by *D. eriantha* (12%) and *E. curvula* "organic insecticide on the base of the coat and as overspray" (11%). The *E. curvula* enhanced with "organic insecticide on the base of the coat" and "plain coat" had higher basal cover (15% and 17%, respectively) compared to the former seed types. The basal cover for the *Cynodon dactylon* plants of the enhanced seed types (15%) was much higher than for the non-enhanced seed types (0.33%). This can be attributed to the small tuft size of *Cynodon dactylon*'s growth forms (Appendix G). However, no significant differences were noted after statistical analysis of the data, for any of the seed types (Appendix I (d)).

A certain trend existed between the density, frequency and basal cover results (Figures 3.8 to 3.10). In the case of the *Chloris gayana* seed types, the plants from the enhanced seed type had a higher density and frequency in comparison with the plants from the non-enhanced seed type. This was, however, not the case in the basal cover results of this species, where the species from non-enhanced seed types had a higher basal cover in comparison with the enhanced seeded species. A trend was noted between the density, frequency and basal cover from the seed types of the *Cynodon dactylon* and *D. eriantha* species, which was not the case for the different *E. curvula* treatments. The results of the natural field trials showed that the plots with the enhancement with "organic insecticide on the base of the coat and as overspray" had the highest density, the same frequency as the rest of the treatments, but the lowest basal cover. The plots of the non-enhanced *E. curvula* had the highest frequency and basal cover.

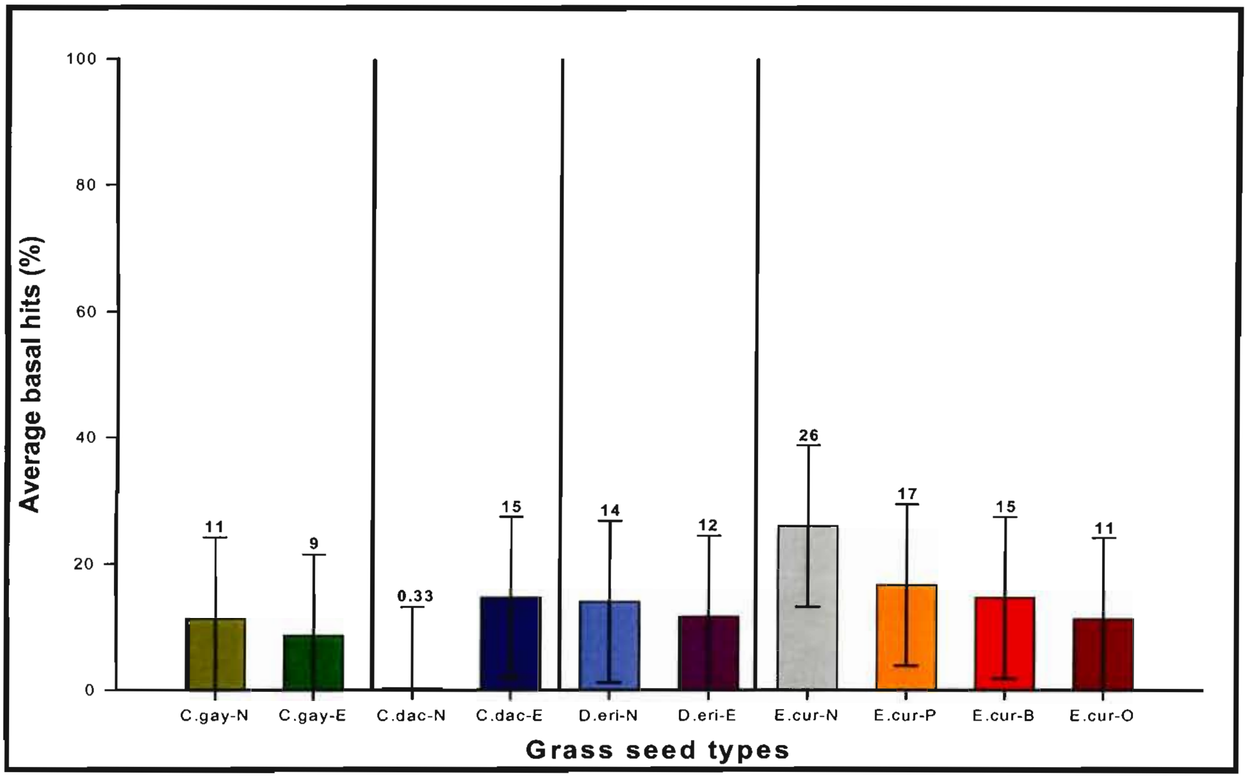


Figure 3.10: Average basal cover (%) per plot surveyed after 9 months under natural field conditions (See Table 3.1 for abbreviations).

It therefore seems that the basal cover, representing the tuft sizes of the grass species, was lower in most species cultivated from enhanced seed.

3.4.4 Abundance of weeds

The abundance of weeds was determined in the plots to establish whether any competition might have occurred with the growth of the different grass species.

Table 3.4: List of weeds observed in the natural field trials.

	Weed species
a.	*Amaranthus viridis*
b.	*Chenopodium album*
c.	*Conyza canadensis.*
d.	*Digitaria sanguinalis*
e.	*Oxygonum dregeanum* var. *canescens*
f.	*Pseudognaphalium luteo-album*
g.	*Schkuhria pinnata*
h.	*Trifolium repens*
i.	*Verbena bonariensis*

The different species of weeds were not species specific recorded. Table 3.4, however lists all the types of weed species that occurred in the natural field trials. The total abundance of the weeds per sample plot is described.

As expected, in the plots where the density and frequency of the seed type of the selected grass species was high, the average frequency of the weeds was low, and vice versa. This is due to the competition factor between the grass and the weed species. The higher the weed abundance, the lower the frequency and density values for the grass species (Figures 3.8 & 3.9).

Weeds in the plots cultivated with non-enhanced and enhanced *Chloris gayana* seed types

In the plots cultivated with the non-enhanced seed type of the *Chloris gayana*, the average frequency of weeds per plot was 29% (Figure 3.11). A lower frequency of weeds occurred in the plots of non-enhanced *Chloris gayana* species which had a higher average density and frequency (Figure 3.9).

The average frequency of weeds in the plots with enhanced *Chloris gayana* (13%) was lower in comparison with the average frequency of weeds in the non-enhanced seeded type plots. This result is also comparable with the high frequency of the enhanced seed type (87%) in the respective plots (Figure 3.9).

Weeds in the plots cultivated with non-enhanced and enhanced *Cynodon dactylon* seed types

The average frequency of weeds in the plots with *Cynodon dactylon* non-enhanced seed type was statistically significant higher (72%) in comparison with that in the enhanced seed type (9%) ($p<0.001$) (Figure 3.11 & Appendix I (c)). The average frequency of the plants of the enhanced seed type was much higher in comparison with the plants from the non-enhanced seed type. This is a logical trend when comparing the average density and frequency of a species and the abundance of weeds in a certain area.

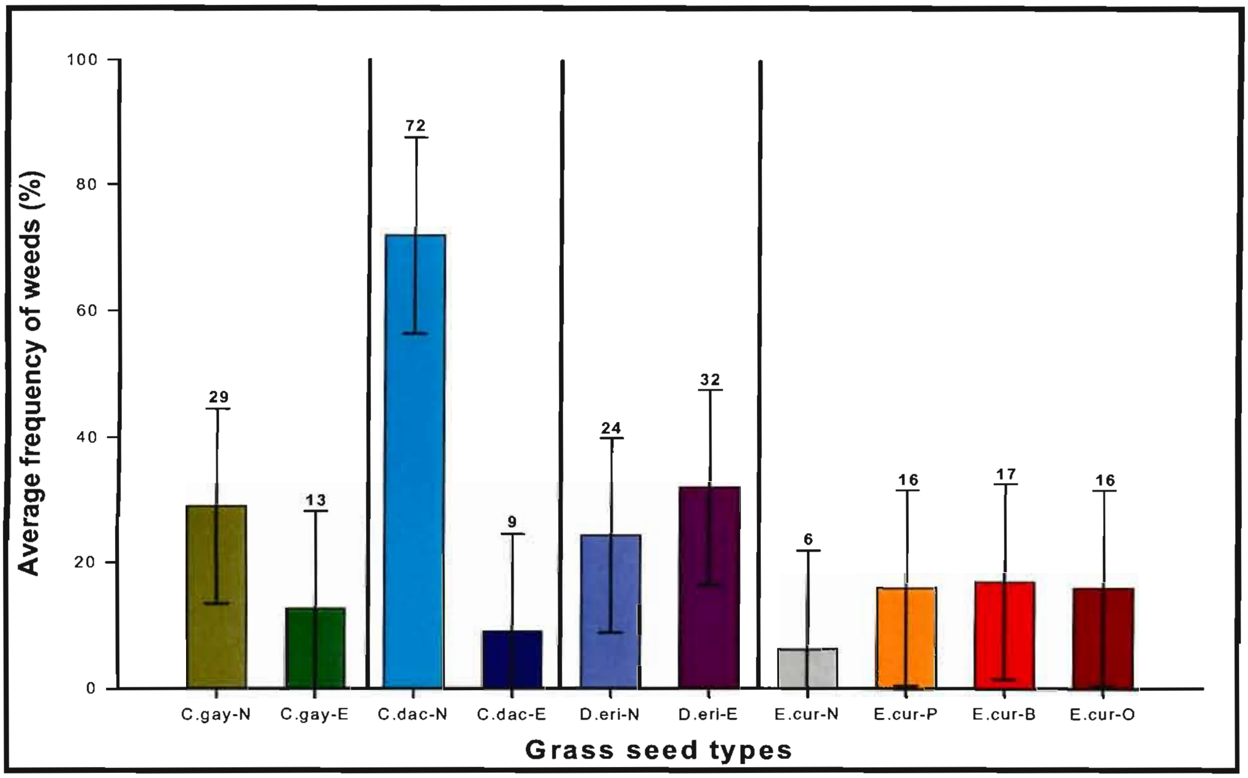


Figure 3.11: Average frequency of weeds (%) per plot surveyed after 9 months under natural field conditions (See Table 3.1 for abbreviations).

Weeds in the plots cultivated with non-enhanced and enhanced *D. eriantha* seed types

The plots of enhanced *D. eriantha* seedling types had a higher average frequency of weeds species (32%) present in the plot when comparing the results with those obtained in the plots of non-enhanced *D. eriantha* (24%) (Figure 3.11). The high frequency of weeds occurring in the plots cultivated with the enhanced seed type reflects the percentage of competition between the weeds and the grasses, as well as the lower density, frequency and basal cover in the plots which is evident in Figures 3.8 to 3.11.

Weeds in the plots cultivated with non-enhanced and enhanced *E. curvula* seed types

E. curvula enhanced with "organic insecticide on base of the coat", "organic insecticide on the base of the coat and as overspray" and "plain coat" had a higher frequency of weeds present within the plot (17%, 16% and 16%, respectively), which may contribute to the lower occurrence of the species in these plots (Figure 3.9). The average frequency of weeds was higher in comparison with the average frequency of weeds in the plots cultivated with the non-enhanced seed type (6%). With regards to the density of the plants in the *E. curvula* plots, the highest density was noted at the plants from the seed type enhanced with "organic insecticide on the base of the coat and as overspray", followed by the plants of the non-enhanced seed type, the enhancement with "organic insecticide on base of the coat" and then the "plain coat" enhancement. No correlation exists between the average frequency and density, although the average frequency of weeds correlates with the frequency of the *E. curvula* data.

The only seed types where the average frequency of the weeds differed statistically were for *Cynodon dactylon*, where in the plots cultivated with the non-enhanced seed type had a statistically higher weed frequency in comparison with the enhanced seed type of the same species ($p<0.001$). The results for the other species did not differ statistically.

3.4.5 Dry matter production

As described in Chapter 2 (Materials and Methods), the dry matter production was determined by randomly placing three, 1 m x 1 m quadrates per plot and harvesting the plant material approximately 5 cm above the ground. The plant material was dried for 24 hours at 60°C and subsequently weighed. The results were expressed as grams per square metre (Figure 3.12) as well as in kilograms per hectare (Table 3.5).

<u>The dry matter production in the plots with non-enhanced and enhanced *Chloris gayana* seeded plants</u>

The average biomass yield of the plants in the plots with enhanced *Chloris gayana* seed (226.07 g) was statistically ($p=0.008$) higher than the biomass yield of the plants of the non-enhanced seed type of *Chloris gayana* (50.48 g) (Figure 3.12). These results correlate with the results obtained in the density and frequency surveys, where the density and frequency of the plants in the plot with enhanced seed type of *Chloris gayana* were higher in comparison with the plants in the non-enhanced seed type plots. Another trend can be made with regards to the average frequency of weeds in these plots. The average frequency of weeds was lower in the plot with enhanced seed types than in the plots with non-enhance seed types, relating to the competition between the selected species and the weeds (Appendix I (e)).

The dry matter production in the plots with non-enhanced and enhanced *Cynodon dactylon* seeded plants

The plants from the *Cynodon dactylon* non-enhanced seed type (15.34 g) had a lower dry matter production in comparison with the enhanced seed type (40.25 g) (Figure 3.12). The same trend was noted in the average density and frequency, basal cover and average frequency of weeds for the different seed types. The dry matter production of the plants had no statistical difference when comparing the enhanced and non-enhanced seed types.

The dry matter production in the plots with non-enhanced and enhanced *D. eriantha* seeded plants

The non-enhanced seed type of *D. eriantha* (123.96 g) was the only seed where the plants of the non-enhanced seed type had a higher dry matter production than the enhanced seed type. The dry matter production of the plants of the enhanced seed type of *D. eriantha* was 65.63g (Figure 3.12). The higher dry matter production of the plants from the non-enhanced seed types in comparison with the plants from the enhanced seed types correlates with the higher density, frequency and basal cover and the lower average frequency of weeds obtained from the surveys.

The dry matter production in the plots of non-enhanced and enhanced *E. curvula* seeded plants

The *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" yielded 248.28 g of dry matter, followed the non-enhanced seed type with a slightly higher biomass production (220.98 g) than the enhancement with "organic insecticide on base of the coat", yielding 213.27 g dry matter. The "plain coat" seed type of *E. curvula* yielded the lowest biomass production (187.57 g).

Overall, the enhanced seed types of all the species had a higher dry matter production under the natural field conditions, except for the *D. eriantha* seed types, where the dry matter production of the non-enhanced seed type was higher. The only significant differences, however, were observed in the *Chloris gayana* and *Cynodon dactylon* seed types.

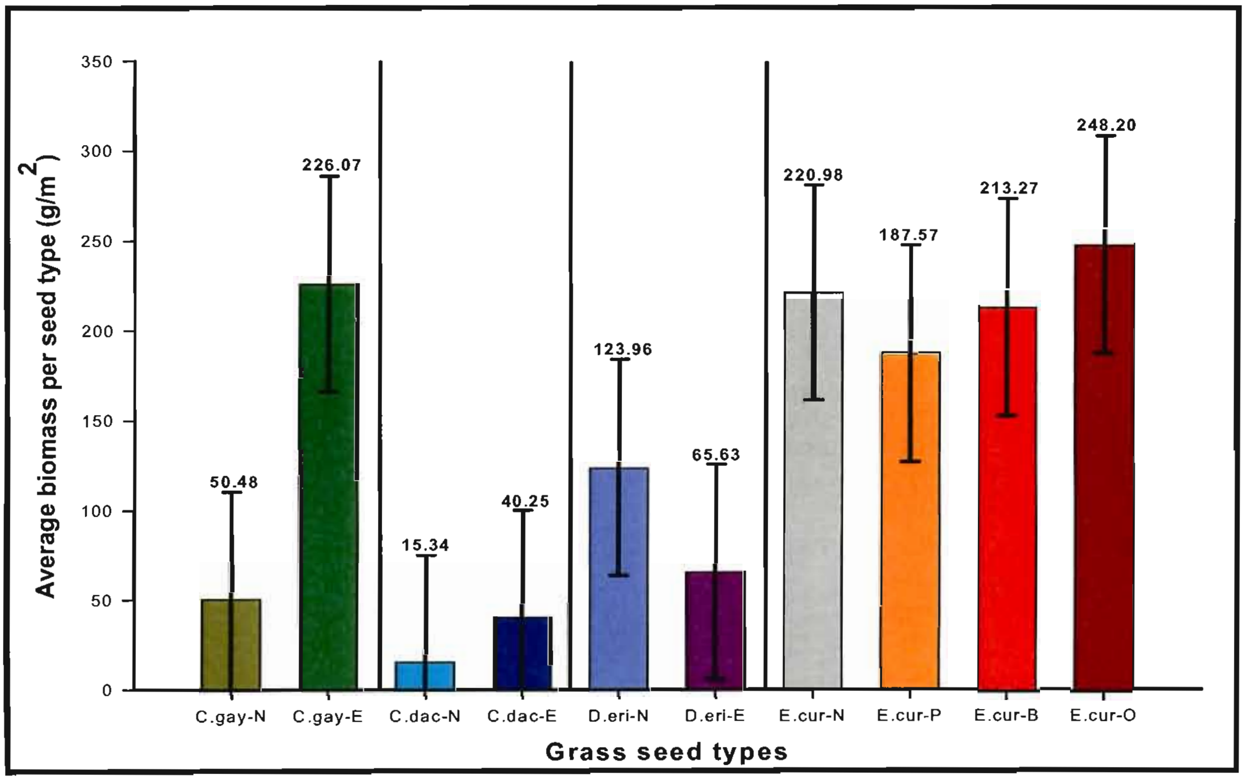


Figure 3.12: Average biomass (g) per m² surveyed after 9 months under natural field conditions (Abbreviations – see Table 3.1).

The dry matter production in g/m² (Figure 3.12) was converted to kg/ha (See Chapter 2 for conversion). These values are given in Table 3.5. According to Table 3.5, the highest dry matter production per hectare was obtained for the plants from the enhanced seed types of *Chloris gayana* (2261 kg/ha) and *E. curvula* enhanced "organic insecticide on the base of the coat and as overspray" (2483 kg/ha).

Table 3.5: The average dry matter production per seed type of the selected grass species expressed as kilograms per hectare.

Seed types of the selected grass species	Dry matter production (kg/ha)
Chloris gayana (non-enhanced)	505
Chloris gayana (enhanced)	**2261**
Cynodon dactylon (non-enhanced)	153
Cynodon dactylon (enhanced)	**403**
D. eriantha (non-enhanced)	**1240**
D. eriantha (enhanced)	656
E. curvula (non-enhanced)	2210
E. curvula (plain coat)	1876
E. curvula (base and overspray)	2133
E. curvula (overspray)	**2483**

There was a trend between the average density of the enhancements and the dry matter production, where at the plots with the *E. curvula* seeded plants, the highest density was noted at the plants from the seed type enhanced with "organic insecticide on the base of the coat and as overspray", followed by the plants of the non-enhanced seed type, the enhancement with "organic insecticide on base of the coat" and then the "plain coat" enhancement. No trend existed between the average frequency and dry matter production. The average frequency of weeds correlated with the frequency of the *E. curvula* data, but not with the dry matter production or density data.

3.4.6 The Importance Value

The Importance Value (IV) of a species is calculated by the sum of the relative density, relative dominance (defined as the mean basal area per species) and relative frequency (Kent & Coker, 2003). When the importance value of a species is known, the species or the treatments can be arranged in a sequence in the order of importance indicating the best possible seed type of the selected grass species to be used in re-seeding practices.

The quantitative factors leading to the importance value, either density, frequency and/or basal cover can also be determined for each grass species (Table 3.6).

The enhanced seed type of *Chloris gayana* had a higher importance value (123) in comparison with the non-enhanced seed type (92). This is also true with regards to the seed types of *Cynodon dactylon*, where the enhanced seed type had an importance value of 140 and the non-enhanced seed type had an importance value of only 36.33 (Table 3.6).

When ranking the importance value of each species in sequence, the non-enhanced seed type of *E. curvula* was ranked at number one, with the highest importance value (175), and the non-enhanced seed type of *Cynodon dactylon* was ranked the lowest, with an importance value of 36.33.

The non-enhanced seed type of *E. curvula,* which had the highest IV (175), also had the highest relative basal cover, density and frequency, except for the relative density of the

E. curvula enhanced with "organic insecticide on the base of the coat and as overspray" with 60 plants/m².

Table 3.6: The importance values of the grass species, calculated as the sum of the relative density, relative frequency and relative basal cover.

Seed type of the selected grass species	Relative Basal cover	Relative Density	Relative Frequency	Importance Value	Importance Ranking (all species)
Chloris gayana N	11.00	11.00	70.00	92.00	9
***Chloris gayana* E**	**9.00**	**27.00**	**87.00**	**123.00**	**6**
Cynodon dactylon N	0.33	8.00	28.00	36.33	10
***Cynodon dactylon* E**	**15.00**	**34.00**	**91.00**	**140.00**	**4**
***D. eriantha* N**	**14.00**	**23.00**	**76.00**	**113.00**	**7**
D. eriantha E	12.00	14.00	68.00	94.00	8
***E. curvula* N**	**26.00**	**55.00**	**94.00**	**175.00**	**1**
E. curvula P	17.00	25.00	84.00	126.00	5
E. curvula B	15.00	47.00	84.00	146.00	3
E. curvula O	11.00	60.00	84.00	155.00	2

The overall IV for *E. curvula* "organic insecticide on the base of the coat and as overspray" was also high (155), with high frequency values, but lower basal cover. The lowest IV was calculated for the non-enhanced *Chloris dactylon* seed type of which the relative basal cover, density and frequency had the lowest values of all the grass seed types.

Hence the results from calculating the Importance Value, *E. curvula* non-enhanced seed type (1), *E. curvula* "organic insecticide on base and as overspray" (2) and *E. curvula* seed type enhanced with "organic insecticide on base" (3) are recommended for ecological amendments with regards to cultivated pastures and restoration and rehabilitation of degraded rangelands and mining areas. This is only based on the relative basal cover, relative density and relative frequency of the species and the biomass production should also be an important factor to consider when deciding on a relative grass species to use in these matters.

3.4.7 Soil analyses

Soil analyses were carried out in 2005 and as recommended by soil specialist no soil amelioration was needed. Soil analyses were again carried out by the NWU in June 2007, after a year of plant establishments. The summary of the type of analysis and results for the soil analyses are given in Table 3.7.

Table 3.7: Soil analyses carried out by the NWU in 2007. NWU 1 representing replicate plot 1, NWU 2 representing replicate plot 2 and NWU 3 representing replicate plot 3 (Figure 2.9 – Chapter 2).

SAMPLE / TYPE OF ANALYSIS			2007 NWU 1	2007 NWU 2	2007 NWU 3
pH (KCl)			6.16	6.54	6.41
P	(Bray 1)	mg/kg	3.74	5.18	12.23
K	(NH_4 Ac)	mg/kg	100.50	95.50	252.00
Ca	(NH_4 Ac)	mg/kg	1066.00	1323.50	1529.50
Mg	(NH_4 Ac)	mg/kg	462.50	651.00	716.00

As mentioned in Chapter 2 (Materials and Methods) these analyses included pH (KCl), the quantity of phosphorous (P) (Bray 1), as well as the quantity of potassium (K), calcium (Ca) and magnesium (Mg) (Ammonium Acetate test) which were carried out.
The soil analyses showed after a year of plant establishment the pH (KCl), Ca and Mg content of the soil is within acceptable range for cultivated lands. A drastic fluctuation was noted in the replicate plots for the P and K contents in the soil (Table 3.7), where the quantity of phosphorous increased from replicate plot 1 to 3, and major differences exist between replicate plot 1 and replicate plot 3, and replicate plot 2 and replicate plot 3. After the establishment of a cultivated land, it is not applicable to make soil ameliorations to alter the nutrient content. For example, in the case phosphate, if amelioration takes place, the phosphate will not be mixed thoroughly in to the soil, because of the established plants. The phosphate content in the soil range from 3.74 – 12.23 mg/kg, which is much too low, for the minimum level of phosphate required in the soil is 25 mg/kg.

Nutrient requirements for each species are as follows:

Chloris gayana is a good grower in various types of soil, including light loamy sand texture to soil with a heavy texture (Dickson *et al.*, 2004). The most suitable pH level for the *Chloris gayana* is a pH of 5.5 – 7.0 (KCl) and a phosphorus (P) level of 15 mg P/kg must be maintained.

According to Dickson *et al.* (2004), little is known about the pasture values and nutrient needs of *Cynodon dactylon* (indigenous couch grass).

In the case of *D. eriantha*, growth can take place in all types of soil (Buys, 2002). Liming must be applied if the pH (KCl) is lower than 4.5, which was not the case in these soils. As with the *Chloris gayana* species, a potassium (P) level of 15 mg P/kg must be maintained on dry land pastures (Dickson *et al.*, 2004).

E. curvula prefers sandy soils, but can grow on many soil types, including acid soils. Liming of the soil is only necessary if the calcium (Ca) and magnesium (Mg) content of the soil is low. Phosphorus (P) is very important to this grass, especially for root development, and the levels must be kept between 15 and 20 mg P/kg, depending on the rainfall in the area. Potassium (K) levels must not be less than 100 mg K/kg when the grass is planted.

Although the three replicate plots were situated in close proximity to each other, the soil composition and drainage (run-off) could have had an influence on the results obtained in the project. The differences would have been minimal, as all the plots were more or less on a flat surface. These minor differences between the plots were not considered during the analysis.

3.5 Multivariate Analysis

The Principal Component Analysis (PCA) ordination was used to determine correlations between the enhanced and non-enhanced seed types of the selected grass species used in this study (Ter Braak, 1988).

The Centred and Standardised Principal Component Analysis (PCA) ordination was carried out on the fresh and dry above- and below-ground biomass production, which took place in the Hygromix growth medium under controlled conditions (Figure 3.13).

3.5.1 Dry matter production under controlled conditions

All the biomass parameters for the above-ground and below-ground, fresh and dry biomass are strongly positive correlated to each other (Figure 3.13).

Although the fresh above- and dry above- and below-ground biomass parameters were slightly more correlated in comparison with the fresh below-ground biomass, the PCA ordination carried out on the biomass production (Figure 3.13) indicated a strong positive correlation between all these parameters.

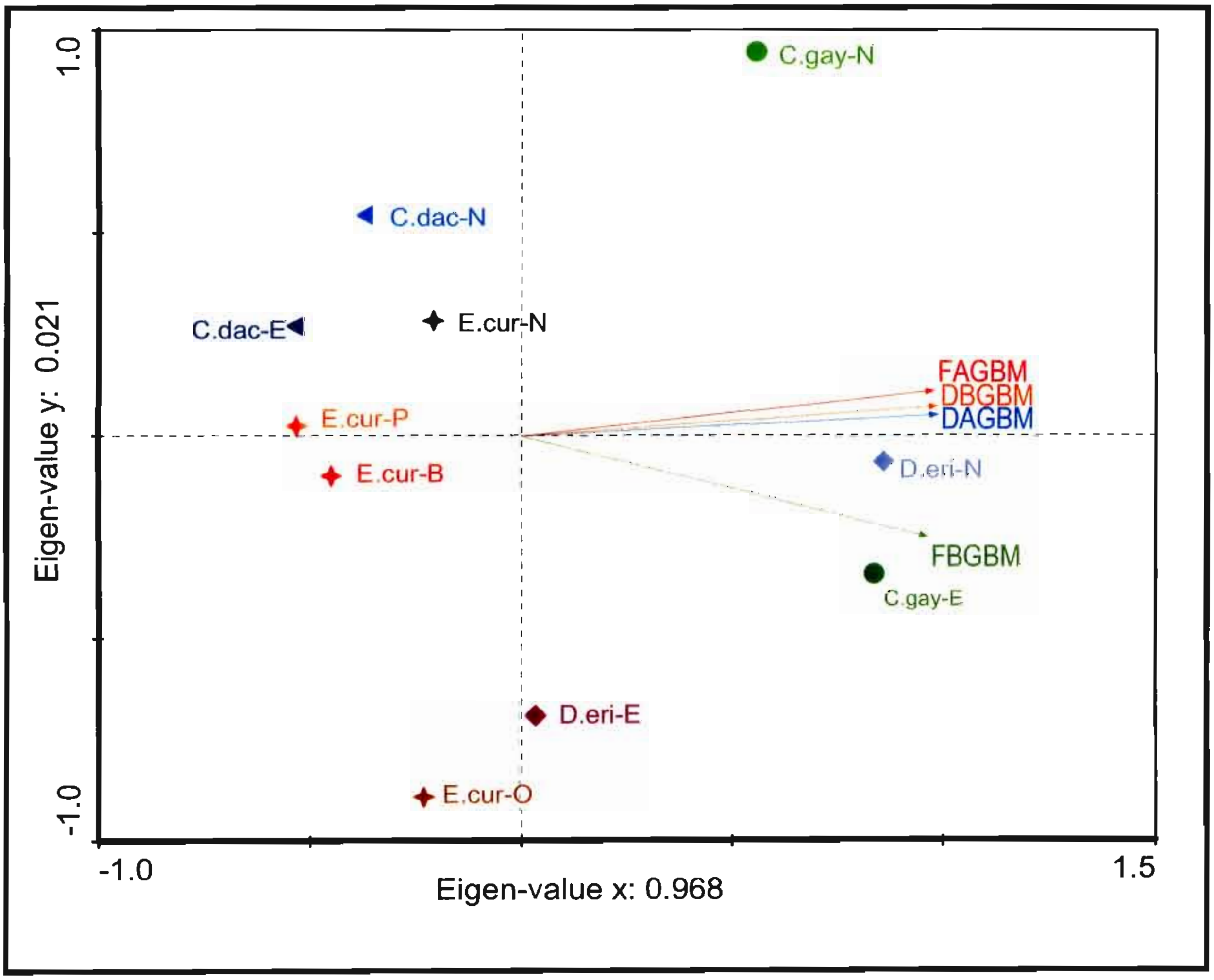


Figure 3.13: The Principal Component Analysis (PCA) of the fresh and dry, above- and below-ground biomass production of each of the seed types of the selected grass species. The Eigen values for the X-axis is 0.968 and for the Y-axis 0.021. (FAGBM – Fresh above-ground biomass, DAGBM – Dry above-ground biomass, FBGBM – Fresh below-ground biomass, DBGBM – Dry below-ground biomass) (See Table 3.1 for species abbreviations).

A strong positive correlation is evident in Figure 3.13, where *D. eriantha* non-enhanced and *Chloris gayana* enhanced correlated with all the parameters of the above and below, fresh and dry biomass. This correlation was confirmed by the high biomass values as indicated in Figures 3.6 and 3.7. In Figure 3.6, *D. eriantha* non-enhanced seeds yielded the highest fresh above- and below-ground biomass and *Chloris gayana* enhanced seeds the second highest. The *Chloris gayana* enhanced seed type yielded the highest dry above- and below-ground biomass (Figure 3.7) and *D. eriantha* non-enhanced produced the second highest biomass.
All the other seed types of the selected grass species had a fairly low fresh and dry, above- and below-ground production, which is evident in the negative correlation showed in Figure 3.13.

The non-enhanced seed type of *D. eriantha* had the strongest correlation towards the parameters, and this was in compliance with *D. eriantha* non-enhanced seed type being the only seed type with a statistically significant difference of the dry above- and below-ground biomass when comparing it to the enhanced seed type of *D. eriantha.*

3.5.2 Natural field trials

The results of the PCA-ordination indicated a good correlation of all the vegetation surveys where the density, frequency, number of basal hits, as well as dry matter production were determined by the natural field trial surveys. *E. curvula* non-enhanced seed types had a strong positive correlation with the number of basal hits and frequency surveys, which corresponds to the value given in Figures 3.8 – 3.10.

The *E. curvula* seed type enhanced with "organic insecticide on the base of the coat" had a strong positive correlation with the dry matter production and density determinations. This corresponds to the values given in Figures 3.8 – 3.10 and 3.12, where the *E. curvula* "organic insecticide on the base of the coat and as overspray" seed type yielded the highest biomass and had the highest density in comparison with the other seed types.

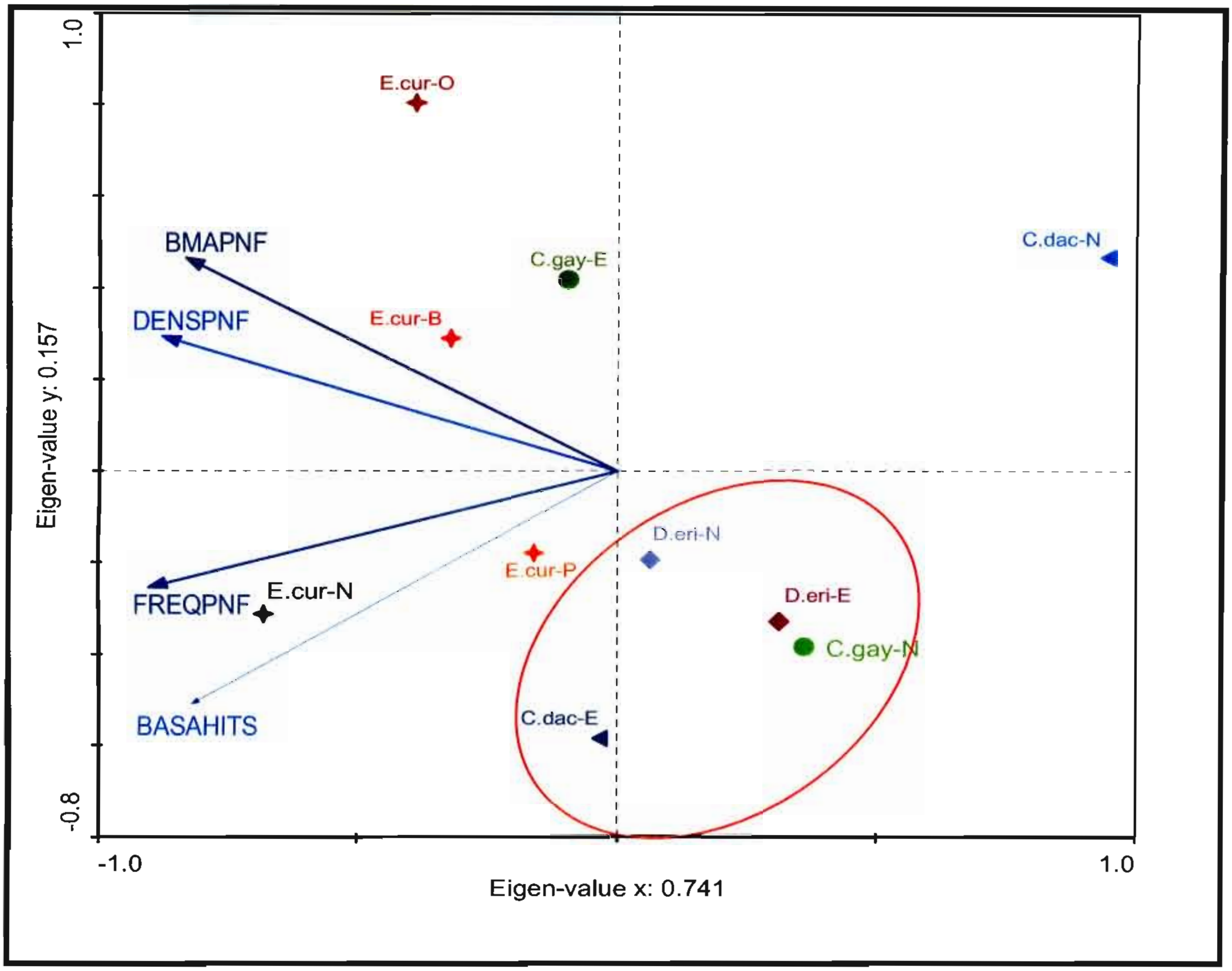


Figure 3.14: The Principal Component Analysis (PCA) of the field surveys carried out under natural conditions with regards to density (DENSPNF), frequency (FREQPNF), biomass (BMAPNF) and basal hits (BASAHITS) of the seed types of the selected grass species. The Eigen value for the x-axis (0.741) indicates a strong positive correlation for all the parameters (See Table 3.1 for species abbreviations).

The correlation of *D. eriantha* non-enhanced and enhanced, *Chloris gayana* non-enhanced and *Cynodon dactylon* enhanced seed type is less positively correlating with all the parameters (Figure 3.14).

3.5.3 The comparison of the average individual biomass under controlled and natural conditions

Although the biomass calculations under controlled (glasshouse) conditions and those obtained from the natural field trials were not conducted at the same time or for a comparable period of growth, the results were compared to point out any similarities between these two biomass production ratios.

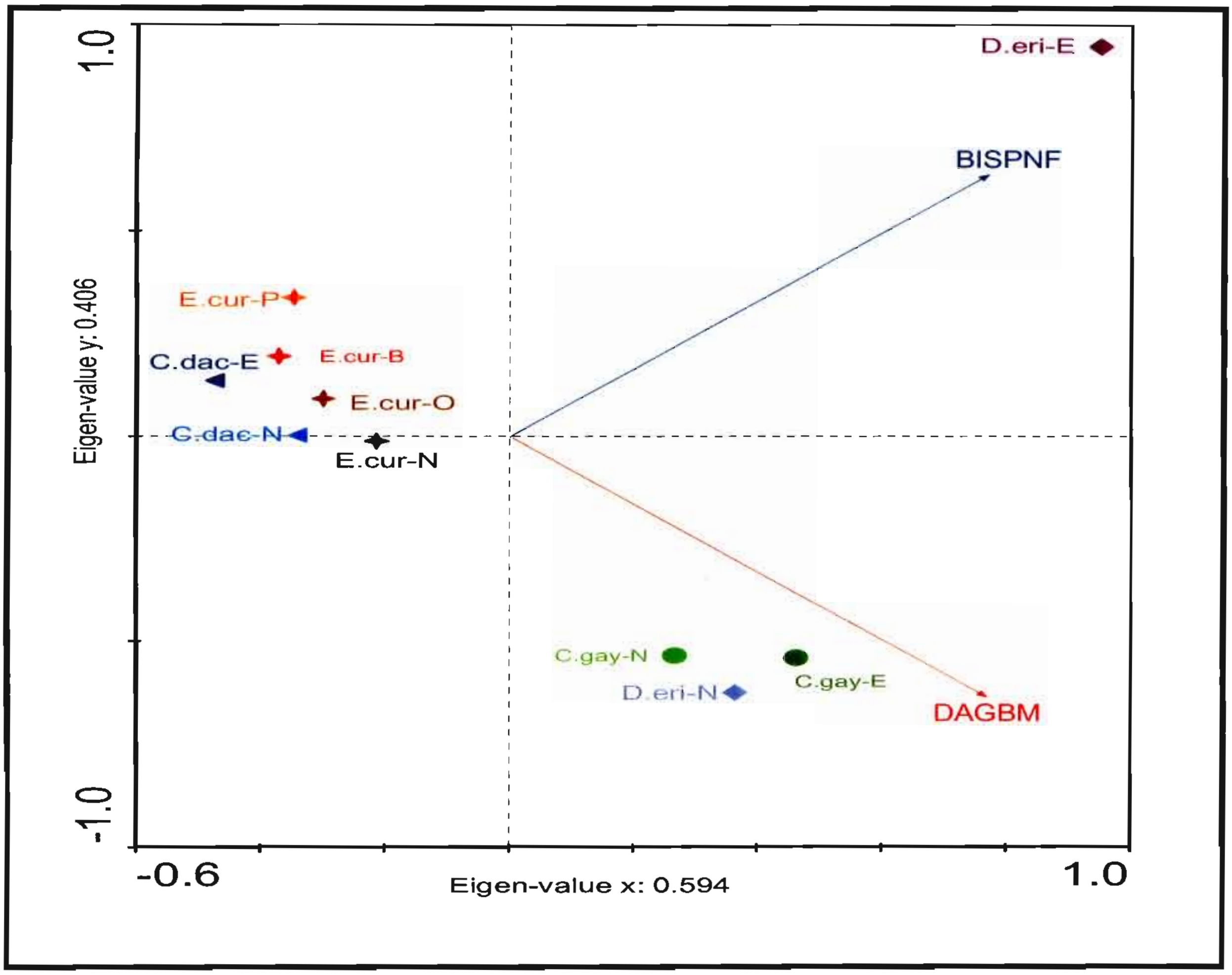


Figure 3.15: The Principal Component Analysis (PCA) of the comparison of the average dry above-ground biomass production, recorded under controlled (DAGBM) as well as natural conditions (BISPNF), calculated per individual. The Eigen value for the X-axis is 0.594 and for the Y-axis 0.406 (See Table 3.1 for species abbreviations).

According to the X-axis of the PCA-ordination, there was a slight positive correlation with regards to the biomass comparison of the glasshouse and natural field trial data (Figure 3.15).

This positive correlation is especially true for the enhanced *Chloris gayana* and non-enhanced *D. eriantha* and *Chloris gayana* seed types with regard to the average individual biomass accumulated under controlled conditions and the *D. eriantha* enhanced seed types for the biomass production under natural conditions. Only the *D. eriantha* enhanced seed type had a positive correlation with the measurements obtained under the natural field conditions (Figure 3.15).

3.6 Root development and structure of vascular tissue

The structure of the vascular tissue of seedlings grown from enhanced and non-enhanced seed was compared under the light microscope. Attention was given to the transition region between the root and the shoot of enhanced and non-enhanced seedlings. The transition region connects the vascular system of the root and the shoot. It is crucial that the vascular system of the root and the shoot is continuous in this region as any interference with the normal differentiation of the vascular tissue will adversely affect the survival of the seedling. Conversely, any treatment that may enhance the differentiation and connectivity of the vascular tissue, may promote the survival of the seedling. The transition region is of importance to a young seedling, as it is the region of the plant axis where the contrasting arrangement of the vascular systems of the shoot and root are joined together (Esau, 1965; Fahn, 1990).

The arrangements of the primary vascular system of the root and shoot differ. These differences are noted in the structure and by the direction of radial development of the vascular tissue. In the primary plant body of the root the pericycle borders directly on the inner surface by the phloem and xylem groups. Phloem groups are arranged on the periphery of a solid vascular cylinder of xylem tissue and they are separate. Xylem groups radiate like the spokes of a wheel from the solid vascular cylinder of the xylem. The xylem groups are separate units on the periphery of the vascular cylinder (Fahn, 1990).

The vascular system of the shoot of monocotyledous plants such as grasses, consists of scattered vascular bundles in the ground tissue of the stem. In the roots the protoxylem is exarch (when protoxylem is on the outside and the metaxylem differentiates centripetally) and the xylem and phloem alternates, while the stem's protoxylem is endarch (when protoxylem is on the inside and the differentiation of the metaxylem proceeds towards the periphery) and the xylem and phloem arrangement is collateral (Fahn, 1990). The differences in the arrangement of the vascular tissue of these organs (roots and shoots) may have implications with regard to the direction of cell differentiation and these variations may be seen in the transition region.

The transition region unites the root and the shoot in such a way as to ensure that there are no hydraulic constrictions in the pathway of the xylem (Fahn, 1990). A gradual change occurs from the root to the shoot and a particular vascular tissue pattern is noted in this region which reflects the differentiation of vascular tissue and structure in the plant (Esau, 1965; Fahn, 1990). The transition region is also the connection between the root and the cotyledons (Esau, 1965). The connection involves spatial adjustment between the two vascular systems of the root and shoot, with several directions of differentiation in the horisontal plane. Therefore intermediate or transitional features between the root and the shoot are evident in this region. The connection between the root and shoot is established through the hypocotyl (Esau, 1965). The area in the transition region where the sections were mostly made during this study was the basal part of the hypocotyl, close to the primary root. Emphasis was placed on possible differences in xylem and phloem differentiation and maturation during the early stages of seedling growth in this region.

The seedlings were all 16 days old and sections were made through their transition regions for each of the different treatments. The sections were made though the transition region because the latter is a short and well defined region and morphologically easy to distinguish and locate. Sections made through this region should provide meaningful comparative results, pointing out differences in vascular tissue differentiation among the different seed enhancement treatments, if present. Other less clearly defined regions, such as an entire root or shoot, may pose some problems with regard to the difficulty of locating comparative areas in similar stages of differentiation or maturity among different seedlings.

According to the literature there are inherent differences in complexity between the structures of the transition region in different plant species (Fahn, 1990). Therefore, the transition between the conducting systems of the root and shoot were not studied in depth during this investigation. The complexity and variability of the structure of the transition region in different plant groups may contribute to slightly variable results and according to Esau (1965) a proper understanding of the transition region is only possible if the plant part is studied throughout its development. The transition region is representative of a connection between an organ with an axial vascular system (root, i.e. a solid cylinder of vascular tissue) and a vascular system that consists of individual bundles and develops in relation to leaves (shoot). The transition region therefore is also the relation between the vascular system of the root and the traces of the first foliar organs of a plant (Esau, 1965). The vascular system in the transition region is very important with regard to the xylem transporting water from the roots to the shoot and phloem transporting sugars and minerals to the rest of the plant (Fahn, 1990).

In monocotyledon plants such as grasses, the single cotyledon and length of the lowermost internode affect the transition between the vascular tissues of the root and shoot (Esau, 1965; Fahn, 1990). This was not noted in the section made in this study. Usually, part of the vascular system of the root is in connection with the vascular system of the cotyledons and the other part is connected to the first foliage leaf, and in these connections typical transition features are common (Fahn, 1990).

The transition region of the various treatments on the *D. eriantha* seed are represented in Figures 3.16 to 3.20, while the transition region of the non-enhanced seed type of *D. eriantha* is represented by Figure 3.21.

<u>Diameter of the vascular cylinder</u>

According to the measurements of the vascular cylinder, the normal treatment of the *D. eriantha* seed type with a vascular cylinder diameter of 146.4 µm (Figure 3.16) had the smallest vascular cylinder diameter, while the largest vascular cylinder diameter was measured on the cylinder of the *D. eriantha* with extra organic growth stimulant treatment seed type – 234.94 µm (Figure 3.19). The vascular cylinder diameter of the *D. eriantha* seed type with dormancy breaking was 223.56 µm (Figure 3.17), while the vascular cylinder diameter of the *D. eriantha* seed type with phosphate treatment was 207.16 µm (Figure 3.18). The *D. eriantha* seed type treated with organic insecticide had a vascular cylinder diameter of 173.70 µm (Figure 3.20). The non-enhanced (without any treatment - control) seed type of *D. eriantha* had a vascular cylinder diameter of 182.22 µm (Figure 3.21). Although a trend exist in the measurements showing that the seed type treated with extra organic growth stimulant had a greater vascular tissue diameter, more seedlings need to be inspected, for only one seedling per treatment were used because of the qualitative investigation in the differentiation of the vascular tissue. A correlation normally exits between the diameter of the vascular cylinder and the number of protoxylem groups and the presence and absence of pith (Fahn, 1990).

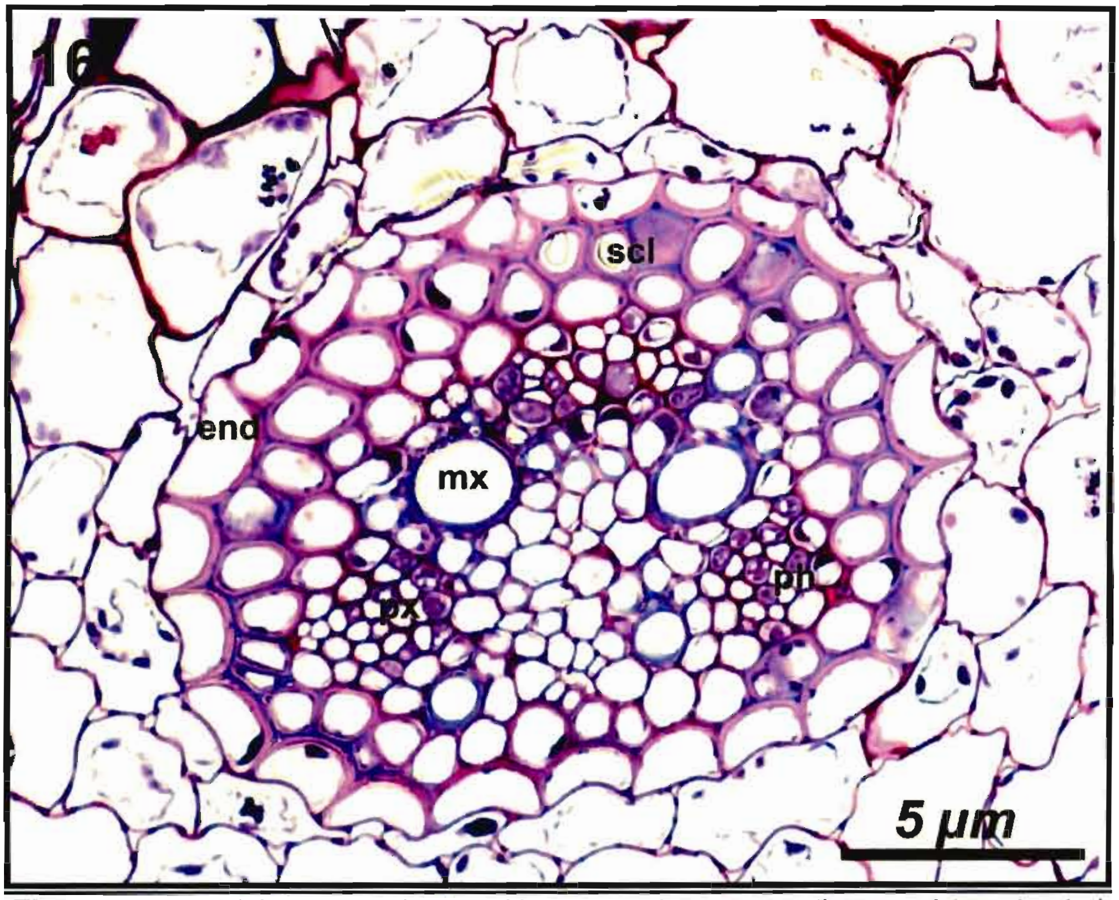


Figure 3.16: *Digitaria eriantha* transition region in cross section, seed type treated with normal treatment (LM). Vascular tissue with the endodermis (end) surrounding the sclerenchyma (scl). Metaxylem (mx), protoxylem (px) and phloem (ph) arranged alternating.

When the diameter of the vascular cylinder is large, a pith (undifferentiated centrally situated procambium (Esau, 1965)) is usually present and the number of metaxylem groups is large. In the current study four metaxylem groups are present in most cases, but vary in spite of the constancy of number of metaxylem groups. The pith is noted in some of the sections resembling more of a shoot vascular arrangement (Figure 3.21).

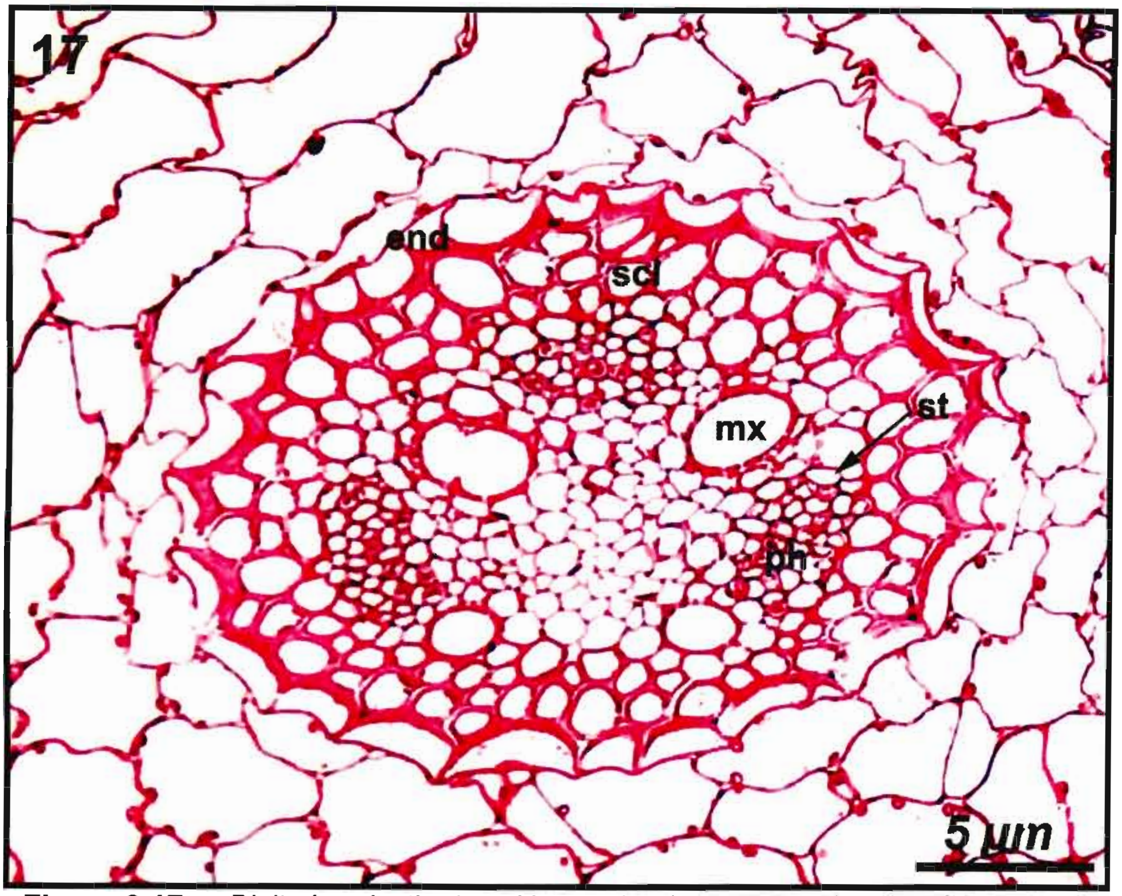


Figure 3.17: *Digitaria eriantha* transition region in cross section, seed type treated with dormancy breaking (LM). Vascular tissue with the endodermis (end) surrounding the sclerenchyma (scl). Metaxylem (mx) and phloem (ph) arranged alternating. Phloem (ph) with sieve-tubes (st) present.

Sclerenchyma

The transition region is a very delicate region and therefore the sclerenchyma has an important supportive function in this region. The sclerenchyma tissue on the inside of the endodermis is thickened and well defined in all the treatments (Figure 3.16 – 3.21). Sclerenchyma layers are present in all the treatments towards the inside of the endodermis. The function of the sclerenchyma in the case of the root and shoot is to protect and support the vascular cylinder. No significant differences regarding the number of cell layers, cell size, wall thickness, cell content, arrangement and position of the sclerenchyma was noted among the different treatments. The differentiation of the sclerenchyma with secondary cell walls and absence of cell content in the cells is an indication that the sections were made nearer to the shoot than the root.

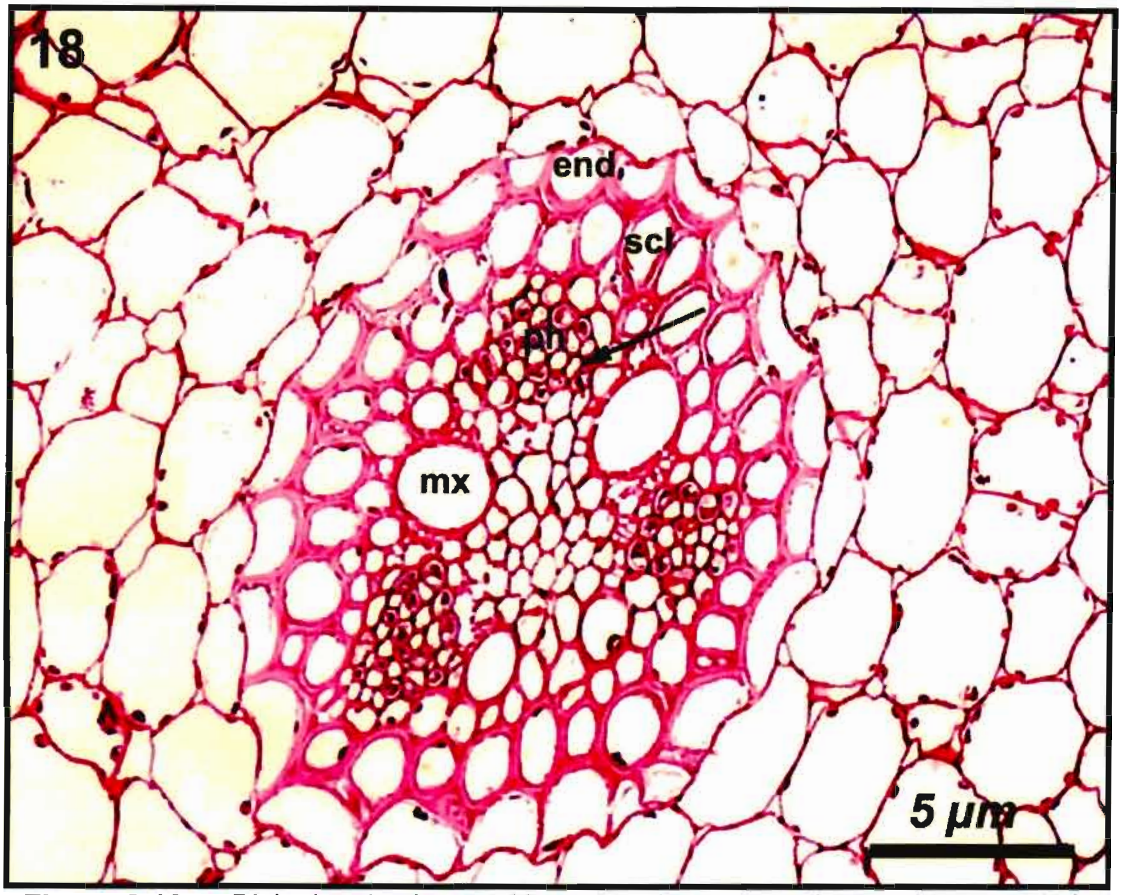


Figure 3.18: *Digitaria eriantha* transition region in cross section seed type treated with phosphate (LM). Vascular tissue with the endodermis (end) surrounding the sclerenchyma (scl). Metaxylem (mx) and phloem (ph) arranged alternating. Phloem (ph) with sieve-tubes (arrow) present.

Phloem

Sieve-tube members, companion cells, fibres and parenchyma are the cell types of the primary phloem. The sieve-tube members are living cells and arrange in vertical rows, forming the sieve tubes. The function of these sieve tubes is the translocation of organic substances. The cells that remain in close contact with the sieve-tube members are known as the companion cells (Fahn, 1990).

In all the treatments (Figures 3.16 – 3.21) the phloem groups are fully differentiated and mature and thus the function of the phloem is fulfilled by the particular cell types within the phloem. Sieve tubes are well defined with sparse cytoplasmic contents, assisting in the transport of organic substances. The sieve-tube members, companion cells and fibres are noted in the protophloem (Figures 3.16 – 3.18). Sieve-tube members are long and narrow cells in the phloem and sieve areas could only be distinguished with great difficulty.

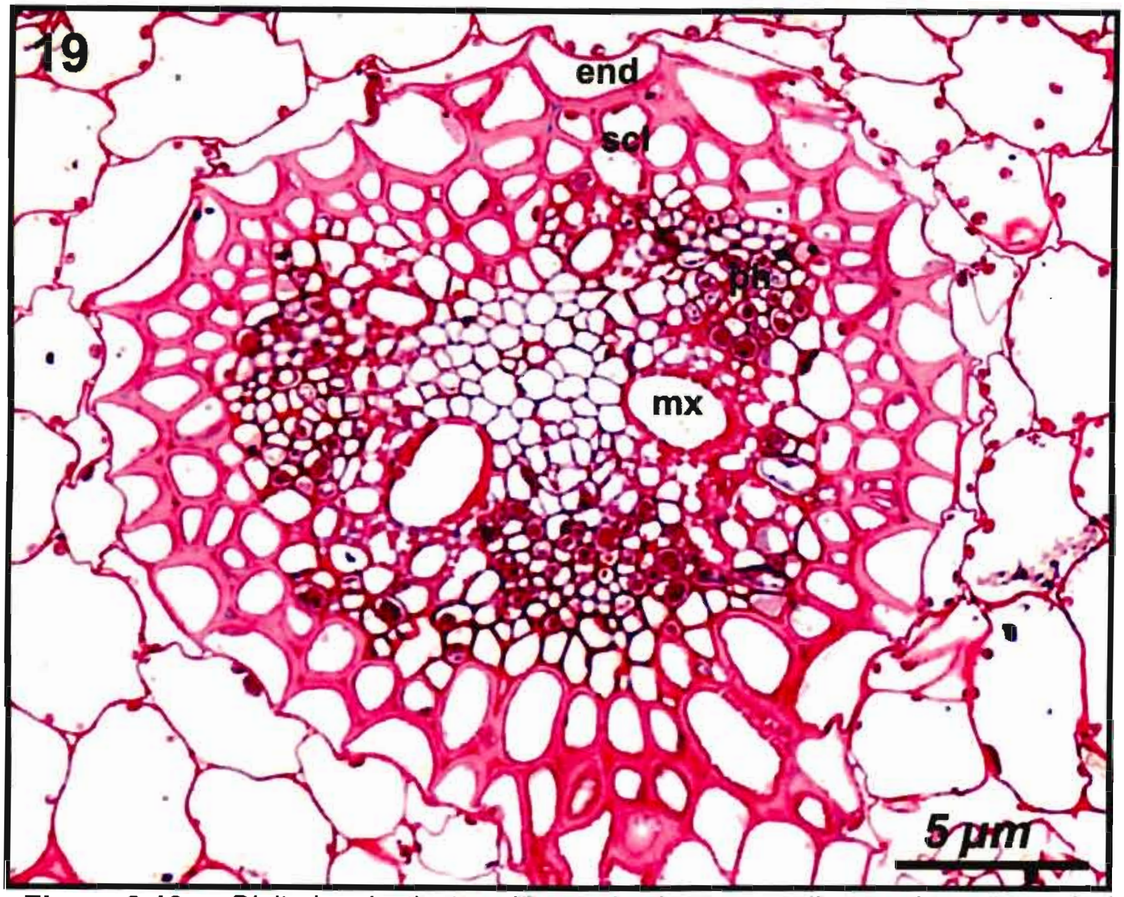


Figure 3.19: *Digitaria eriantha* transition region in cross section seed type treated with extra organic growth stimulant (LM). Vascular tissue with the endodermis (end) surrounding the sclerenchyma (scl). Metaxylem (mx), protoxylem (px) and alternates with phloem (ph).

Number and position of protophloem and protoxylem groups

Primary phloem consists of protophloem and metaphloem, the protophloem being the phloem which develops during the earlier stages of seedling development. In the case of the xylem, the primary xylem consists of protoxylem and protophloem. The vascular tissue of the young elongating parts of the plant is known as the protophloem and protoxylem (Fahn, 1990).

The differentiation of the phloem is centripetal and the protophloem is situated closest to the endodermis, while the metaphloem is situated closest to axis of the root. In monocotyledons a difference is noted between the narrow protoxylem cells and the early metaxylem cells, which is somewhat wider. The late metaxylem cells are the widest cells and these parameters are useful in comparative studies (Esau, 1965). No significant variation in the diameter of the tracheary elements of the proto- and metaxylem between the different treatments are noted.

The number of protoxylem strands in roots is more than three and this is known as polyarch, which is characteristic of monocots (Fahn, 1990). Variations in these features may be found even within the same plant due to the age of the seedling as well as environmental factors, such as water and nutrient availability that may influence these features.

There were however variations in the transition region, which differ from seedling to seedling. The normal treatment (Figure 3.16), dormancy breaking treatment (Figure 3.17), phosphate treatment (Figure 3.18), extra organic growth stimulant treatment (Figure 3.19) and the no treatment type (non-enhanced) of the *D. eriantha* seed type already had polyarch protoxylem arrangement, characteristic of monocotyledonous plants. The organic fungicide treatment (Figure 3.20) seed type of *D. eriantha* did not have the conspicuous polyarch protoxylem arrangement yet, which can be attributed to sections through the transition region being more towards the root than the shoot in the sections made in this treatment.

Cytoplastic characteristics of cells

In order to determine the maturity of the elements the condition of the protoplasts must be known. When the secondary walls are developed, the tracheary elements do not contain any living nuclei and cytoplasmic structures. Tracheary elements in all the treatments are devoid of cell content and the secondary walls are present, thus the tissue is fully differentiated.

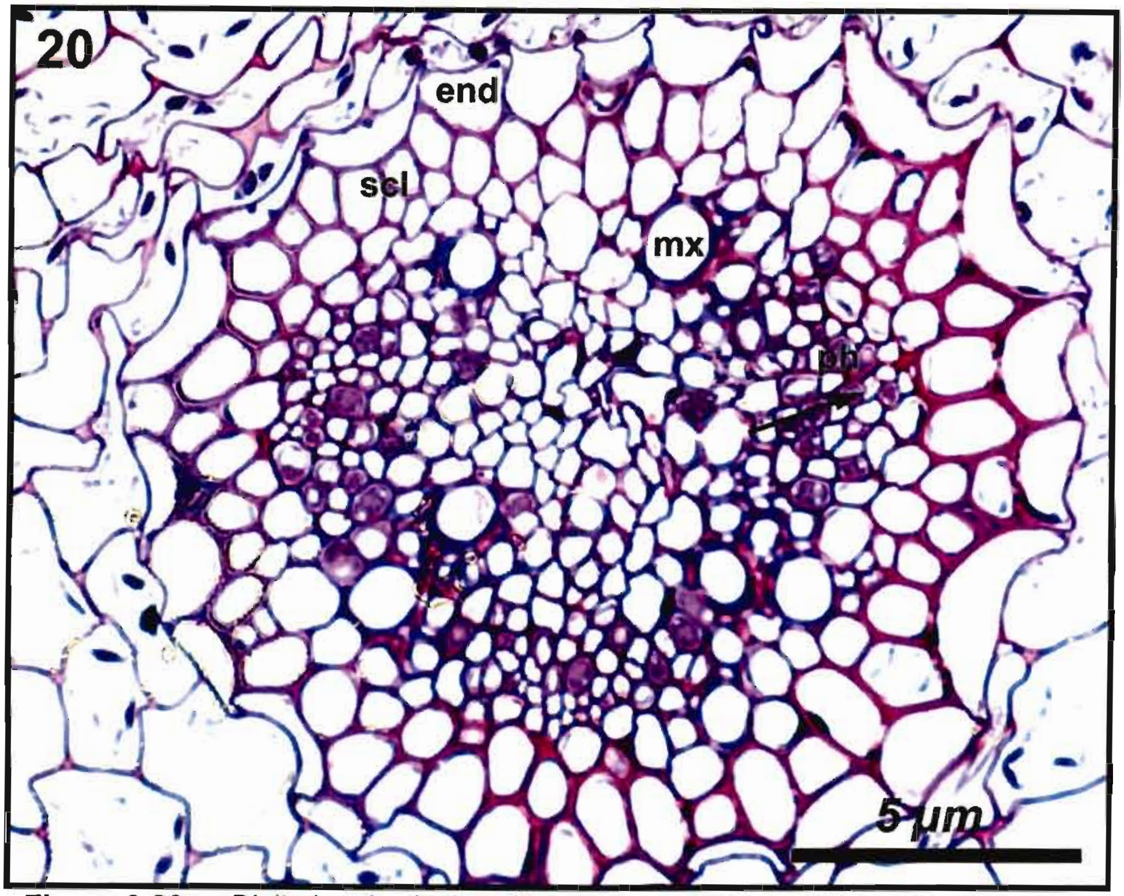


Figure 3.20: *Digitaria eriantha* transition region in cross section seed type treated with organic fungicide (LM). Vascular tissue with the endodermis (end) surrounding the sclerenchyma (scl). Metaxylem (mx) and phloem (ph) arranged alternating with sieve-tubes (arrow) present.

<u>Rate of phloem differentiation – mature sieve tubes</u>

In all the treatments (Figure 3.16 – 3.21) the sieve tubes are fully developed and functional. In Figures 3.16 – 3.20, the phloem groups alternate with the xylem groups. The section was made more to the root than the shoot in the transition region. In Figure 3.19, a pith is present, which may indicate that this section was made nearer to the shoot than the root. Large well developed metaxylem elements were noted in Figure 3.20, indicating that the section was made more towards the root than the shoot. However, Figure 3.21 resembles more of a shoot than a root, because the phloem is almost on the same radius than the xylem, which is typical of co-lateral vascular bundles present in the shoot.

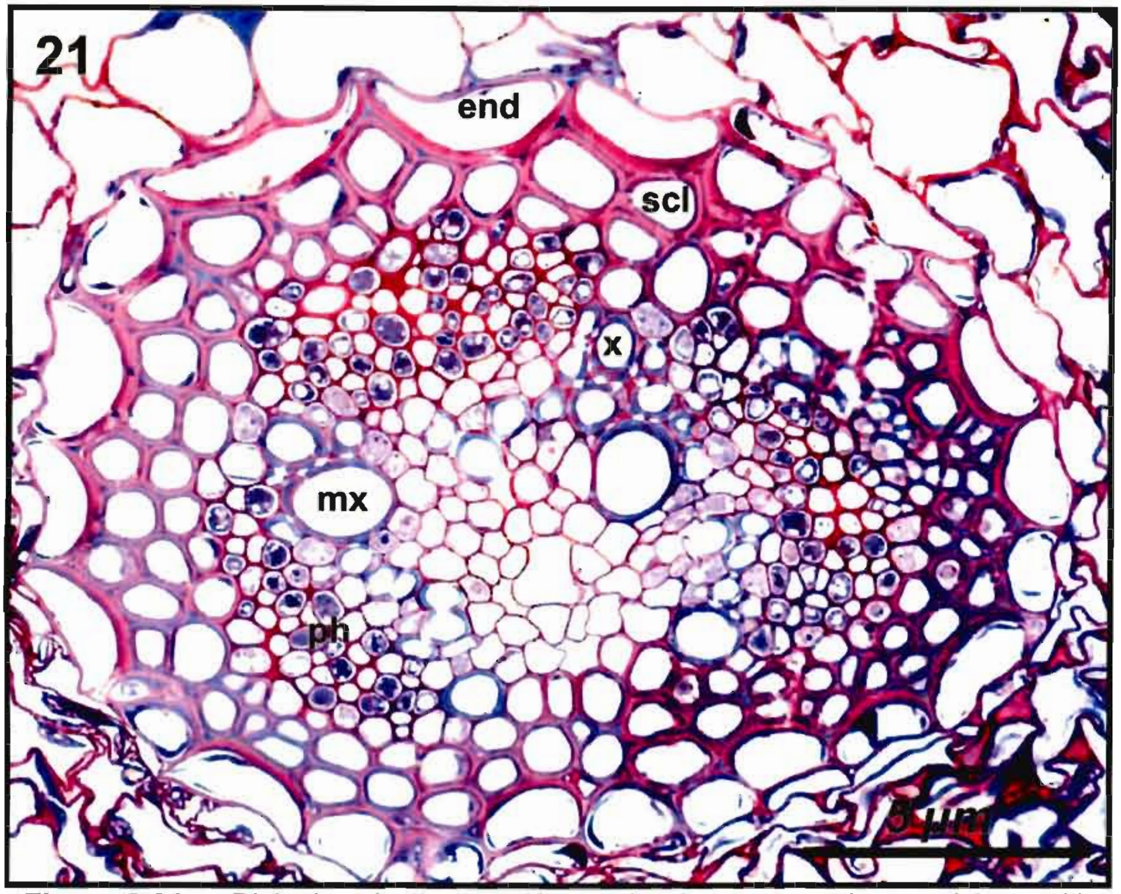


Figure 3.21: *Digitaria eriantha* transition region in cross section seed type with no treatment (non-enhanced) (LM). Vascular tissue with the endodermis (end) surrounding the sclerenchyma (scl). Metaxylem (mx), and xylem (x) and phloem (ph) arranged alternating.

All the tracheary elements (of xylem) and sieve elements (of phloem) associated with the transition region are fully differentiated. No differences could be noted between the transition regions of the non-enhanced (Figure 3.23) and different enhancement treatments of the *D. eriantha* seed type (Figure 3.16 – 3.22). Because there are no visual variability in the number of protoxylem groups in the transition region of the different seedlings, it can not be said that the vascular tissue of the seed types that were enhanced are better adapted for water conduction.

3.7 Seed predation

The seed predation trials were carried out on several plots as a pilot study to determine an applicable method to be used in this study. Figure 3.22 represents the preliminary results of one of the pilot studies carried out in July 2007. This pilot study was carried out to determine whether the covering of the seed by either closed or open hoods or the presence of soil in the petri dish, had an influence on seed put out in the seed predation trials (see Chapter 2). The preliminary results could serve as an indication of which predation trials could be used in this study or similar studies in the future.

Seed predation in the plot cultivated with non-enhanced and enhanced seed types of *Chloris gayana*

When comparing all the methods used in the seed predation trials, the seed predation on the non-enhanced and enhanced seed types of *Chloris gayana*, the highest seed predation was noted on the non-enhanced seed type. The average seed predation was noted in non-enhanced seed type of *Chloris gayana*, in the "soil and open" (SO) (0.19%) trial. The enhanced seed type had a seed predation of 0.08% in the SO trial. In the "no soil and open" (NSO) trial the non-enhanced seed type had a 0.14% seed predation and the enhanced seed type a seed predation of 0.11%. The highest seed predation in the "soil and closed" (SC) seed predation trials took place in the plots where the non-enhanced (0.2%) seed type were established. The enhanced seed type of *Chloris gayana* had a lower seed predation in the SC trial compared to the non-enhanced seed type. In the "no soil and closed" (NSC) trials the non-enhanced (0.14%) seed type had a higher seed predation than the enhanced seed type (0.06%).

Seed predation in the plot cultivated with non-enhanced and enhanced seed types of *Cynodon dactylon*

The average seed predation in the SO trials in the plots cultivated with *C. dactylon* was higher on the non-enhanced (0.32%) seed type than on the enhanced seed type (0.28%). In the NSO trial the non-enhanced seed type had a 0.14% seed predation and the enhanced seed type a seed predation of 0.12%. In the case of the SC trials the highest seed predation in the plots where on the non-enhanced (0.23%) seed type and the seed predation on the enhanced seed type was only 0.18%. The non-enhanced (0.33%) seed type of *Cynodon dactylon* had a lower seed predation in the NSC trial compared to the enhanced seed type (0.48%). The latter was the only trial were higher seed predation took place on the enhanced seed type.

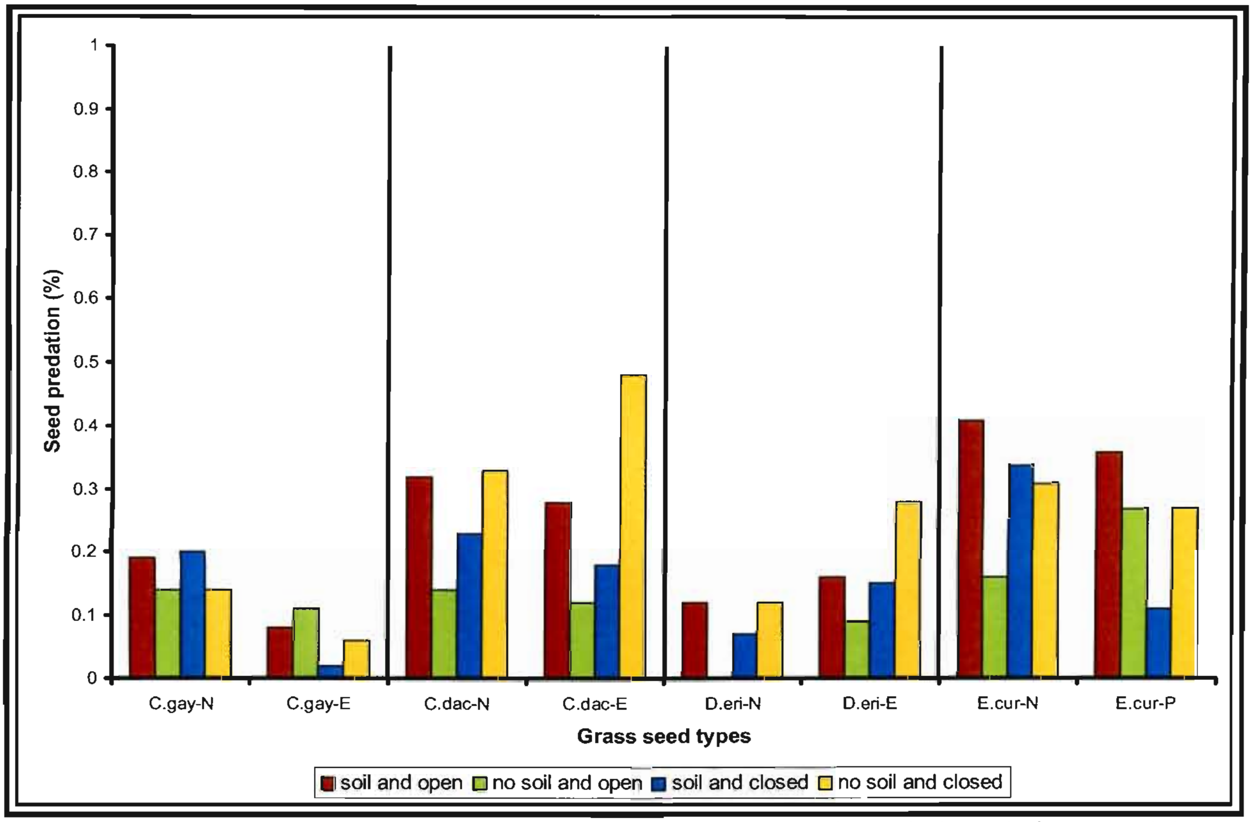


Figure 3.22: The average seed predation in the pilot study period carried out in July 2007. Note that a minimum predation took place (less than 1%, in all the seed types) – see text.

<u>Seed predation in the plot cultivated with non-enhanced and enhanced seed types of *D. eriantha*</u>

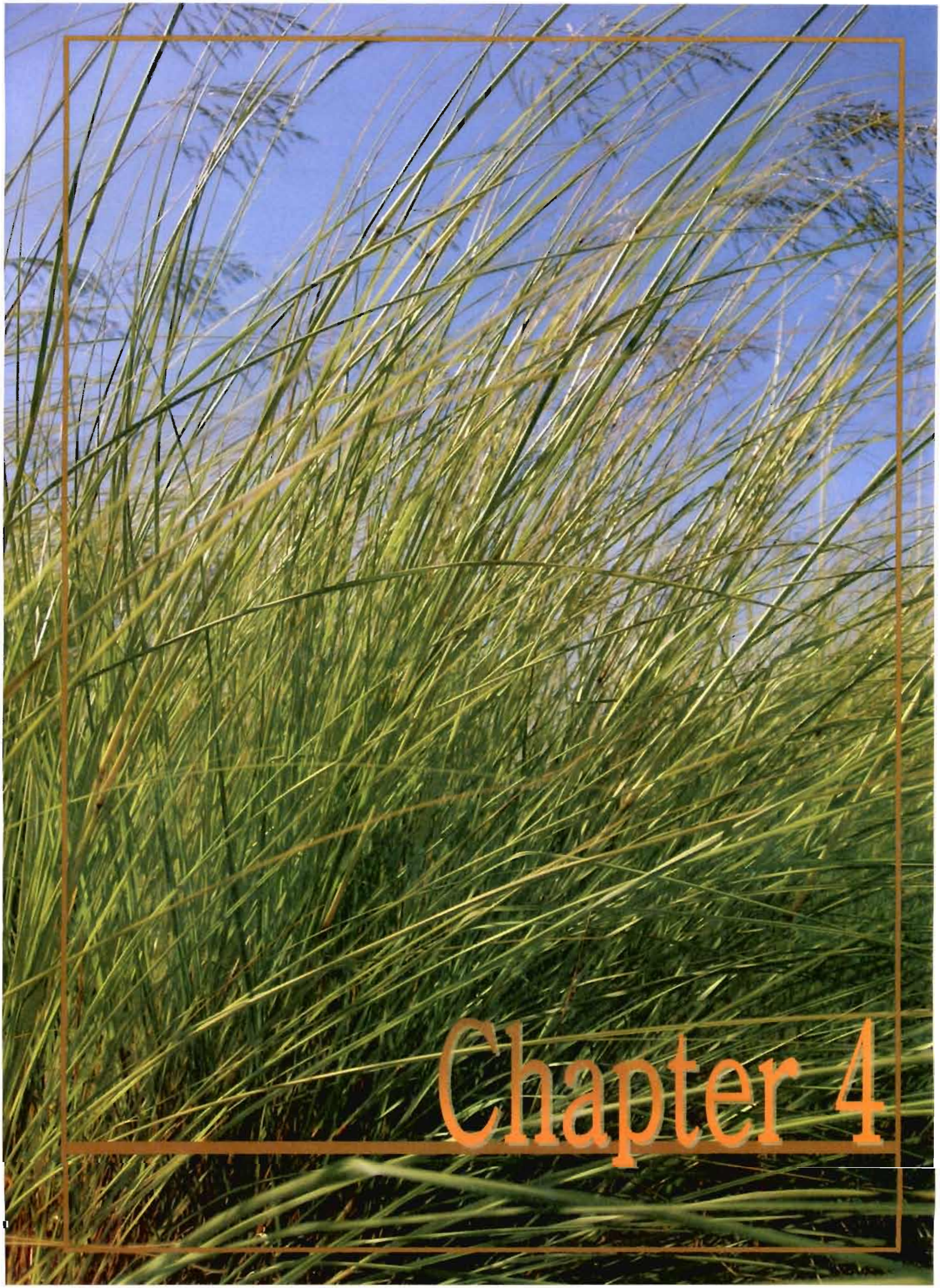
When comparing all the methods used in the seed predation trials, the seed predation on the non-enhanced and enhanced seed types of *D. eriantha*, the highest seed predation was noted on the enhanced seed type. The average seed predation was noted in enhanced seed type of *D. eriantha*, in the SO trail (0.16%). The non-enhanced seed type had a seed predation of 0.12% in the SO trial. In the NSO trial the enhanced seed type had a 0.09% seed predation and no seed predation was noted on the non-enhanced seed type (0%). The highest seed predation in the SC seed predation trials took place in the plots where the enhanced (0.15%) seed type was established. The non-enhanced seed type of *D. eriantha* had a lower seed predation in the SC trial compared to the non-enhanced seed type (0.07%). In the NSC trials the enhanced (0.28%) seed type had a higher seed predation than the enhanced seed type (0.12%).

Seed predation in the plot cultivated with non-enhanced and enhanced seed types of *E. curvula*

The average seed predation in the SO trials in the plots cultivated with *E. curvula* was higher on the non-enhanced (0.41%) seed type than on the "plain coat" seed type (0.36%). In the NSO trial the "plain coat" seed type had a 0.27% seed predation which was higher when compared to the seed predation on the non-enhanced seed type (0.16%). In the case of the SC trials the highest seed predation in the plots where on the non-enhanced (0.34%) seed type with a lower seed predation on the "plain coat" seed type (0.11%). The non-enhanced (0.31%) seed type of *E. curvula* had a higher seed predation in the NSC trial compared to the "plain coat" seed type (0.27%).

The pilot study took place in the winter season, which is known for decreased ant activity. The seed predation was less than 1 % for all the seed types, and no concrete evidence exists to support that the seeds that were not present after the trial period were actually taken by ants. Recommendations for future seed predation studies are given in Chapter 4.

The following Chapter summarise the study in the form of concluding remarks as well as recommendations towards future studies.



Chapter 4

CHAPTER 4

CONCLUSION AND RECOMMENDATIONS

4.1 Introduction

In degraded rangelands where the biomass production for grazers has to be increased, grasses are mostly used. Advance Seed Company in Krugersdorp, South Africa uses a specific coating to enhance the germination and growth percentage of all types of species, including grasses. Active restoration applications often include re-seeding practices to improve the cover, density and biomass of the vegetation and control erosion in degraded areas. Re-seeding can be carried out in different ways and, depending on the habitat, environmental factors and aim of the restoration, grass seed of different species can be used.

The main aim of the study entailed the evaluation and comparison of selected enhanced and non-enhanced grass seed types of *Chloris gayana, Cynodon dactylon, D. eriantha* and *E. curvula* under various conditions in controlled laboratory and glasshouse trials and trials in the natural field. These seeds were to be used in re-seeding practices in restoration applications. In the case of the *E. curvula* seed types, the seed was enhanced with three different enhancements, namely "plain coat", "organic insecticide on the base" and "organic insecticide on the base and as overspray".

The specific objectives included comparing germination and establishment percentages, above- and below-ground biomass under controlled and natural conditions, differences in the structure of the vascular tissue as well as the seed predation of enhanced and non-enhanced seeds.

The evaluation of the vascular structure in the transition region of the seedlings took place on a different seed lot of *D. eriantha*, with different enhancements. These seed were enhanced with treatments which included a normal treatment, a dormancy breaking treatment, a phosphate treatment, a treatment with organic growth stimulants, an organic fungicide treatment and seed with no treatment.

The hypothesis stated that, when selected enhanced and non-enhanced grass seed types are compared, it is expected that the enhanced grass seeds will have a better seed purity, germination and establishment percentage, as well as higher above- and below-ground biomass yields. The roots of the enhanced seed are expected to develop better and the structure of the vascular tissue is expected to be better adapted for especially water conduction. The seed predation of the enhanced seed is also expected to be lower.

PART 1

4.2 Germination percentages of selected grasses

4.2.1 Seed purity and seed germination

The purity of the selected grass seed species were very high and all above 95%, which is an indication of the quality of seed supplied by Advance Seed Company. A general conclusion could therefore be drawn that seed purchased from a registered seed merchant, whether enhanced or not, are of a high quality, because it is required by legislation to test the seed before it can be commercially sold. According to the Plant Improvement Act (No. 53 of 1976) - Provisions relating to seed and seed samples (Appendix A – Table 4), only a certain percentage of inert matter, other seed and weeds may be present in a seed lot and from the results obtained, all the selected grass seeds obtained from the seed merchant contained less than the minimum inert matter, other seed and weed species (Appendix F). Therefore, a guarantee with regards to the seed purity and germination potential of the seed was given by the seed merchant. When a batch of seed does not meet these regulations, the seed company can be declined not to sell the seed, which could lead to a financial mishap in the company, causing the company to lose money. When companies sell the seed which have not met the standard regulations of the Plant Improvement Act (No. 53 of 1976), the company can lose its accreditation and ultimately their clientele.

In practice, when seeds are tested six months after harvesting, dormancy breaking methods are not applied when the germination tests are carried out (Personal communications: Louise Kotze). In this study, two germination tests were carried out, one with the application of dormancy breaking methods and another without dormancy breaking methods. No remarkable differences were noted between the germination test with dormancy breaking and that without, for the seed tested were harvested in October 2005 and tested in May 2006. This confirms that no dormancy breaking is needed approximately six months after harvesting the seed. The only statistical difference between the germination percentages of enhanced and non-enhanced seed was noted in the dormancy breaking germination test between the enhanced and non-enhanced seed types of *Chloris gayana* and *E. curvula.* The non-enhanced seed type of *Chloris gayana* had a higher germination percentage (28%) in comparison with the enhanced seed type (12%). With regards to the *E. curvula* seed types, the non-enhanced seed, enhancement with "organic insecticide on base of coat" and enhancement with "organic insecticide on base of coat and as overspray" all had a statistically higher germination percentage (55%, 47% and 57%, respectively) than the "plain coat" *E. curvula* seed type (7%).

Taking the statistical significance into account and referring to all the germination tests, including those carried out by Advance Seed Company, the non-enhanced seed types of *Chloris gayana* and *Cynodon dactylon* had a higher germination percentage in comparison with the enhanced seed types of these respective species. In the case of the *D. eriantha* and *E. curvula* species overall, the enhanced seed type of *D. eriantha* and *E. curvula* enhanced with "organic insecticide on base and as overspray" had higher germination percentages than the non-enhanced seed types and other enhancements.

If the seed purity is compared to the germination percentage, the conclusion can therefore be drawn that a high seed purity does not necessarily imply that the germination of the seed will also be high.

4.3 Glasshouse trials

4.3.1 Establishment of selected grass seedlings in different growth mediums

The establishment percentage of the seedlings was compared in two growth mediums, i.e. Hygromix growth medium and Coco Peat Moss medium. As with the germination tests, the establishment of the seed types of the selected grass species were the same in both the mediums and a trend are noticed when comparing the results with the results of the germination tests. The establishment of the non-enhanced seed types of *Chloris gayana* (Hygromix medium 31%; Coco Peat Moss 19%) and *Cynodon dactylon* (Hygromix medium 37%; Coco Peat Moss 52%) was better in both the mediums in comparison with the enhanced seed types of the species, while the enhanced seed type of *D. eriantha* had a higher establishment percentage (Hygromix 26%; Coco Peat Moss 32%) than the non-enhanced seed type (Hygromix medium 13%; Coco Peat Moss 9%) in both mediums. The *E. curvula* enhanced with "organic insecticide on base and as overspray" had higher establishment percentages (Hygromix medium 60%; Coco Peat Moss 51%) than the non-enhanced (Hygromix 27%; Coco Peat Moss 37%) and other enhancement treatments of the *E. curvula* seed types ("plain coat": Hygromix medium 10%; Coco Peat Moss 12% and enhancement with "organic insecticide on the base of the coat": Hygromix medium 36%; Coco Peat Moss 41%). The results indicated whenever a Hygromix growth medium or Coco Peat Moss are used as growth medium, the non-enhanced of *Cynodon dactylon* and the enhanced seed type of *D. eriantha* as well as the *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray" recommended for establishment purposes.

4.3.2 Biomass production

In all the biomass production assessments, i.e. fresh and dry, above- and below-ground biomass, the plants from the enhanced seed type of *Chloris gayana* yielded higher biomass in comparison with the non-enhanced seed type. The plants of the non-enhanced seed types of *Cynodon dactylon* and *D. eriantha* had a higher average biomass production than the enhanced seed types in all the biomass production assessments. In the case of the plants from the *E. curvula* seed types, the non-enhanced seed type yielded a higher average biomass in the fresh above-ground and dry above- and below-ground biomass production. The highest average fresh below-ground biomass was yielded by the *E. curvula* enhanced with "organic insecticide on the base of the coat and as overspray".

When comparing all the species used in this study, the highest average fresh above-ground biomass production was noted at the plants of the non-enhanced seed type of

D. eriantha (82.78g), while the highest average fresh below-ground biomass production was in the plants from the enhanced seed type of *Chloris gayana* (32.97g).

The plants from the *Chloris gayana* enhanced seed type yielded the highest average dry above-ground biomass (27.55g) and the plants of the non-enhanced *D. eriantha* seed type yielded the highest average dry-below ground biomass (27.11g).

4.4 Natural field trials

4.4.1 Density of the individuals of species and dry matter production

The plants of the enhanced seed types of *Chloris gayana, Cynodon dactylon* and *E. curvula* (enhanced with "organic insecticide on base and as overspray" had higher density and dry matter biomass production (27 plants/m^2, 34 plants/m^2 and 60 plants/m^2 respectively) than the plants of the non-enhanced seed types. The plants of the non-enhanced seed type of *D. eriantha* had a higher density and dry matter production in the plots (plants/m^2 and 656g) in comparison with the enhanced seed type (14 plants/m^2 and 124g).

4.4.2 Basal cover and the frequency of grasses and weeds

A higher frequency of grass plants and a lower frequency of weeds were recorded in the plots of the enhanced seed types of especially *Chloris gayana* (frequency 87%; weeds 13%) and *Cynodon dactylon* (frequency 91%; weeds 9%) than in the plots of the non-enhanced *Chloris gayana* (Frequency 17% and weeds 29%); *Cynodon dactylon* (Frequency 28%; weeds 72%) seed types. The basal cover of the plants of the non-enhanced seed type of *Chloris gayana* (11%) and plants in of the enhanced seed type of *Cynodon dactylon* (15%) was higher in comparison with the basal cover in the plots of the enhanced seed type of *Chloris gayana* (9%) and the non-enhanced seed type of *Cynodon dactylon* (0.33%). This could be due to the different tuft sizes of the grasses tested. The grasses in the plots of the non-enhanced *D. eriantha* (frequency 78%; basal cover 14%) and *E. curvula* (frequency 94%; basal cover 26%) seed types had a higher frequency and basal cover, with a low frequency of weeds (*D. eriantha* 24% and *E. curvula* 6%) occurring in these plots.

Very little correlation existed between the basal cover, frequency and density. The low basal cover recordings were mainly because of the low number of sampling points for this type of sampling. Basal cover sampling requires at least 2000 recording points per plot (Tidmarsh & Havenga, 1955), which was not carried out in this trial.

4.5 Root development and structure of vascular tissue

For this trial, a different batch of *D. eriantha* was supplied by Advance Seed Company. The seed was treated with five different treatments, which included a normal treatment, a dormancy breaking treatment, a phosphate treatment, an extra organic growth stimulant treatment and an organic fungicide treatment. Together with the treated seed, seed with no treatment (control) was also used in these trials. From the results in Chapter 3, no beneficial effect on the development of the xylem and phloem tissue in the transition region of seedlings of enhanced and non-enhanced seed types of *D. eriantha* were noted. The enhancements, thus did not affect the normal differentiation of the vascular tissue negatively. The conducting tissues appear to be normal in all the different treatments. The enhancements did not have any effect on the genetic material, which is responsible for the development of all the tissue within a plant.

4.6 Seed predation trials

In the pilot surveys, the seed predation was lower than 1%. Although this low value was due to the low temperature of the season in which these trials took place, an overall higher seed predation (in the "soil and open", "no soil and open", "soil and closed" and "no soil and closed") was noted in all the non-enhanced seed types, with the exception of the *D. eriantha* seed.

Unforeseen rains, lower night temperatures and time constraint contributed to the fact that the trials could not be repeated in the warmer seasons, as was planned.

To get a more reliable indication of seed predation, the trials should be repeated seasonally. It is also recommended that the environment in which the seed are set in the seed predation trials, should represent the most natural environment, i.e. placing the seed in petri dishes containing soil from the plots and using a sieve-covering, rather than the metal cover, to eliminate the influence rising temperature within the metal cover.

PART 2

The concluding remarks per seed type tested are as follows.

4.7 The *Chloris gayana* seed types

Although the enhanced seed type of *Chloris gayana* had higher seed purity (97.6%) than the non-enhanced seed type (96.1%), the *Chloris gayana* non-enhanced seed type had a higher germination percentage in both mediums in the laboratory as well as in the glasshouse trials.

Under controlled conditions, the biomass production of the plants of *Chloris gayana* of the enhanced seed type had higher fresh and dry, above- and below-ground biomass in comparison with the non-enhanced seed type.
In the natural field trials, the plants in the plots planted with the enhanced seed type of *Chloris gayana* had a higher average density (27 plants/m^2), frequency (87%) as well as biomass production (226.1g) in comparison with the plants of the non-enhanced seed type (density: 11 plants/m^2; frequency: 17%; biomass: 50.5g). The average basal cover was higher in the non-enhanced (11%) plots. The non-enhanced seed type also had a higher average frequency of weeds (29%) and higher seed predation took place in these plots.

When the above statements are taken into consideration, the results reflect that the enhanced seed type of *Chloris gayana,* rather than the non-enhanced seed type, could be recommended for use in the natural field environment.

4.8 The *Cynodon dactylon* seed types

In the laboratory experiments, which included the seed purity and germination tests, the non-enhanced seed type of *Cynodon dactylon* had higher seed purity (non-enhanced

100%; enhanced 99.4%) as well as higher germination percentages than the enhanced seed type of the species.

The plants from the non-enhanced seed type also yielded a higher average fresh and dry, above- and below-ground biomass in the controlled glasshouse trials.

In the natural field trials, however, the plants of the enhanced seed type yielded a higher average density (34 plants/m^2), frequency (91%), basal cover (15%) and dry matter production (40.25g). The most weeds were recorded in the non-enhanced (72%) seed type plots and overall the highest seed predation took place on the non-enhanced seed type of *Cynodon dactylon.*

Although the non-enhanced seed type performed better in the laboratory and glasshouse trials, in practice the seed needs to be sown in the natural field, and accordingly it would be advantageous to sow the enhanced seed type of the *Cynodon dactylon* seed.

4.9 The *D. eriantha* seed types

The enhanced seed type of *D. eriantha* had a higher germination and establishment percentage in comparison with the non-enhanced seed type, although the seed purity of the non-enhanced seed type (98.3%) was higher than that of the enhanced seed type (97.4%).

In the glasshouse trials, the non-enhanced seed type yielded higher fresh and dry, above- and below-ground biomass than the enhanced seed type.

The biomass production under the controlled conditions corresponded with the natural field trials, where the non-enhanced seed type had a higher average density (23 plants/m^2), frequency (78%), basal cover (14%) and dry matter production (123.96g) in comparison with the enhanced seed type. The highest weed frequency (32%) was noted in the plots of the enhanced seed type of *D. eriantha*, where the highest seed predation was noted.

In conclusion, the best *D. eriantha* seed type to use in the natural field would be the non-enhanced seed type.

4.10 The *E. curvula* seed types

In the case of the *E. curvula* seed types, the non-enhanced seed type, together with the enhancement with "organic insecticide on the base of the coat" and "organic insecticide on the base and as overspray", had 100% seed purity. The germination percentages (laboratory) and establishment percentage (glasshouse) indicated that the *E. curvula* enhanced with the "organic insecticide on the base and as overspray" had higher germination and establishment percentages in comparison with the other enhancements.

The fresh and dry above-ground and dry below-ground parts of the non-enhanced *E. curvula* seed type yielded a higher biomass than the all the enhanced types of *E. curvula.* It is only in the case of the fresh below-ground biomass where the "organic insecticide on the base and as overspray" enhancement of *E. curvula* yielded the highest biomass of the seed types of the selected grass species.

In the natural field trials, the plants of *E. curvula* enhanced with "organic insecticide on the base and as overspray" had a higher average density (60 plants/m^2) and dry matter production (248.28g) than the non-enhanced and other enhanced seed types of *E. curvula.* However, the highest average frequency (94%), basal cover (26%) and seed predation were present in plots of the plants in the non-enhanced plots of *E. curvula.*

The highest frequency of weeds was noted in the plots of the *E. curvula* seed type enhanced with "organic insecticide on the base of the coat" (17%).

In practice, either the seed enhanced with "organic insecticide on the base and as overspray" or the non-enhanced seed type can be used in re-seeding activities. The seed type enhanced with "plain coat" had the lowest values in all the trials and can therefore be excluded from any restoration and rehabilitation practices.

With regards to the surveys carried out in the natural field trials, an Importance Value (IV) was calculated for each species, which indicated that the non-enhanced seed type of *E. curvula* (175) had the highest IV and the non-enhanced seed type of *C. dactylon* (36.33) had the lowest IV of all the grass seed types tested.

4.11 Final conclusion

After the analyses of the all the results of this study, such as germination and establishment percentages, as well as all the data from the natural field trials and not only the calculated importance values and comparing the enhanced and non-enhanced seed type of each species – as mentioned in Chapter 3, it is advised that the enhanced non-enhanced and "organic insecticide on base of coat and as overspray" enhancement of *E. curvula* should be used in re-seeding practices. Of the other species, the enhanced seed types of *Chloris gayana* and *Cynodon dactylon* and the non-enhanced seed type of *D. eriantha* can be recommended in the same practices. It is not advised to use the "plain coat" enhancement of the *E. curvula* species in restoration and rehabilitation practices, as this seed type showed lower results in most trials. The difference in the costs of the enhanced and non-enhanced seed types could also influence the final decision of what seed type one will use in restoration and rehabilitation practices. This will however be influenced by the organisation or farmer that will apply re-seeding practices, as the aim and the available resources may differ between them.

Many of the degraded lands occur in areas of formerly disadvantaged communities and it is not easy for these land users or farmers to cultivate or restore their degraded land. To establish pastures and restore land has high financial implications (Morris, 2001). Seed is very expensive and the application of active restoration technologies, which often also implies the use of agricultural machinery and labour, increases the costs. Although the number of seed per seed mass is lower, using enhanced seed types could lower the cost if the germination, establishment, density, frequency and biomass production of these species are higher than the non-enhanced seed types. In this study, it was found that the enhanced seed types of *Chloris gayana* and *Cynodon dactylon* with regards to the natural field trials were better than the non-enhanced seed types. Communal farmers, commercial farmers and managers of conservation areas will all benefit from the results of this project, as their costs to purchase seed could be reduced due to better seed type selection and increased germination and establishment percentages.

Overall, not all the enhanced seed types had a better seed purity, germination and establishment percentage, as well as higher above- and below-ground biomass yields than the non-enhanced species. The non-enhanced seed type of *Chloris gayana* had a higher germination and establishment percentage in the laboratory and the glasshouse compared to the enhanced seed type. The seed from the enhanced seed type of *Chloris gayana* had a higher biomass (fresh and dry, above- and below-ground) in the glasshouse, in comparison with the non-enhanced seed type. The non-enhanced seed type of *Cynodon dactylon* and a higher seed purity, germination and establishment percentage and biomass yield under controlled conditions than the enhanced seed type of *Cynodon dactylon.* Under the controlled conditions, the non-enhanced seed type of *D. eriantha* had higher seed purity and biomass (fresh and dry, above- and below-ground) compared to the enhanced seed type. However, the enhanced seed type of *D. eriantha* had a higher germination and establishment percentage under the controlled conditions which do not correlate with the higher seed purity and biomass yielded by the non-enhanced seed type of *D. eriantha*. The *E. curvula* seed type enhanced with "organic insecticide on the base and as overspray". No differences in the development of the structure of the vascular tissue were observed between all the treatments. In most cases, the seed predation of the enhanced seed was lower, but further trials are advised.

4.12 Shortcomings and Recommendations of study

- The germination tests should only be carried out by one analyst to exclude any inaccuracy in the results. With regards to similar studies in this field the time frame of germination test should be taken into consideration, for the seeds dormancy are affected over time. Although no dormancy breaking methods are needed after the seed reaches the age of 6 months or more, this aspect has to be investigated further.

- In future it is advised to only use the dry biomass productions in the glasshouse trials, for the fresh (wet) biomass value mainly includes water and soil from external sources, which distorts the data and makes direct conclusions difficult or inaccurate. Also, only the dry matter production calculated from the natural field trials is used to calculate the grazing capacity of a grass species and it is therefore unnecessary to determine the fresh biomass, because many variations can be noted in the results.

The seedlings also need to be transferred to individual bags with growth medium at an earlier stage, to ensure that the competition factor is eliminated and to ensure that the roots are more easily up-rooted. Planting the seedlings in individual bags prevent roots of neighbouring plants from getting entangled. Depending on the objective of the study, the growth medium must be monitored to control any leaching of minerals that may take place. To ensure that other nutrients and minerals do not have an influence on the controlled conditions, the tap water should be replaced with distilled water in all trials.

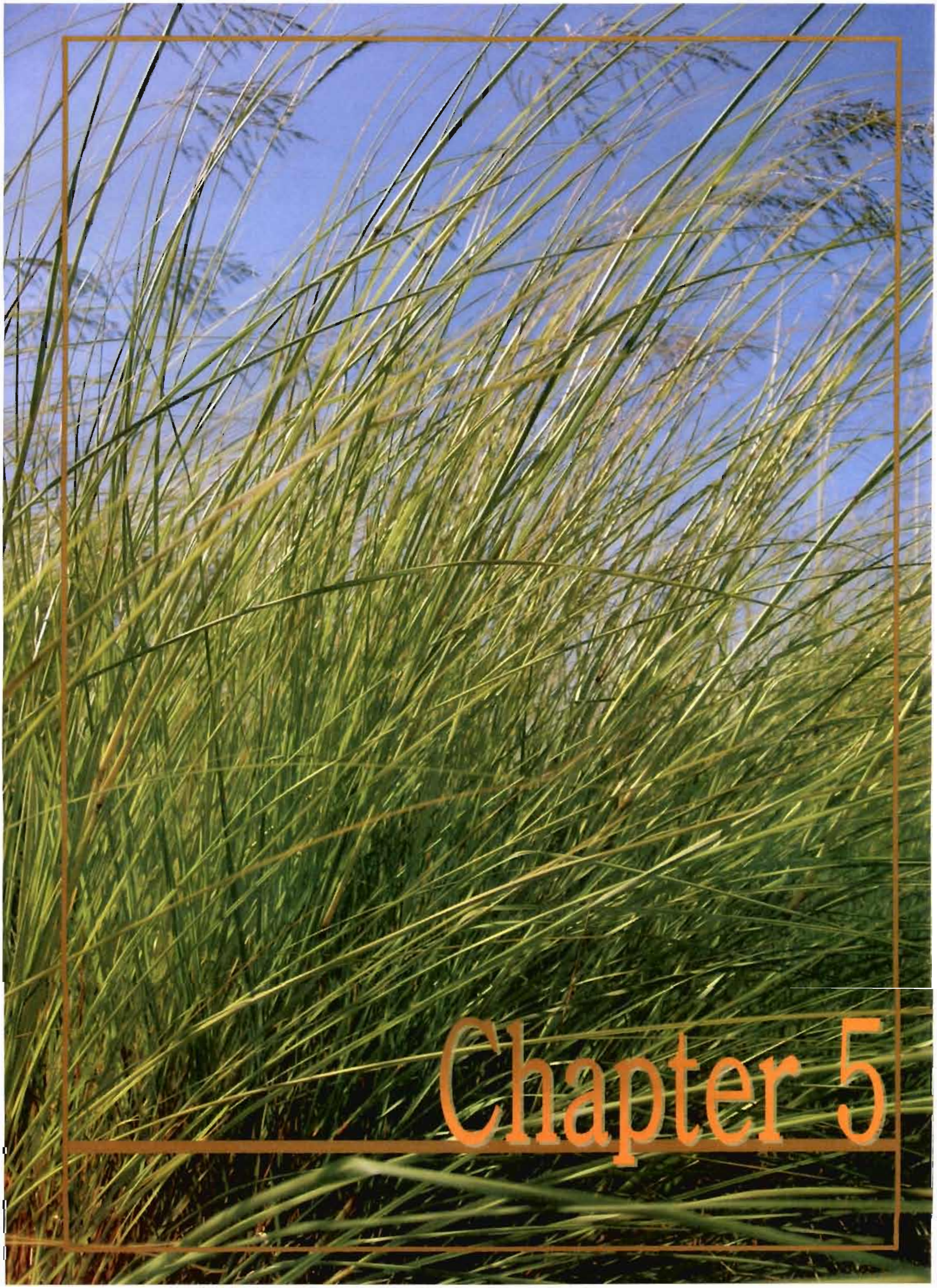
- With regards to the natural field trials, it would be better to have more repetitions (for statistical purposes), although limited resources must also be taken into account. Together with the repetitions, more surveys need to be done in the future to test the long-term productivity and to have more data available to draw more accurate conclusions with regards to the use of enhanced and non-enhanced seed types in re-seeding restoration practices. In order to apply the correct soil ameliorations, soil analyses need to be done directly before re-seeding or seeding activities take place. The soil analyses should be carried out in each sub-plot and monitored over time. Leaf analyses are recommended to determine the uptake of the soil nutrients and the water content within the leaves, which may have an influence on biomass.

- More basal hit counts have to be recorded to give a better answer regarding the basal cover of the tufts, which can then be compared to the frequency and density data.

- No differences with regards to the transition region, which are important in the development of the vascular tissue in the uniting the shoot and roots were noted on an anatomical level. It is, however advised to study more individuals seedlings per treatment, for the quantitative aspect regarding the conducting tissue of the seedlings. Also a study of the root on a physiology level, especially with regards to the influence of the contents of the enhancements on the development of the seedling are also advised to be carried out in future studies.

- After the pilot trials, it is advised to make a few adjustments in the seed predation study.

These adjustments include to only using the "open" cover, with the seed set out in petri dishes filled with soil from the site. The "closed" covering created a hot environment that may have influenced the ant activity under and around the cover. The petri dishes that did not have a soil layer may be foreign to the ants and the petri dishes could have been avoided by the ants, because of the obstruction of the petri dish without the soil in the experiment. The sieve covering should also be placed very carefully over the petri dishes in order to ensure the minimum disturbance in the vicinity of the trial which might have a negative influence on the ant activity. Pitfalls need to be set out to determine the ant population, other predators and different species present on the experimental lands where the seed predation trials take place. These pitfalls can also be placed out in the undisturbed fields to compare the species diversity with that of the cultivated lands where disturbance took place.



Chapter 5

CHAPTER 5

APPENDIX

B. (i) Weighted sample - example

Ref no.	
Seedkind:	
Replicates	Mass
1	
2	
3	
4	
5	
6	
7	
8	
Calculate the following:	
Variance = $\frac{n(\Sigma x^2) - (\Sigma x)^2}{n(n-1)}$ x = weight of each replicate in grams n = number of replicates Σ = sum of	
Standard deviation (s) = √variance	
Coefficient of variation = $\frac{s \times 100}{X}$ X = mean weight of 100 seeds	
If the coefficient of variation does not exceed 6,0 for chaffy seeds, or 4,0 for other seeds the results of the determination can be calculated. If the coefficient of variation exceeds whichever of these limits is appropriate, count and weigh a further eight (8) replicates. Calculate the standard deviation for the sixteen replicates. Discard any replicates which diverge from the mean by more than twice than standard deviation so calculated.	
Final calculation:	
100 seeds =	grams
Therefore: 2500 seeds =	grams

C. (i) Purity analysis - example

No:	%Pure seed	%Inert matter	%Other seed	%Other weed

Seedkind:	Botanical Name:

Submitted sample weight (gram):		
Working sample weight (gram):		
Weight gain/loss (%)		
	Mass in gram	%
Pure seed		
Inert matter (1)		
Other seeds (2)		
Weed seeds (3)		
Total		
1)		
2)		
3)		

Date:	Sign:
Remarks:	

D. (i) Germination test - example

No:	Days:	% Normal	% Hard	% Fresh	% Abnormal	% Dead

Seed kind:			Botanical name:		
Germination Method	Substratum: TP	Temperature: 25°C	Container: Tupperware	Number of Replicates: 4	Seeds per Replicate: 100
Additional recommendations:					
Evaluation intervals:			Date planted:		

Evaluation dates:	A						B						C						D					
	N	H	F	A	D	L	N	H	F	A	D	L	N	H	F	A	D	L	N	H	F	A	D	L
Total																								

Abnormal seedlings (describe): A----------			
B----------			
C----------			
D----------			
Remarks:			
Planted by:	Date:	Evaluated by:	Date:

E. (i) Seed Analysis Report - example

SEED ANALYSIS REPORT

	INFORMATION SUPPLIED BY APPLICANT: SEED KIND: VARIETY: CODE:

ANALYSIS RESULTS

DATE RECEIVED:

DATE TEST CONCLUDED:

WEIGHT OF SUBMITTED SAMPLE (GRAMS):

BOTANICAL NAME:

PURITY (% BY WEIGHT)	PURE SEED	INERT MATTER	OTHER SEED

INERT MATTER:

OTHER SEEDS:

GERMINATION METHOD:	

GERMINATION (% BY NUMBER)	DURATION OF TEST (DAYS)	NORMAL SEEDLINGS	HARD SEEDS	FRESH SEEDS	ABNORMAL SEEDLINGS	DEAD SEEDS

REMARKS:

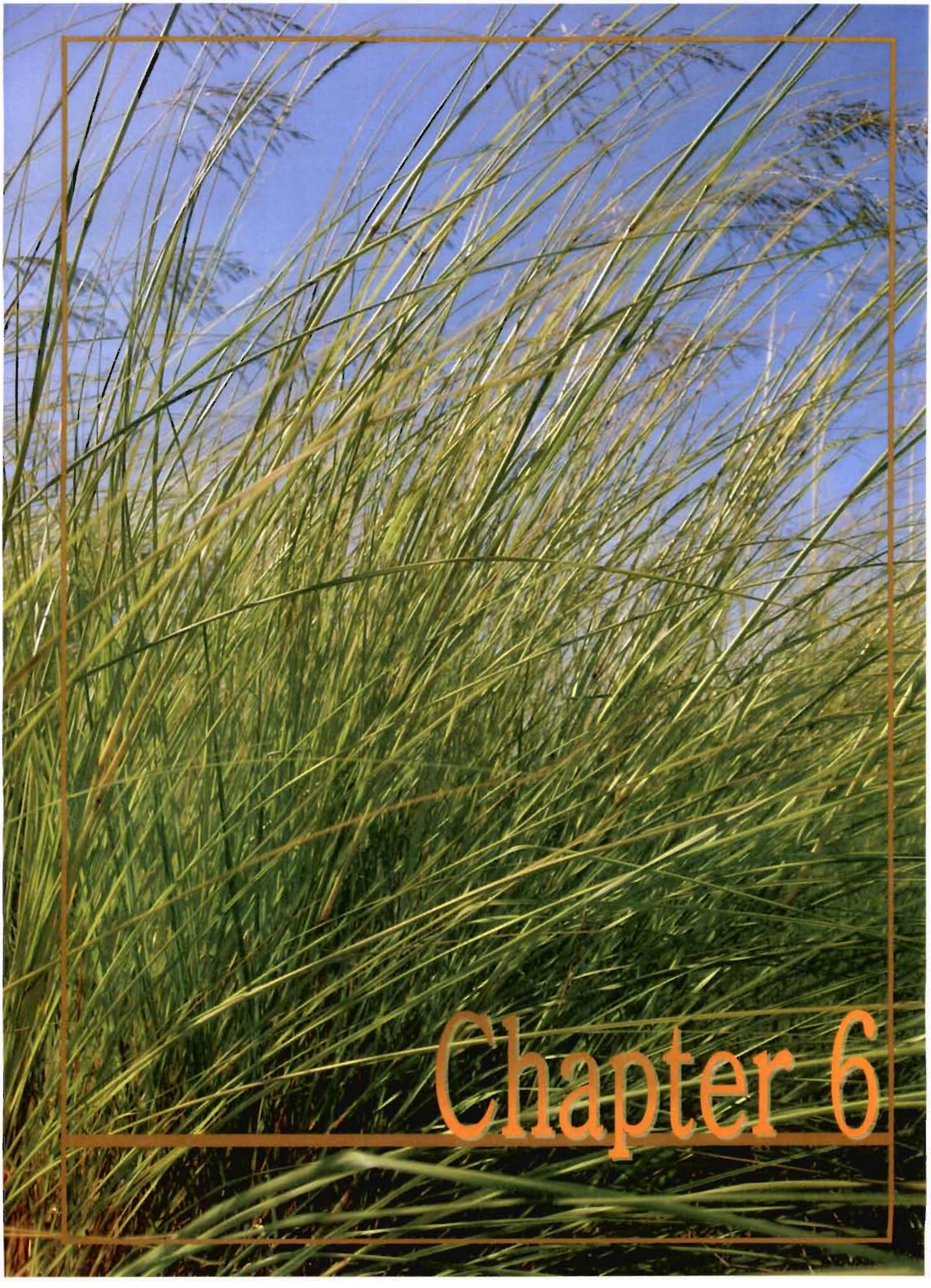
DATE:	SIGN:

H. Natural field data sheet - example

Grass seed type		Block		Sub-plot	

Frequency		Biomass				
ROW						
1	0.5		2.5		4.5	
	1		3		5	
	1.5		3.5		5.5	
	2		4			
2	0.5		2.5		4.5	
	1		3		5	
	1.5		3.5		5.5	
	2		4			
3	0.5		2.5		4.5	
	1		3		5	
	1.5		3.5		5.5	
	2		4			
4	0.5		2.5		4.5	
	1		3		5	
	1.5		3.5		5.5	
	2		4			
5	0.5		2.5		4.5	
	1		3		5	
	1.5		3.5		5.5	
	2		4			
6	0.5		2.5		4.5	
	1		3		5	
	1.5		3.5		5.5	
	2		4			
7	0.5		2.5		4.5	
	1		3		5	
	1.5		3.5		5.5	
	2		4			

Density				
ROW			Weeds estimation	
1				
2				
3				
4				
5 6				
7				



Chapter 6

CHAPTER 6
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