Agroecological studies of *Desmanthus* – a tropical forage legume

Thesis submitted by
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in November 2005

for the degree of Doctor of Philosophy
in Tropical Plant Sciences
within the School of Tropical Biology
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STATEMENT ON SOURCES

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José Henrique de Albuquerque Rangel

ABSTRACT

The use of forage legumes in tropical regions to improve the efficiency of animal production from grazing has been limited, largely because of the lack of economic incentives. There is a clear need, therefore, to investigate the existing gene pool of tropical forage plants to assess their potential for pasture improvement. Therefore, the present study evaluated the agronomic and ecological aspects of plant development in a set of genotypes of the genus *Desmanthus* and their relationships with the components of the surrounding environment, at different stages of growth, in a series of laboratory and field experiments.

Accessions of the genus *Desmanthus* formed permanent soil seed banks that ranged from 281 to 1303 seeds/m^2, with a large variation between genotypes, in experiments on a duplex soil on the Douglas Campus of James Cook University, Townsville. Genotypes originally collected in Argentina had larger seed banks than those of other tested genotypes, but a small number of surviving plants.

Fire increased seedling recruitment in almost all observed genotypes. Temperatures observed during controlled grass-fires reached a maximum of 300 ºC at the soil surface, 80 ºC at 10 mm depth, and around 30 ºC at 30 mm depth suggesting that all seeds located at soil surface were killed, those at 10 mm depth were probably softened, and those at 30 mm or more in soil had no alteration in their seed-coat permeability.

Changes in strophiolar structure and germination, in response to the variation of oven temperatures ranging from 25 ºC to 120 ºC were observed in seeds of nine genotypes of *Desmanthus*. There were two groups with different patterns of responses: genotypes in which strophiolar structures were not significantly affected by temperatures below 80 ºC; and genotypes with significant changes in the strophiolar structures when temperature rose to 60 ºC.

Seedlings of 8 accessions of the *Desmanthus* complex, growing directly under trees in open savanna woodland had higher values of means for number of leaves/plant, height of plant, and number of plants surviving than seedlings growing between trees. Three years after sowing, all plants from the between-canopy environment had died, while many plants of accessions TQ88, CPI 79653, and CPI 91162 were thriving under the tree canopy.

Plants of *D. virgatus* CPI 78382 and *D. leptophyllus* TQ 88 growing in soils collected from under and between canopies had significantly increased their seedling emergence, by increasing shade levels and watering frequency. A low number of seedlings died in both genotypes, growing in soil from under the canopy but, plant deaths drastically increased in seedlings grown in soil from between the canopy. Growing in soil collected from under-canopies, plants allocated most of their dry matter to the production of aerial, rather than the underground parts, however, when grown in soil from between-canopies environment the largest proportion of the total dry matter was diverted to the underground parts. This diversified behaviour of biomass allocation for shoot and root in the two soils is thought to be controlled by the contents of nutrients in soil.

Seven accessions of the *Desmanthus* complex, sown into a pasture as seeds or seedlings, under two levels of competition with the natural vegetation, showed to have differentiated behaviour according the different treatments. Plant establishment and dry matter yields of plants sown by seed into unaltered vegetation were significantly reduced by competition.

The effect on liveweight changes and wool growth of Merino sheep of 200 g hay of four different forms of the *Desmanthus* complex included as a supplement to a diet of 600 g Mitchell grass (*Astrebla* spp.) was compared with 200 g hay of *Stylosanthes hamata* cv. Verano. Verano and *D. virgatus* CPI 79653 supplemented diets had the highest dry matter digestibility (46.52% and 44.94% respectively). All the legume-supplemented diets produced significantly more wool than the control. Clean wool growth was significantly correlated with nutritional parameters. The levels of nitrogen and sulphur present in some *Desmanthus* genotypes shows the potential of these plants in promoting wool growth.

AKNOWLEDGEMENTS

I am most grateful to Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA, Brazil) for the financial support to carry out this research project.

I would like to express my gratitude to my supervisors Dr Robert L. Burt and Dr Ross Coventry, the first for his guidance and encouragements during the period the work was carried out and initially written, and the second by his wise and constructive suggestions in the final written work.

Grateful acknowledgments are made of the valuable help and advice given by members of the academic staff of the School of Tropical Biology of James Cook University. In particular I wish to thank my "Aussie brother" Chris Gardiner, for his help in field and laboratory work and in all my needs in Australia, and to Professor D. Griffiths for the accurate reading and precise suggestions on a draft of the manuscript.

I wish also to extend my sincere thanks to Dr J. Hogan of the CSIRO Davies Laboratory at Townsville, for providing facilities and advice in the first steps of my research program at CSIRO Landsdown Research Station.

I express sincere thanks to my friend Dr Peter Durr for his valuable help on numerous occasions.

Finally, I would forever thank the wonderful people of Australia for the friendship and affection given to myself and my family.

This thesis is dedicated in memory of my parents Fernando and Cely, and to my brothers Vital, Fernando, and Mario, my sister Catarina, my wife Salete, my sons Fernando, Andre, and José, and my daughter Jean, from whom I always had the greatest of the love and support.

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Glossary of Terms

Abscission scar or h**ilum**. The scar at the point of attachment of the seed to a funicle (Cutler 1978; Raven *et al.* 1985).

Acid detergent fibre. The insoluble residue left from boiling a substance in a solution of acid detergent for 1 hour and filtering. Consists mainly of cellulose, lignin, and silica (Lassiter and Edwards 1982); the cellular wall components of forages (cellulose, lignin, and minerals) not soluble in acid detergent (Silva and Queiroz 2002).

Ad libitum. Applied to feeding animals meaning allowed feed in accordance with desire (Lassiter and Edwards 1982).

After-ripening. It is a process necessary for the completion of certain metabolic changes in seeds before germination is possible (Debenham 1971, Murdoch and Ellis 1992). It depends upon the environment and is usually accelerated at high temperatures (Gardener 1975).

Agroforestry system. It is a farming system that integrates crops and/or livestock with trees and shrubs (Sanchez 1999; Beetz 2002).

Alley-crop. It is a method of growing perennial species, usually shrub or tree legumes, together with annual crops **(**Stirzaker and Bunn 1996**).**

Bipinnate. Is said of a compound leaf when secondary leaflets (**pinnules**) arising along a secondary rachis (**rachilla**), the primary (compound) leaflets being termed **pinnae** (Debenhan 1971).

Boss. See Strophiole.

Brigalow. Country where brigalow, a species of acacia (*Acacia harpophylla*), is the main vegetation. “Extensive stands of Brigalow forests occurred on clay soils of

South West and Central West Queensland. Now mostly cleared for agriculture" (Griffith University 2005).

Browsing. When animals such as deer or goats browse, they feed on plants, especially on their young twigs or leaves, in an unhurried way (Sinclair 1988).

Caatinga. It is a type of Brazilian Northeast forest which is deciduous during the hot and dry season and includes a number of thorny species (Sinclair 1988).

Caudex, or flange. The perennial base of an otherwise herbaceous plant (Swartz 1971).

Cerrados. Is the name given to the Brazilian savanna. Around 85% of the large plateau of Brazilian central region was originally dominated by cerrados landscape, representing some thing like 1.5 to 2 millions of km^2, or 25% of the country surface (Pivello 2005).

Chartaceous. Of parchment or paper-like texture, usually devoid of green (Debenham 1971).

Clean wool. Processed wool, free of grease, soil particles, and vegetable matter (DPI 2004).

Cohabitant species. Mixtures of different plant species growing on the same patch of land (Happer 1977d).

Coated seed. Seed having an integument cover composed of layers (Swartz 1971).

Crown. The persistent base of a tufted grass (Swartz 1971).

Cuticle. A fatty and fat-derived layer of cutin in the seed outer wall, serving as a barrier to water and gas exchange but permeable to the diffusion of aqueous solutions through cracks and ridges to a limited degree (Debenham 1971, Cutler 1978); layer of wax or fat covering the external wall of epidermal cells (Raven *et al*. 1985).

Dorsi-ventrally flattened. Flat at both dorsal and ventral sides (Debenham 1971).

Endosperm. The multicellular food-storing tissue consisting chiefly of starches and oils, providing nutrient for the developing embryo formed inside a seed of flowering plants, following the double fertilization of the embryo-sac by the second sperm nucleus (Debenham 1971, Swartz 1971); a nutrient tissue formed within the embryo-sac of the spermatophyta (Cutler 1978).

Epidermal, Prism, or **Malpighian cells**. The outermost cells layer of primary tissues of the plant, sometimes comprising more than one layer (Cutler 1978). In the seed coats of certain plants (specifically in the legumes) a layer of radially elongated cells, which are palisade-like but devoid of intercellular spaces, may be present. These cells have been termed *Malpighian cells* after the investigator who first described them (Fahn 1974).

Evapotranspiration. The loss to the atmosphere of moisture from both the soil (evaporation) and its vegetative cover (transpiration) (Answer.com 2005) (Physical Geography 2004).

Falcate. With the shape of a lamina when flat and curved (like a reaper's hook, or sickle) (Debenham 1971).

Fire-recruitment syndrome or **refractory seed syndrome.** The condition by which certain seeds require the occurrence of a fire, for germination from the soil seed bank (Kelley 1991).

Flange. A projecting edge on an object used for strengthening it or for attaching it to another object (Sinclair 1988).

Forbs. Herbs others than grasses or sedges (Debenham 1971, Swartz 1971).

Funiculus. The stalk attaching an ovule to the placenta (Cutler 1978).

Greasy wool. Wool as it is shorn before washing or sorting (Wimburne 1982).

Hardseededness or **seed-coat dormancy**. The condition of having a sed coat that is impermeable to water (Fahn 1974).

Hilum, or **abscssion scar**. The scar at the point of attachment of the seed to a funicle (Cutler 1978; Raven *et al.* 1985).

Hour-glass cells. A single layer of cells forms the hypodermis, which is also, called hourglass cells, pillar cells, osteosclereids or lagenosclereids, depending on their pattern of cell wall thickness and shape. They are usually larger than adjacent cell layers and are separated by wide intercellular spaces, except under the hilum cleft where they are absent (Souza and Marcos Filho 2001).

Hypocotyl. The short stem of an embryo seed plant, the portion of the axis of the embryo seedling between the attachment of the cotyledons and the radicle (Swartz 1971); the part of the axis marking the transition of root and stem development (Raven *et al.* 1985).

Innate dormancy, or **primary dormancy.** The physiological inhibiting mechanism of germination in the embryo; physiological dormancy **(**El-Keblawy 2006**).** The process of growth of an embryo to a stage fit for the germination process to occur, has not been completed while the embryo was still born on the parent plant (Haper 1977b)

In vivo dry matter digestibility. The apparent digestibility of the dry matter in animal fodder. The difference between the dry matter intake and its faecal excretion (Lassiter and Edwards 1982).

Lens, boss, or **strophiole**. See strophiole.

Llanos. Spanish American term for prairies, specifically those of the Orinoco River basin of North South America, in Venezuela and East Colombia. The llanos of the Orinoco are a vast, hot region of rolling savanna broken by low-lying mesas, scrub forest, and scattered palms. Elevation above sea level never reaches more than a few hundred feet. During the dry season (November to April) the land is sear, the grass

brown, brittle, and inedible; during the rainy season much of the area is inundated (Answers.com 2005).

Malpighian cells, palisade macrosclerid cells, **epidermal**, or **prism.** Plant cell on the surface of a leaf or other young plant tissue, where bark is absent. The exposed surface is covered with a layer of cutin (Biology Dictionary – Biology on Line 2005).

Metabolizable energy. The gross energy value of a food from which energy losses in faeces, urine, and gaseous products of digestion have been subtracted (Lassiter and Edwards 1982).

Micropyle. It is a structure located close to the hilum and represents the former passage for the pollen tube through the integument of the ovule (Tran and Cavanagh 1984); a small opening between the integuments at the free end of an ovule (Cutler 1978).

Mulga. A vegetation community of wide occurrence in the arid parts of Australia in which the shrub mulga (*Acacia aneura*) usually is a dominant (Debenham 1971).

Neutral detergent fibre. The cellular wall components of forages (cellulose, hemi-cellulose, lignin, protein, and minerals) not soluble in neutral detergents (Silva and Queiroz 2002); the part of a feed that is not soluble in boiling neutral detergent solution (3% sodium laurel sulfate); mostly cellulose, lignin, silica, and hemicellulose on the cell walls (Lessiter and Edwards 1982).

Obligate seeder. Group of plants that can regenerate only by the recruitment of seedlings from the soil seed bank (Bell 1985; 1994; Pate *et al.* 1990).

Palisade macro-sclereid cells. A layer of elongated cells in plant seeds set at right angles to the surface of the seed (Swartz 1971); because the shape and the thickness of the Malpighian cells they are also termed macrosclereids (Fahn 1974).

Papillate. Surface with superficial protuberances (Swartz 1971).

Parenchyma sclereid layer. A layer of tissue composed by cells with lignified, thick and pitted walls, involving the cotyledons of legume seeds (Swartz 1971; Van Staden *et al*. 1989).

Paripinnate. Is said of a pinnate leaf when there is no terminal leaflet of the rhachis, i.e. the rachis ends in a leaflet-pair (Debenhan 1971).

Plant plasticity. The potential of plants to adapt to a large array of growth conditions and even to temporarily suspend active metabolism in order to withstand environmental conditions, not suitable for 'normal life'. The responsive adaptations of plant species to their environments lead to differences in growth rate and productivity and to differences in water or nitrogen use efficiency ('plant plasticity'). Ultimately adaptation will result in increased survival and reproduction (Experimental Plant Science 2005).

Pleurogram. A seed structure often present and complete in the Mimosaceae, rarely present and open in the Caesalpinaceae. It is visible in immature seeds as a localized area where the epidermal cells are shorter than the cells from other areas, and the area at the base of the shorter cells is filled in by parenchyma cells derived from periclinal division in the young hypodermal cells. In the mature seeds the pleurogram is visible as a fissure extending completely through the epidermis (Van Staden *et al.* 1989).

Prism, palisade macrosclerid cells, **epidermal**, or **Malpighian cells.** Plant cell on the surface of a leaf or other young plant tissue, where bark is absent. The exposed surface is covered with a layer of cutin (Biology Dictionary – Biology on Line 2005).

Residual hardseededness. Amount of hard seeds remaining after 21 days of germination test (the author).

Resprouters. Group of plants that regenerates from buds located in underground organs (Bell 1985; 1994; Pate *et al.* 1990).

Rugulate. With a wrinkled surface, marked by irregular raised or depressed lines (Debenham 1971, Swartz 1971).

Rugulate-papillate. Wrinkled surface with superficial protuberances (Swartz 1971).

Sclerid or sclereid. A unit of sclerenchyma, a cell with a lignified, thick and pitted wall and usually devoid of, or with very little protoplasm (Debenhan 1971). A sclerotic or stone cell, a sclereid (FAO 2006)
Glossary www-ididas.iaea.org/IDIDAS/w3.exe$GloSearch?ID=15462

Seed imbibition. The first step in seed germination is **imbibition**. In this process, water penetrates the seed coat and begins to soften the hard, dry tissues inside. The water uptake causes the grain to swell up. The seed/fruit coat usually splits open allowing water to enter even faster. The water begins to activate the biochemistry of the dormant embryo (Koning 1994).

Seed softening. Natural or enforced breakdown of seed-coat dormancy in legume seeds (Mott *et al.* 1981).

Seed-coat dormancy, or **hardseededness**. See hardseededness.

Seedling recruitment. Emergence of seedling from seeds stored on a soil seed bank, normally occurred after a fire event (Bebawi and Campbell 2002).

Sessile. Is said of a structure when borne without a supporting part, e.g. the petiole of a leaf, the filament of an anther, the pedicel of a flower (Debenhan 1971).

Shade tolerance. Plant adaptation to reduced levels of incident sunlight (Benjamin *et al.* 2005) http://www.aciar.gov.au/web.nsf/att/JFRN-6BN8Y2/$file/pr32chapter16.pdf

Silvipastoral system. A class of agroforestry system characterized by the presence of animals grazing between or under the canopies (Sanchéz 1999).

Soil saturation. Wet soil in which all the pores are filled with water (Foth 1990).

Soil seed bank. Pool of seeds that, having some type of dormancy after release from the plant are incorporated into the soil and stay stored for undetermined periods (Grime 1979).

Strophiole, Boss, or **Lens**. An excrescence or appendage at or about the hilum of a seed, the caruncle (Swartz 1971); after the fertilization of the ovule, growths termed arils, develop on the surface of the seeds of certain plants. These growths, when they occur on the funiculus (e.g. *Euonymus* and *Acacia* spp.), are often termed *strophioles* and when occurring around the micropyle (e.g. *Ricinus*), are called *caruncles* (Fahn 1974).

Subcuticle. Under the cuticle, epidermis, or outer skin (Suartz 1971; Holmes 1985).

Suberecte. **A**lmost erect, slightly erect or somewhat erect (Botanical Glossary 2005).

Suffruticose. The same as suffrutescent, slightly shrubby (Swartz 1971; Holmes 1985).

Tap-root. The primary persistent root typical of dicots and gymnosperms from which lateral roots are developed in acropetal succession (Debenham 1971); a water storage structure known as a 'xyllopode' (Burkart 1952; Carvalho and Mattos 1974).

Thermocouple. Thermocouples are pairs of dissimilar metal wires joined at least at one end, which generate a net thermoelectric voltage between the open pair according to the size of the temperature difference between the ends, the relative Seebeck coefficient of the wire pair and the uniformity of the wire-pair relative Seebeck coefficient (Temperature.com 2006). www.temperatures.com/tcs.html.

Testa or seed-coat. Represents the hardened integuments of the ovule. In seeds of the legumes it is covered by a very thick cuticle. This and another layer of the testa may prevent the passage of water and air as long as it is undamaged (Fahn 1974).

Water use efficiency. It is the relationship between plant production and water plant water uptake from soil below. Can be expressed as the ratio of plant dry matter yield and the plant water uptake in weight (Durr and Rangel 2003).

Xylopode. A more or less stony, hard, tuberous thickening of the roots and underground parts of shrubs in steppe regions in Brazil (Swartz 1971).

Chapter 1
INTRODUCTION

1.1 Aim of this thesis

The aim of the present study was to explore the adaptation of plants of the genus *Desmanthus*, tropical forage legumes of the family Mimosaceae, to some of the key limiting environmental conditions in the rangelands of the dry tropics of North Queensland. The research also explored the nutritional value of *Desmanthus* as a supplement of roughage diets to improve wool growth in sheep. The botanical names of the accessions of *Desmanthus* used in the present study are based on the studies of Luckow (1993), Pengelly (2000), Pengelly and Conway (2000), and Pengelly and Liu (2001), and listed in the web site of Australian Plant Genetic Resource Information Service (AusPGRIS 2004). The field experiments were carried out at James Cook University, Townsville, Queensland, a centre for research in the dry tropics, on duplex soils which are commonly found in such regions. Plant and animal nutrition studies were conducted at the CSIRO Research Station Landsdown, located 50 km south of Townsville, and at the CSIRO Davies Laboratory, which is adjacent to James Cook University. Factors studied included **soil seed bank**[1] formation, the effect of fire on seedling recruitment from the soil seed bank, the influence of heat in overcoming **seed-coat dormancy**, germination rates, the effect of light, water stress, and soil conditions on plant establishment, and responses to defoliation.

Desmanthus was chosen because of its potential as a plant suited to the improvement of pastures on hard clay soils in dry tropical and subtropical regions: it is the only sown pasture legume genus so far tested that can persist and spread in such conditions in Northern Australia (Cook *et al.* 1993; Gardiner *et al*. 2004). Additional studies were therefore carried out to study the effect of feeding various forms of *Desmanthus* to sheep and to compare the responses with those obtained with *Stylosanthes hamata* cv. Verano, the main forage legume currently sown on sandy-surfaced soils in dry tropical areas.

[1] The specific meaning of certain terms are given in the Glossary at the front of this thesis. The first use of any term listed in the Glossary is indicated in the text by **bold** format.

Because of the need to fit the present research into a 3-year time frame, work on the effect of fire could not be tested on sown stands of *Desmanthus* genotypes. Instead, such work was carried out on small plant stands, or on hay collected originally for other purposes. It is for these reasons that the same genotypes could not always be used throughout the studies reported in this thesis.

1.2 Role of legumes in pastures

Native and introduced forage legumes have, since ancient times, played an important part in improving animal production and enhancing soil nitrogen contents in temperate areas. In tropical regions, however, their use has been less intense, largely because the economic incentives to improve the efficiency of animal production from grazing have been lacking (Williams *et al.* 1985; Peterson 1988). There is a clear need, therefore, to investigate the existing gene pool of tropical forage plants to access their potential for pasture improvement.

Much knowledge about the use of tropical forage legumes has come from studies carried out in Australia, and somewhat less from other tropical countries such as Brazil and Colombia (Burt 1993). Despite the assistance of government authorities and private corporations in supplying graziers with adapted forage legumes for their pastures, only a small number of species have been brought into general use. One of the major problems in developing forage legumes for the tropics is the need to cater for the large environmental diversity encountered. There are often abrupt changes in soil conditions, and rainfall and temperature regimes, over short distances (Johnson and Brown 1986; Wheeler and Freer 1986).

For the wet tropics, despite these limitations, there are a number of potentially useful forage legumes available. They include the genera *Centrosema, Macroptilium, Clitoria, Leucaena, Stylosanthes,* and *Arachis.* Under dry tropical conditions, however, a much narrower range of pasture plant cultivars is available, and these are mostly from the genus *Stylosanthes* (Burt and Williams 1975; Burt 1993).

The 44 species and sub-species of the genus *Stylosanthes* have been referred to by Edye and Cameron (1984) as “the most important source of pasture legumes for

tropical and sub-tropical environments". Indeed, since the accidental introduction into Australia of *Stylosanthes humilis* and the naturalising of its cultivar "Townsville stylo", in the early 1900s, the genus *Stylosanthes* has played a very important role in the Australian pastoral industry, particularly in the low fertile soils of semi-arid areas, where native grasses represent the main source of feed for grazing animals. The legumes provide ruminants with access to green forage with a high digestibility and high protein content for some weeks if not months after senescence of the native grasses, reducing cattle weight losses during the dry period (Burt 1993).

Stylosanthes humilis and *S. guianensis* were for long time, the only two species of the genus to be recognised as pasture legumes. In 1967, a detailed agronomic study of a wide range of *Stylosanthes* species was initiated in Australia by CSIRO in Townsville (Edye *et al*. 1984). As a result of this work, the list of released stylos now has many other new species and cultivars including: *S. hamata* (Verano and Amiga cultivars), *S. scabra* (Seca cultivar) and, more recently, *S. seabrana*. These new stylos are now widely used across the 1.5 million ha of the Australian dry tropics (Clements 1996). In South America, a joint venture of the Centro Internacional de Agricultura Tropical (CIAT) and Empresa Brasileira de Pesquisa Agropecuária (EMBRAPA), developed two other *Stylosanthes* species for tropical pastures: *S. capitata* and *S. macrocephala* (Burt 1993). Both of these are well suited to the edaphic constraints of strong acidity and high aluminum contents of the soils of the **Cerrados** of Brazil and the **Llanos** of Colombia.

Stylosanthes species do not thrive well, however, in sites where the annual rainfall drops below 750 mm, or in heavy clay soils (Burt 1993). Thus, there are currently no introduced legumes suitable for the approximately 26 million hectares of clay soils of the Mitchell grass (*Astrebla* spp.) plains of semi-arid North Queensland, and other similar regions of the world's tropics.

Other disadvantages of the use of *Stylosanthes* spp. is their susceptibility to the fungal disease anthracnose caused by the fungus *Colletotrichum gloeosporioides* (*Glomerella singulata)*, and the low levels of sodium and phosphorus often found in *Stylosanthe*s plant tissues (Burt 1993).

1.3 *Desmanthus* for tropical pastures

The search for new tropical forage legumes to supplement *Stylosanthes* species and cultivars in places where they are subjected to edaphic, climatic, or disease limitations, stimulated an interest in the genus *Desmanthus.* This genus appears to offer the possibility of fulfilling the role of *Stylosanthes* in such places. Consisting of 24 species (Luckow 1993) or 18 species (Burt 1993), *Desmanthus* belongs to the tribe Mimoseae, the family Mimosaceae (Luckow 1993). With only two exceptions, the genus is native to the Americas and the associated offshore tropical islands of the Caribbean. The exceptions are a controversial Madagascan species (*Desmanthus arboraceus*; Burt 1993) and *Desmanthus pernambucanus,* described by Luckow (1993) as a pantropical weed. Pengelly and Liu (2001), using a random amplified polymorphic DNA technique to assess variation in tropical *Desmanthus* plants, suggested that much of the *D. virgatus,* reported as having colonized extensive regions of the tropics, may be *D. pernambucanus*.

The genus shows wide variation both within and between species. Burt (1986) described six forms of *D. virgatus* and these differed widely in both agronomic and morphological characteristics (Fig. 1.1). Some species have strong lignotubers which probably confer resistance to both fire and grazing; an important characteristic for a pasture species.

There is also considerable variation among genotypes in the tannin content of the leaves (R.L. Burt, personal communication). Tannins can bind plant proteins and slow down release of minerals into soil from decomposing plant leaves, a feature of potential importance in semiarid tropical ecosystems where soil organic matter is scarce. How the nitrogen that returned to the soil is released also varies among genotypes. Sulphur, an element often deficient in animal feed in dry tropical areas, is much higher in *Desmanthus* seed than in other forage legumes, but varies between genotypes (Schlink and Burt 1993).

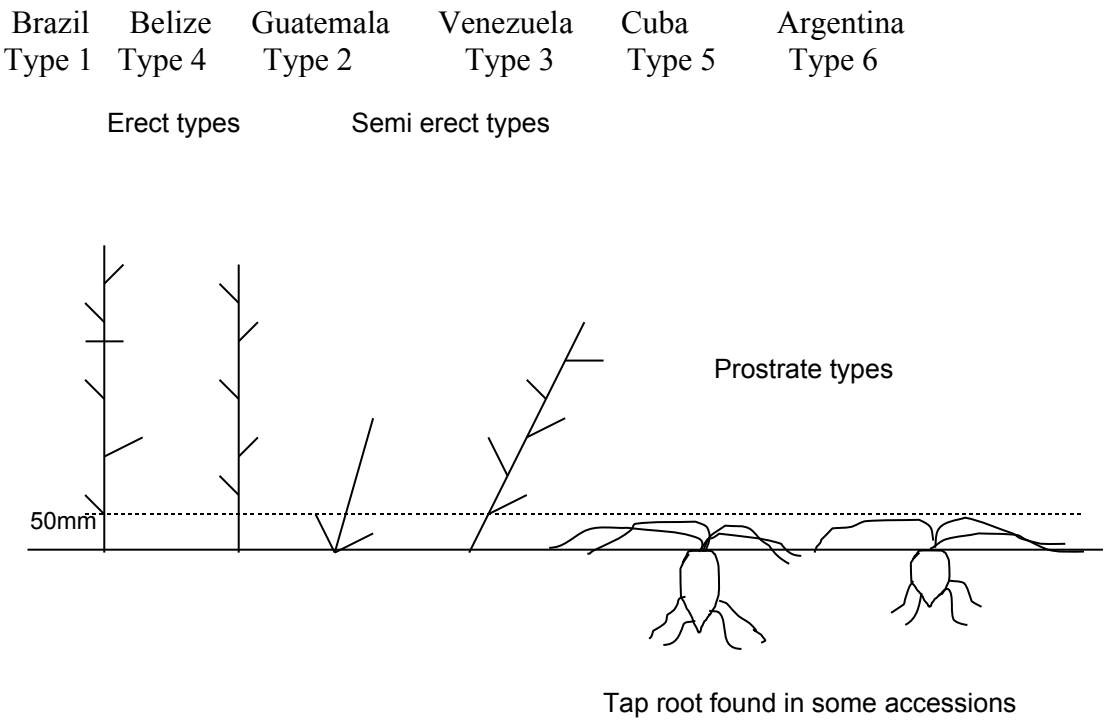

Figure 1.1 Variation among forms of *Desmanthus virgatus* complex, collected in Central and South America (R.L. Burt, unpublished data).

The Figure 1.2, proposed by Partridge (2003) gives a general characterisation of the aerial parts of a plant of *Desmanthus.*

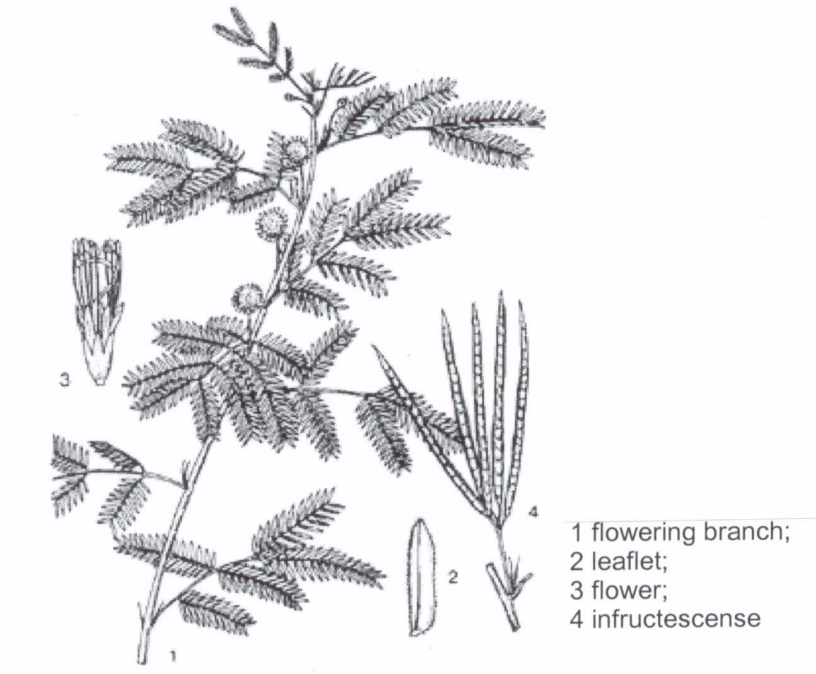

Figure 1.2 Aerial parts of a *Desmanthus* plant. Adapted from Partridge (2003).

R.L. Burt (unpublished data) referred to *Desmanthus* as forming an effective association with rhizobia from the genus *Neptunia,* one of the most common, though

often toxic, legumes on the clay soils of tropical areas, including Australia. According to Turner and Beaman (1953), Windler (1966), Lewis and Elias (1981), and Luckow (1993), *Desmanthus* and *Neptunia* are closely related taxonomically. This parallels the links between *Stylosanthes* and *Zornia*, a legume taxonomically related to *Stylosanthes*, and native to Australia (t'Mannetje 1984).

It should be noted, however, that based on the evidence of a phylogenetic analysis of combined data from chloroplast DNA restriction sites, and the comparative morphology of six genera in the *Dichrostachys* and *Leucaena* groups, Luckow (1997) concluded that *Desmanthus* is a monophyletic genus and not paraphyletic with *Neptunia*. *Desmanthus* is envisaged as more closely related to *Leucaena* and *Schleinitzia* than to any other genus; while *Neptunia* has an ambiguous position relative to the other genera (Luckow 1997).

Some results showing the agronomic potential of *Desmanthus* in both Australia and overseas are currently available. They include:

- Easy accessibility for cattle, the absence of toxic compounds in leaves and stems (Carvalho and Mattos 1974; Battad 1993), and easy harvesting of their pithy stems (Battad 1993), have been pointed as advantages of *Desmanthus* over *Leucaena.*
- In Peninsular Florida, USA, seeds of *Desmanthus illinoensis*, maintained their germinative potential as high as 82% when stored for four years at 16 °C and 40% relative humidity (Call 1985). Seed yield of this species, however, dropped from

1700 kg/ha, in the first year of cultivation, to nearly zero, after 4 years of propagation (Piper and Towne 1988). Epp (1989) suggested that a decline in seed yield over the time of the crop is restricted to some accessions of *D. illinoensis* only, while other accessions can maintain their productivity over successive years of cultivation

- Also in Peninsular Florida, management strategies involving periods of no grazing during the growing season, were shown by Muir and Pitman (1991), as necessary for the persistence of *Desmanthus virgatus*.
- Frequent, but light defoliation was indicated by Trujillo *et al*. (1996) to maximise dry matter productivity of *D. virgatus* accession IRFL 1857 in Bartow, Florida.

- The crude protein and carbohydrate contents of 38% and 35%, respectively found in seeds of *Desmanthus illinoensis* led Kulakow *et al*. (1989) to compare their nutritional value favourably with soybean and to aim the development and use of seeds of *D. illinoensis* to meet human nutritional needs.
- At St. Croix in the Caribbean, there was no observed improvement in total dry matter yield by combining grass with *Desmanthus virgatus* from **alley-crops**, and comparing them with yields from sole grass crops (Adjei 1995). The higher concentration of crude protein in the legume however, would be a definite advantage towards improvement of overall feeding value of forage products (Adjei 1995).
- The nutritional characteristics found by Gardiner and Rangel (1996) in accessions CPI 79653, CPI 92803 and CPI 78382 of *D. virgatus,* and CPI 38351 of *D. leptophyllus*, indicated that these plants offer a valuable feed resource for livestock in all seasons.
- Sulphur contents higher than 9 ppm found in the seeds of the accessions CPI 38351 of *D. leptophyllus* and CPI 92809 of *D. pubescens,* were considered by Schlink and Burt (1993) as indirectly beneficial to the nutrition of animals fed roughage deficient in that element.
- In Australia, *Desmanthus virgatus* CPI 78373 was among the forage legumes which are consistently ranked highly for persistence, regeneration, production, and spread, when tested in the cracking clay soils of north-eastern Queensland, and was considered "promising as pasture or short-term ley" for such soils (Clem and Hall 1994).
- Adaptability, persistence, drought resistance, and grazing/mowing tolerance, were attributed by Gardiner and Burt (1995) to the accessions of *D. pernambucanus* CPI 40071 in Java, Indonesia; *D. leptophyllus* CPI 38351 and TQ 88, *D. pubescens* CPI 92803, *D. virgatus* CPI 37143, and *Desmanthus* sp. Alligator Creek, at James Cook University, north Queensland; and to the accession CPI 78382 of *D. virgatus* in four sites of semi-arid zone of Queensland, Australia (Jones and Rees 1997).
- Long roots found by Veasey and Martins (1991) in *D. virgatus* accession 2027 (Tanquinho da Feira/BA, Brazil) was thought to constitute a physiological adaptation of the plant to dry and poor soil nutritional conditions.

- Deficiencies of S, Mo, P, Cu, and Mn on a black earth soil at Gayndah in southeastern Queensland, and of S, Mo, and P on euchozem soil at Walkamin, in northeastern Queensland, were responsible for the development of chlorosis and low yields of inoculated *Desmanthus virgatus* (Brandon and Date 1998; Spies *et al.* 1998).
- Studies carried out on 8 clay soils of central and southern Queensland, Australia demonstrated that the commercial cultivars of *Desmanthus,* cv. Marc, Bayamo, and Uman, responded to inoculation with *Rhizobium* strain CB3126 when sown into soils with few or no native rhizobia (Bahnisch *et al*. 1998; Brandon *et al.* 1998).
- Nicking the seeds or soaking them in concentrated (17 M) H_2SO_4 for 40 minutes, were found to be the best treatments to break down seed-coat dormancy in seeds of *Desmanthus virgatus* accession 436898 of the USDA-SCS Plant Materials Center, Knox City, Texas, USA (Fullbright and Flenniken 1987).
- Buried to depths greater than 2 cm in clay soils prevent germination of seeds of cv. Marc, Bayamo, and Uman (Brandon and Jones 1998).

Many of the foregoing studies were carried out with species of the *Desmanthus virgatus* complex with no relation to a specific accession number, and do not include all the known plant forms, which may vary from small, fine prostrate herbs to large, **suffruticose**, erect plants. No doubt, the agronomic results obtained will vary with the form of the test plant used, and it is difficult to draw general conclusions. As was noted by Harper (1977a), the behaviour of plants that form pastures must be determined by considerations of fitness of individuals rather than that of the whole population.

1.4 Adaptation of *Desmanthus* to the semi-arid tropics

Before a newly introduced plant is judged to have been adapted to specific local or regional conditions, a series of ecological and agronomic studies should be conducted. The main focus in ecological studies should be on the relationships of the plants with the surrounding environmental components. "The factors limiting plant production should be studied to determine the unalterable constraints of the environment which the introduced species will have to tolerate" (Williams *et al.*

1985). These unalterable constraints clearly include light, temperature, soil chemical and physical characteristics, and water as the most important of the physical components.

The biotic components involve the impact of humans, animals, and neighbouring plants. As McWilliam (1978) has pointed out, these factors are particularly important at certain critical stages of development, such as seed germination and seedling establishment and their interactions play a major role in determining the level of plant adaptation to major climatic and edaphic conditions. The way in which environmental components are related to a pasture plant at different stages of its life cycle is proposed in Fig. 1.3. This model starts at "seed dispersal" where wind, water, and animals play very important roles. Animals can work as seed predators either during dispersal events and after the seeds have been deposited on the soil surface or buried in soils to form the soil seed bank.

The relationship continues at **seedling recruitment** when soil characteristics (especially water and temperature) control seed germination and break down seed-coat dormancy. Animals can also help in the removal of seed-coat dormancy by grazing, cracking, or scarifying seed-coats. When seedling shoots emerge from the soil surface, the phases of establishment, persistence, biomass production, and seed production follow and are controlled by water, temperature, light, and soil fertility; in these phases, plant responses to the effects of trampling and defoliation by grazing animals, and of competition with neighboring plants, are key modifiers of the environmental adaptation of the plant. Resistance to grazing or effective regeneration strategies are key factors affecting plant persistence in mixed pastures where different species are grazed selectively (Williams *et al.* 1985).

1.5 Seeds and soil seed bank, seed dormancy of *Desmanthus*

1.5.1 The importance of seeds

The structure of a biological organ, at any given moment, represents the phenotypic expression of selection, usually after a long period of evolution. Selection acts within the constraints imposed by the developmental processes involved.

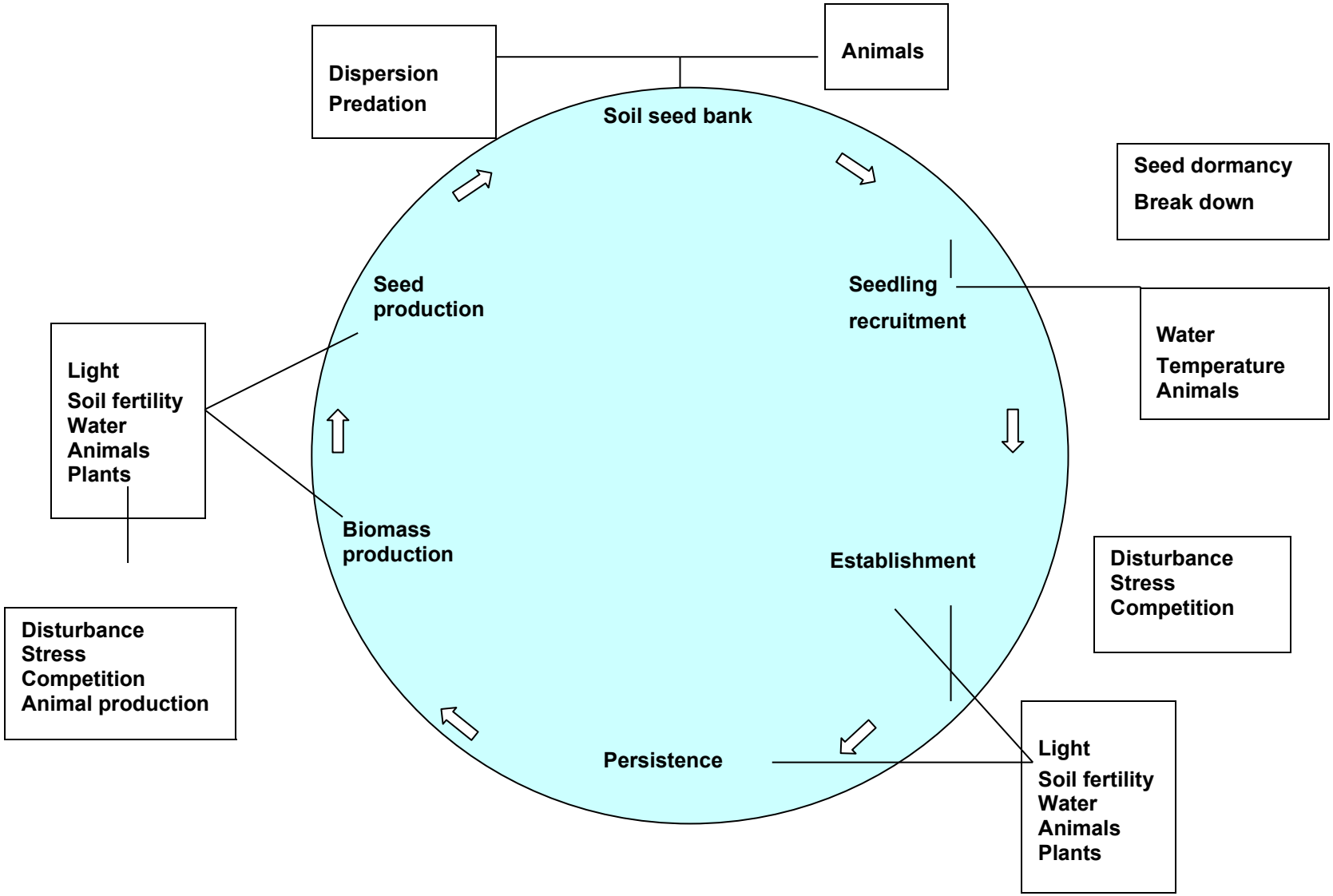

Figure 1.3 Circular model representing the environmental influences on a pasture plant in its different stages of life.

Under a strictly channeled, ontogenetic sequence there is little opportunity for extensive modifications within the sequence (Van Staden *et al.* 1989). On the other hand, an organ subjected to environmental pressure has an enhanced capacity for modification during the development processes (Van Staden *et al.* 1989).

The relatively conservative structure present in a legume seed represents a very successful framework from which many particular adaptations have emerged. The success of the legume family was attributed by Van Staden *et al.* (1989), in part, to the relative versatility of legume seeds in meeting environmental and phylogenetic challenges.

Since vegetative reproduction is seldom successful in the dry tropics, seed is the only propagation method possible to be used to guarantee the perpetuation of a species. It should not be considered, however, as a mere ancillary to plant survival, but as a highly integrated aspect of environmental exploitation (Van Staden *et al.* 1989). According to McKeon and Mott (1984), the nature of the seed plays a vital role for introducing forage legumes into a new environment, and in maintaining their existence in a pasture community.

Seeds of forage legumes, may present great variability in shape, size, color, and structure. A detailed description of the structural variation found in seeds of leguminous plants is given by Van Staden *et al.* (1989). Such a level of detail is beyond the scope of the present study, but the nature of the seeds of *Desmanthus* is outlined in the next section.

1.5.2 Seeds of Desmanthus

In the genus *Desmanthus,* seeds are generally small (Tab. 1.1), ovate and dorsi-ventrally flattened, with the **testa** varying from light brown to nearly black, and marked on both sides by a **pleurogram** (Luckow 1993). Four major patterns on testa surface were described by Luckow (1993): **rugulate**, **rugulate-papillate**, flattened-rugulate-papillate, and raised-rugulate-papillate. In all species of the genus, seeds have well-developed **parenchyma** (seed-coat) and **endosperm** layers, and a conspicuous **sclerid** layer of **hour-glass cells** (Luckow 1993).

Three main structures can be distinguished on the external surface of a *Desmanthus* seed (Fig. 1.4): the **hilum** or **abscission scar**, the **micropyle**, and the **strophiole**, or **boss,** or **lens** (Van Staden *et al.* 1989). The pleurogram, a fourth structure, is found in all species of *Desmanthus*, is very common in the Mimosaceae, rarely in the Caesalpiniaceae species, but is not present in the Fabaceae.

Table 1.1 Variation in the size of seeds of some *Desmanthus* species. Adapted from Luckow (1993).

Species	Length mm	Diameter mm
D. bicornutus	2.5 – 3.7	2.0 – 2.8
D. leptophyllus	2.4 – 3.1	1.7 – 2.4
D. pubescens	2.3 – 3.1	1.8 – 2.5
D. pernambucanus	2.4 – 3.2	1.9 – 2.4
D. virgatus	2.1 – 2.9	1.4 – 2.7

The hilum is the scar left when the seed has been detached from the **funiculus**. According to Tran and Cavanagh (1984), the hilum in the Fabaceae functions as a hygroscopically-activated, one-way valve. During desiccation, it permits the seed to lose water to the external atmosphere (Manning and Van Staden 1987a). There is no evidence that the hilum is a site for water entry during imbibition. The Mimosaceae and Caesalpiniaceae lack the developed hilum complex of the Fabaceae and, consequently, the regulation of the moisture content seems not to be under hilar control. In the Mimosaceae and Caesalpiniaceae families, the pleurogram may functions during desiccation in an analogous although more rudimentary manner, to the hilum. (Van Staden *et al.* 1989). There are three basic shapes of pleurogram in *Desmanthus*: narrowly U-shaped, broadly U-shaped, and broadly bell-shaped (Luckow 1993).

The micropyle is a structure located close to the hilum and represents the former passage for the pollen tube through the integument of the ovule (Tran and Cavanagh 1984). The function of the micropyle in germination is not well defined in the literature. Some authors have related seed-coat impermeability to a closed micropyle, and permeability to an open or semi-open micropyle (Tran and Cavanagh 1984).

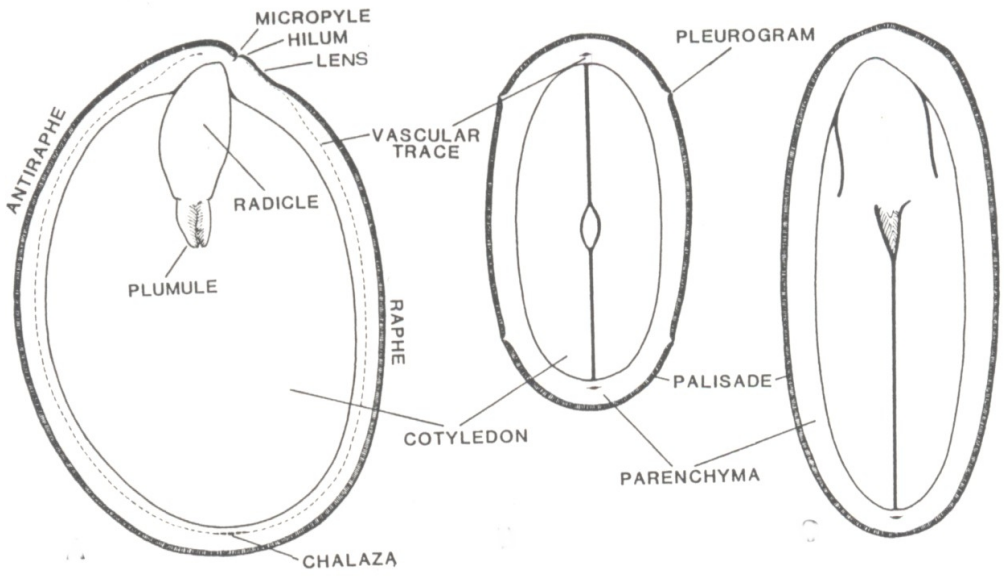

Figure 1.4 Diagram of a *Desmanthus* seed showing main structures. Adapted from Van Staden *et al*. (1989)

The strophiole, or lens, is the major structure involved in water uptake in seeds having impermeable seed-coats. It is described by Rolston (1978) as a mound of tissue near the hilum, but on the opposite side of the micropyle. Seeds of Caesalpiniaceae and Mimosaceae have strophioles varying from insignificant to quite obvious. The strophiole functions as a control structure for the water uptake and this function seems to be specifically related to the legume species possessing an impermeable seed-coat, as in species of *Lupinus* (Quinlivan 1968; Serrato Valenti *et al.* 1989); *Medicago* and *Trifolium* (Ballard 1976); *Stylosanthes* (Ballard 1976; Mott 1979; Hopkinson and Paton 1993); *Acacia* (Tran 1979; Morrison *et al.* 1992); *Albizia* (Dell 1980); *Vigna* (Gopinathan and Babu 1985); *Sesbania* (Manning and Van Staden 1987a); *Indigofera* (Manning and Van Staden 1987b), *Cassia* (Bhattacharya and Saha 1990); and *Desmanthus* and *Leucaena* (Hopkinson 1993b).

When under strophiolar control, seed imbibition is determined by the extent to which the strophiole is open; even in hard, non-dormant seeds, an open strophiole allows water entry in *Vigna minima* (Gopinathan and Babu 1985)*;* in *Cassia* sp. (Bhattacharya and Saha 1990); in *Leucaena leucocephala, Desmanthus virgatus* and *Cassia rotundifolia* (Hopkinson 1993b). Many legumes, however, have water uptake across the

seed-coat without any control of the strophiole. Tran and Cavanagh (1984) believed that this lack of control is predominant in the domesticated legume species, which precludes species genetically selected, or bred for commercial cultivation such as *Glycine max* and *Phaseolus vulgaris*.

Similarly to other legumes, the *Desmanthus* seed-coat has an external, impermeable layer formed by **palisade macrosclerid cells**, commonly called **epidermal**, **prism**, or **Malpighian cells** (Rolston 1978; Dell 1980; Serrato Valenti *et al.* 1989; Luckow 1993). The outer surface of this layer is formed by a **cuticle**, or cuticle and **sub-cuticle**, of variable thickness.

The nature of the impermeability of legume seed-coats varies among species. The presence of water-repellent compounds in the seed-coat of legumes has been described for many different species. The sequential layers of wax, hemicellulose-cellulose complex, arabinan, tannins, and phenols in the cuticle, sub-cuticle and palisade layer, were proposed by Rangaswamy and Nandakumar (1985) as a progressive barrier to water uptake by seeds of *Rhyncoesia minima,* and have close similarities with those of the *Desmanthus* genus.

1.5.3 Seed-coat dormancy in Desmanthus

Seed-coat dormancy or **hardseededness** is present in most tropical legume pasture species, including the genus *Desmanthus* (Hopkinson 1993b), and is found in many woody legumes (Floyd 1966; 1976, Shea *et al.* 1979; Dell 1980; Bell *et al*. 1987a; Crestana and Beltrati 1988; Bhattacharya and Saha 1990; 1992; Keelley 1991; Morrison *et al.* 1992; Cox *et al.* 1993; Bell *et al.* 1993). It prevents cellular rupture of the seed-coat, inhibits water entry into seed, and allows annual pasture legumes, for example, to produce a seed bank reserve in the soil. The seeds of some legumes are known to be particularly susceptible to damage of some sort, at any rate during **imbibition** if the seed-coats are damaged or missing (Bhattacharya and Saha 1990; Gardener *et al*. 1992; Hopkinson 1993a).

The presence of a testa in legume seeds does not, however, necessarily produce an impermeable seed-coat. Permeability of the seed-coat varies among species, cultivars,

and even among different lots of seeds from the same cultivar. There is a relationship between environmental conditions during seed formation and maturation, and impermeability of released or harvested seeds (Quinlivan 1971a; b; McKeon and Mott 1984). Relative humidity and temperature are the major environmental conditions normally cited as affecting hardseededness (see for example Quinlivan 1971a; b; Rolston 1978). Seeds tend to adjust their internal moisture to the lowest relative humidity of the environment to which they have been exposed (Hyde 1954; Barret-Lennard and Gladstone 1964). Impermeability is in turn inversely related to seed moisture contents (Quinlivan, 1971 a; b; Copeland 1976; Rolston 1978). The lower limit of moisture content in which a legume seed can maintain its permeability is between 10% and 15% (Quinlivan 1968; 1971 a; Ballard 1976; Rolston 1978; Argel and Humphreys 1983; Tran and Cavanagh 1984). This relationship between hardseededness and the relative humidity at seed formation was not found by Argel and Humphreys (1983) in seeds of *Stylosanthes hamata* cv Verano. Similarly, seeds of 32 species of native Australian legumes were reported by Morrisson *et al.* (1992) to have moisture contents between 5.9% and 10.6%, and still had predominantly permeable, viable seeds.

The decline of **innate dormancy,** or primary dormancy in a seed after harvest, or after natural release from the plant, is very important both economically and ecologically. The process of dormancy release is termed "**after-ripening**" (Murdoch and Ellis 1992). After-ripening depends upon the environment and is usually accelerated at elevated temperatures (Gardener 1975). Such responses to temperature are well documented in commercial pastures legumes (Quinlivan 1961; Cameron 1967; Torssel and McKeon 1976; Mott *et al.* 1993; McKeon and Mott 1984).

The **softening** of hard seeds of *Stylosanthes humilis*, *S. hamata, S. scabra* and *S. viscosa* was found by Mott *et al.* (1981) to be related to the number of days during which the soil temperature rose above 50-55 ^{0}C. This was documented for Katherine, Townsville, and Narayen, located in tropical and sub-tropical Australia, and a predictive model was proposed for these sites. In seeds of some members of the Gramineae there is close relationship between seed germination and temperature as: "the logarithm of the mean dormancy period is a negative linear function of temperature" (Murdoch and Ellis 1992). This sort of prediction however was not accepted by some workers as being

useful to predict germination in stored seeds of commercial grasses, in that the "manipulation of storage environment offers little promise, and temperature has no material hastening in loss of seed dormancy" (Hopkinson 1993 a).

Three main types of seed-coat dormancy patterns were detected by Morrisson *et al.* (1992) in their examination of native Australian species of the families Fabaceae and Mimosaceae:

- Type A species - have a relatively small non-dormant fraction of their ripe seeds, which is maintained through time. Seeds of *Desmanthus* spp. can be included in this type (Hopkinson 1993 b);

- Type B species - have a relatively large non-dormant fraction of their ripe seeds at maturity and also is maintained through time;

- Type C species - have a relatively small non-dormant fraction when the seeds are ripe, but this fraction increases with time.

Hardseededness can be considered in agronomic and ecological contexts. Agronomically, harvesting and storage conditions may govern the dormancy and viability of pasture seeds. Such seeds are required to germinate when sown and knowledge of the mechanisms regulating dormancy under storage is essential. Ecologically, many plants including forest and forage species rely on seed germination and seedling recruitment from soil seed banks for the long term survival of a plant stand (see, for example Gardener 1975).

A diversity of treatments has been used to overcome seed dormancy and these enhance the entry of water into the seed, increasing the percentage of germination in commercial seeds. The treatments were separated by Tran and Cavanagh (1984) into wet treatments and dry treatments. Sulphuric acid, alcohol, acetone, oxidising agents, hot or boiling water, and liquefied gases are in the wet category; while scarification, impacting, high pressure, dry heating, and electromagnetic waves are in the dry category. The efficiency of each method has been tested in different legume species with a wide range of results.

The loss of seed-coat impermeability has been attributed to many causes and varies with the treatment used to break impermeability. According to Hopkinson (1993 b), a treated hard seed becomes permeable because of fractures to the testa, or cleaving of the strophiole. The reversibility of the process of seed softening by a natural resealing of the strophiole was observed by Hopkinson (1993 b) in members of the family Fabaceae, but not in species of the Mimosaceae.

4.4 Soil seed banks

Most of the seeds reaching the soil after dispersal either have some type of dormancy or can acquire dormancy in their new habitat (Harper and Benson 1966; Harper 1977 b). The activation of seeds stored in the soil depends upon stimuli to break down their dormancy or to promote their germination. In some specific environments fully supplied with essential conditions for germination, two main groups of plants can be recognized. One group produces seeds that will germinate soon after release from the plant and never will form a seed bank; the second group has seeds that exhibit dormancy and which will become incorporated into the soil seed bank and "can be detectable in the habitat at all times during the year and may represent an accumulation of many years" (Grime 1979). These two groups are extremes and "between them there are species and populations in which the seed bank, although present throughout the year, shows seasonal variation in size" (Grime 1979). The role of soil seed banks in vegetation dynamics is well understood and closely connected with disturbance (Thompson 1992).

Two main types of soil seed banks are defined by the time over which the seeds of a species or a group of species remain in the habitat in a viable condition. In persistent seed banks, released seeds remain in the soil in a viable condition for periods longer than one year. In transient seed banks, none of the released seed remain in soil in a viable condition for periods longer than one year (Grime 1979; Thompson 1992).

The lack of correlation between the presence of some species and the distribution of their seeds in the soil seed bank in grasslands has been stressed by many authors (Harper 1977 c; Grime 1979; Rabinowitz 1981; Johnson and Anderson 1986; Thompson 1992; Balun 1993). In such conditions, the greatest numbers of seeds are

located at the soil surface or close to it (Thompson 1992). The use of annual estimations of the size of the seed bank, in many cases carried out after the season of seedling recruitment, is probably the reason why only apparently poor seed banks have been found in some grasslands (Thompson 1992).

Harper (1977 c) reported the occurrence of a higher proportion of seeds in seed banks of grasslands dominated by annual grasses compared with those dominated by perennial species. A positive correlation between number of seeds in the seed bank and frequency of grazing activities was also shown by Harper (1977 c). The effect of grazing animals in increasing the size of seed banks was explained by Harper (1977 c) as the result of enforced seed dormancy in animal faeces, and by the trampling effect upon the seeds facilitating their incorporation in the soil.

The burial mechanisms of seed populations in soil are rather obscure (Harper 1977 c). Small seeds can easily be washed down into pores and fissures in the soil profile by rain or can be buried by ants or other the soil microfauna. Under grazing conditions, animal trampling and disturbance seem to be the principal causes of seed burial (Harper 1977 c; Grime 1979; Thompson and Grime 1979; Thompson 1992).

Desmanthus is a pasture plant only recently introduced into many countries and published works on the soil seed dynamics of its varieties and accessions are scarce; they are limited to the tropical and subtropical regions of Australia (Borrows and Porter 1993). These authors studied the influence of grazing regimes upon the regeneration and survival of *D. virgatus* CPI 78382 in a cracking clay soil at Gayndah, Queensland, Australia. A larger number of seeds were found in the soil from grazed than ungrazed plots. Seedling recruitment of *D. virgatus* CPI 78382 was much higher under grazing and was significantly related to rainfall events (Borrows and Porter 1993). These results were similar to those obtained by other authors working with other species (Harper 1977 c; Ward and Jennings 1990; Kinucan and Smeins 1992; Chambers 1993; Tremont 1994).

In the dry season, under the effect of high solar temperatures, the break down in dormancy of a hard-seeded population occurred very slowly (Probert 1992). With a

brief exposure to high temperatures, as a result of fire, break down may be more rapid (Probert 1992).

The position of the seeds within the soil profile, coupled with the intensity and duration of the fire, are critical determinants of whether or not hard seed break down occurs (Floyd 1966; 1976; Walker *et al.* 1981; Probert 1992). Seeds of *Acacia longifolia* at a depth of 3 cm were unaffected by fire; at 2 cm depth, a high proportion of seeds were rendered permeable without affecting viability; those on the soil surface were all killed (Pieterse and Cairns 1986). Fire temperatures at ground level reached 145 °C and 266 °C respectively in annually burned and 5-year burn sites in grasslands of Kansas and Florida, U.S.A. (Gibson *et al.* 1990).

The intensity and duration of heat affects different plant populations differently. Relatively brief exposure of a few minutes to temperatures of about 100 °C caused more than 80% of the hard seeds to break down in a number of species (Keeley 1987), whereas bulked seeds of the *Stylosanthes* genus needed only 15-30 seconds when exposed to temperatures of 40-50 °C (Mott 1979). Leguminous species of the wet sclerophyll forests at New South Wales, Australia, required different heat intensities and durations to break down hardseededness (Floyd 1976). The effect of fire intensity upon the regeneration of leguminous species was very well demonstrated by Shea *et al.* (1979) in Western Australia. Low intensity fires did not raise the soil temperatures significantly at depths below 0.5 cm and were inefficient in breaking hardseededness in seeds below that depth. In high intensity fires, temperatures at 2 cm depth reached 170 °C, and killed seeds located in this and upper layers; but they broke hardseededness of seeds located in layers between 2 cm and 4 cm. These high intensity fires reduced hardseededness in *Acacia extensa, A. myrtifolia, A. pulchella, A. strigosa* and *Mirbelia dilatata* by, on average 75%, at 90 °C (Shea *et al.* 1979). Fire was highly efficient in increasing the germinability of the soil seed bank of *Adenostoma facsciculatum,* but had very little effect in the *Ceanothus greggii* seed bank in mixed chaparral of San Diego, Southeastern U.S.A. (Zaamit and Zedler 1988). On the other hand, however, a range of temperatures considered ideal for the stimulation of germination of dry seeds in Mediterranean ecosystems were lethal for buried seeds of 31 tropical tree species in the Amazon rainforest (Brinkmann and Vieira 1971).

Increase of temperature does not seem to be the only fire-associated factor responsible for the release of dormancy in seeds. Smoke contains substances which can play a very important role in the promotion of germination of dormant seeds. The germination of over 70 plant species in Australia and California was reported by Roche *et al.* (1994) as being smoke-responsive. Variation in grass population and plant communities was also pointed out by Campbell *et al*. (1995) as affected by different levels of CO^2. The butenolide was pointed out by Flematti *et al.* (2002) as a chemical component present in plant-and cellulose-derived smoke, which promotes germination of a number of species. Smoke, however, has not been reported to affect hard seed coat dormancy in legumes.

1.6 Regenerative strategies of *Desmanthus*

The adaptation and regenerative strategies of plants in habitats subject to fire have been discussed by many authors (Floyd 1976; Keeley and Zedller 1978; Bell and Koch 1980; Bell *et al.* 1987b; Bell 1985; 1994; Pate *et al.* 1990; Hansen *et al.* 1991; Keelley 1991; Moreno and Oechel 1991; Bell *et al.* 1993; Pate 1993; Brewer and Platt 1994). Plant species having a "**fire-recruitment syndrome**" or "**refractory seed syndrome**", whose seeds require the occurrence of a fire for germination from the soil seed bank, are largely lacking the opportunities for population expansion in the absence of fire or other disturbance (Keelley 1991). Plants may be categorised into two types: "**obligate seeders**" and "**resprouters**" (Bell 1985; 1994; Pate *et al.* 1990). Obligate seeder plants adjust regeneration by the recruitment of seedlings from the soil seed bank, whereas resprouter plants regenerate from buds located in underground organs. Resprouters can also regenerate from seed, but less intensively than the obligate seeders.

Seeders and resprouters differ in the partitioning of assimilates between their aerial and underground structures. Seeders allocate much of assimilates to reproductive aerial structures, and less to underground organs. In resprouters, assimilates are diverted to root reserves and underground organs for vegetative regrowth. High concentrations of macronutrients are also allocated by seeders for shoots and reproductive parts, and very little for the roots. Resprouters allocate about equal amounts of Mg, K, N, and P in

shoots and roots, higher amounts of Ca in roots than shoots, and very little macronutrient in reproductive parts (Pate *et al*. 1990; Hansen *et al.* 1991).

The partitioning of stored starch between the shoot and root, together with the morphology and growth of the plant components, may also be used to distinguish seeders and resprouters. Seeders show a noticeably larger shoot and a more laterally extensive root system than resprouters; the latter typically possess a thickened **tap root** in which the concentration of starch generally greatly exceeds that of a seeder (Pate *et al.* 1990; Pate 1993). Some genera of the Leguminosae have been recorded as obligate seeders and resprouters (Pate 1993) with the leguminous species, *Acacia extensa*, reported as behaving as a facultative resprouter/seeder (Pate *et al.* 1990).

Some species of *Desmanthus* have been reported as having a similar root structure to that of the resprouter plants (Burt 1986; 1993; Gardiner 1992). This type of structure in *Desmanthus* has variously been described as a "**crown**" or "thickened tap-root", or less specifically as a "**caudex**" or "**flange"** (Burt 1993), and as a water storage structure known as a "**xylopode**" (Burkart 1952; Carvalho and Mattos 1974). The anatomical origin of the root in *Desmanthus illinoensis* was said to be derived from the hypocotyls and includes the root/stem transition (Latting 1961). No attempt has yet been made to describe species of *Desmanthus* as resprouter plants. No literature was found describing the partitioning of starch and minerals between roots and shoots of *Desmanthus*. However, if a resprouter strategy can be confirmed, associated with the prolific seed yield presented by some of the *Desmanthus* species (Piper 1986; Piper and Towne 1988; Piper *et al.* 1988; Adjei and Pitman 1993), these species should probably be classified as facultative seed/resprouter plants. This form would seem to have much to offer agronomically, and for the dry tropics, it is quite unlike the high seed-producing reseeder *Stylosanthes hamata* or the suffruticose reseeder *S. scabra.*

1.7 Environmental constraints to the growth of forage legumes

Seeds landing in habitats never before experienced by their parents can expose the emerging seedlings to all kind of hazards. This situation explains many opportunistic adaptive specialisations (Grime 1993).

The abundance of primary elements necessary for plant survival, light, water and mineral nutrients is the initial need of the emerging new plant from its surrounding environment, and will determine the success or the failure of an introduced plant to colonise a new site. If one or more of these components restricts the photosynthetic process, it induces "stress" in the plant (Harper 1977d; Grime 1979; 1993). Plants are highly susceptible to stress during their establishment phase, because of their limited capacity to explore the available resources of, primarily, water and nutrients. Vigorous seedling growth is important for both, initial establishment and adequate regeneration (Mott *et al.* 1976; Gardener 1978).

The driving force influencing the pattern of vegetation in open grassland habitats is the ability of a species to capture and efficiently use the available resources while coping with the effects of competition from other plants and disturbances such as grazing, cutting, fire, fertilising, and watering. In open woodland habitats an important factor is the adaptability or tolerance of the herbaceous understorey plants to shade and other microenvironments under the tree canopy.

1.7.1 Shade tolerance in tropical forage legumes

Tropical pasture species do not require more than 10-15% of midday illuminance to grow and this is independent of any genetic differences between species; it is referred to as **shade tolerance** (Ludlow *et al.* 1974).

Most information concerning the shade tolerance of tropical legumes has been obtained from intercropping systems in high rainfall environments (Ludlow 1980; Wong and Wilson 1980; Eriksen and Whitney 1981; Chen and Othman 1984; Bazill 1987; Shelton *et al.* 1987; Chen 1989; Shelton and Stur 1991). Apart from some conflicting results between authors and differences in cultivars of the same species, high shade tolerance was frequently found in species of the genera *Desmodium*, *Centrosema*, and *Calopogonium.* Species and cultivars of the genus *Stylosanthes* represent the category of low shade tolerance, except for cultivars of *Stylosanthes humilis* that show high relative yields at intermediate level of shade (50% sunlight) (Wong *et al.* 1985 b, Wong 1991)

The association of increasing levels of shade with repeated grazing has also been the frequent cause of loss of tropical legumes under plantation crops (Chen and Ahmad 1983). Wilson (1988) pointed out that the morphological changes that occur in shaded plants, with lower accumulations of reserve carbohydrates, serve to exaggerate the problems of plant recovery after grazing. The reduction in the percentage of more palatable plants in grazed pastures is undoubtedly due to animal selectivity, but may be confounded with low shade tolerance and *vice versa*. During drought, the reduction of the plant investment in dry matter to roots with decreasing light intensity is disadvantageous for seedling plants (Cooper 1966). In such conditions, seedlings have a restricted root development and cannot use water in deeper soil layers. This issue is of great importance to drought tolerance characteristics of plants in dry habitats.

1.7.2 Soil water content in shaded habitats

Contrary to the generally held assumption that woody plants have predominant use of subsoil water, and herbaceous plants use water in the top soil, competition for water between these two plant categories may operate over a wide range of the soil strata (Walker *et al.* 1972; Tiedemann and Klemmedson 1973; Kellman 1979; Connor 1983; Knoop and Walker 1985; Castellanos *et al.* 1991; Smith and Stuart 1994).

The water content of soils under shade environments was frequently noticed to be higher than in open areas (Maranga 1984; Smith *et al.* 1987; Ovalle and Avendano 1987; Wilson and Ludlow 1991; Belsky 1994; Belsky *et al.* 1993a; b), but some conflicting results may be found in the literature (Stuart-Hill *et al.* 1987).

If the hypothesis of shade leading to a higher soil water content and increasing productivity, can be proved, it is likely to be of importance in regions where water is a limiting factor rather than in wetter regions (Lauenroth 1979).

Another aspect of the tree canopy and water interaction concerns the rainfall interception by the tree. Such interception may not greatly affect the understorey vegetation of wet tropical regions where rainfall is not commonly a growth-limiting

factor. In dry tropical environments, however, it can operate as a major driving force for the growth of understoreys vegetation (Durr and Rangel 2000).

1.7.3 Effect of trees on soil nutrients

The presence of organic matter as plant litter, animal dung, and urine, is a key feature in grazing systems. In a grazing system composed exclusively of herbaceous plants with their roots distributed in more-or-less the same soil layer, plants capture nutrients from the soil organic matter and return them to the soil as decomposing plant litter and debris; living plant roots cannot reach the available nutrients leached to deeper soil layers. As a result of the losses of nutrients in leached or eroded soils, and by animal products withdrawn and exported, the system soon becomes deprived of nutrients with parallel decreases in pasture yields and animal production. This situation also favours the invasion of weedy plants better adapted to nutrient-deficient soils but of very low forage value. A system formed of herbaceous and arboreous plants may have tree roots that can extract nutrients from layers deeper than those explored by grasses and herbaceous legumes and can return such nutrients to the soil surface as plant litter. Prinsley (1991) used this assumption to explain the enhanced productivity of agroforestry systems in Australia. If the presence of trees does not depress pasture yields, compared with that of pastures in open habitats, an extra input of litter from the trees is available for animal consumption or for enhancing soil fertility through soil organic matter decomposition. Organic matter can also return to the soil as woody stems and bark from the trees, but the high lignin content of such non-palatable material delays the decomposition process. Nutrients leached to deeper soil layers may also be available to tree roots. An advantage of trees over the understorey plants is associated with an increase in levels of soil nitrogen (Wong and Wilson 1980; Ericksen and Witney 1981; 1982; Wilson *et al.* 1980; Wilson and Wild 1991 a; b).

Many workers support the theory of soil enrichment by recycled organic matter and mineral nutrients under both leguminous and non-leguminous trees (Ebersohn and Lucas 1965; Radwanski and Wickens 1967; Tiedemann and Klemmedson 1973; Bernhard-Reversat 1982; Virginia 1986 Belsky *et al.* 1989; 1993a; b; Isichei and Muoghalu 1992; Smith and Swart 1994). The increase of nutrients in soil, especially

nitrogen may also lead to increases in **water use efficiency** of understorey plants (Ludlow *et al.* 1974; Erikson and Whitney 1981; Durr and Rangel 2000).

1.8 *Nutritional value of Desmanthus*

Despite a reasonable number of published works and researches presently undergoing in Australia and overseas focused on the agronomic potential of *Desmanthus* spp., there is a lack of information relating to its nutritional value and influence on animal performance. Available results are of somehow inconsistent:

- In Cambodia native goats supplemented with *Desmanthus virgatus* foliage had lower daily weight gain (8.9 g/day) than goats supplemented with cassava foliage (44.9 g/day), or banana leaves (17.3 g/day) (Sokerya and Rodriguez 2001). The daily foliage intake of *D. virgatus*, cassava, and banana were respectively 276, 308 and 195 g DM/day.

- Also in Cambodia Ly and Samkol (2001) testing the replacement of 17% of a basal diet for pigs by *Desmanthus virgatus* leaf meal concluded: "the apparently low nutritive value of desmanthus leaf meal makes it unsuitable as an alternative protein source in pig diets" - "the use of desmanthus leaf meal in diets for pigs could only be justified if methods to increase its nutritive value could be successfully developed".

- In Bangkok steers fed a (*Desmanthus virgatus* + total mix) diet had higher growth rate (1.17 kg/day) then steers fed the control total mix diet (0.94 kg/day) or a (*Leucaena leucocephala* + total mix diet) (1.09 kg/day) (Khy *et al.* 20000 . In Thailand the addition of 2 kg/head/day of fresh *Desmanthus virgatus* leaves to the 0.8% of body weight control (*Brachiaria ruziziensis* + concentrate) diet increased the daily weight gain of Australian Brahman steers from 0.56 to 0.89 kg/day (Sukkasame and Phaikaew 2001).

1.9 The approach of this thesis

The characteristics of the range of 13 *Desmanthus* genotypes used in the present study are set out in the next chapter, which also describes the location, climate, vegetation, and soils of the main study area at James Cook University, Townsville.

The viability of *Desmanthus* seeds in the soil seed banks of the study area is assessed in Chapter 3, and the germination responses of seeds to controlled heating in a laboratory oven are used to evaluate the responses of the soil seed banks to heat from controlled burns and sun irradiation in the field.

Shade is a major issue in the establishment and early growth of many forage legumes in pasture swards (Wong and Wilson 1980; Wong *et al*. 1985 b; Wilson 1988; Wilson and Ludlow 1991; Wong 1991). The results of experiments aimed at determining the growth responses of *Desmanthus* species to different levels of shade in the field, both under- and between tree canopies of an open eucalyptus woodland at the study site, are set out in Chapter 4. These resultant experiments were extended in a pot trial, discussed in Chapter 5, to evaluate the impacts of different light, soil, and watering regimes on the early growth of the *Desmanthus* genotypes.

Field trials were also conducted to compare the efficacy of establishment of *Desmanthus* into an existing pasture sward by using a preliminary herbicide treatment, or by sowing scarified seed and transplanted seedlings into untreated pasture plots. These trials are discussed in Chapter 6.

The value of adding a legume-based supplement (*Stylosanthes hamata* cv.Verano, or a range of *Desmanthus* genotypes) to a Mitchell grass (*Astrebla* spp.) diet for sheep has been assessed in Chapter 7. Using sheep housed in metabolic cages and fed various diets, changes in live weight and increases in wool growth were found to be effective measures of the importance of several of the *Desmanthus* genotypes in the animal diet.

The results of each of the experimental approaches are presented in the appropriate chapters of the thesis and some of the supporting data are presented in the Appendices. The overall results of the study are brought together in Chapter 8.

A variety of statistical analyses of the numerical data has been used, most of which have been derived from the “SPSS” and “Statistix”, statistical packages for personal computers. A 95% confidence level has been employed throughout the thesis in discussing the statistical significance of all the results. The tables summarising data

that support various parts of this thesis, including the results of the analyses of variance, are included in the Appendices; references in the text to any data in tables in the Appendices are indicated by "A." as a prefix to the table number.

8.10 Scientific papers and conference presentations arising from the present study

The following papers have been prepared during the present study. They are reproduced in Appendix 3.

Rangel, J.H. de A. 2001. Competitividade de quatro gramíneas e seis leguminosas forrageiras tropicais em resposta a freqüência de desfolhamento. *Revista Científica Rural*, **6** (1), 13-21.

Rangel, J.H de A., Schlink, A.C. and Gardiner, C.P. 1996. Simulation of wool growth by Desmanthus virgatus: a legume for tropical savannas. In, J.Brown (ed), *The Future of Tropical Savannas: An Australian Perspective* (Poster Papers), CSIRO Publishing, 41-42.

Rangel, J.H de A. and Gardiner, C.P. 1996. Effect of fire on seed germination of *Desmanthus virgatus* accessions. In, J.Brown (ed), *The Future of Tropical Savannas: An Au stralian Perspective* (Poster Papers), CSIRO Publishing, 43-44.

Chapter 2

MATERIAL AND METHODS

2.1 Morphological characteristics of the *Desmanthus* genus

Most species of the genus are herbaceous, perennial, much-branched, and suffruticose at the base with stems arising from a woody tap root. Woody species have a variety of forms, ranging from the *Acacia*-like habit with several trunks and flat top to weak tree form with a single trunk and weeping branches. The leaves are alternate, **bipinnate** and **paripinnate**. Inflorescences are heads or condensed spikes borne 1 – 2 in the axils of the leaves (Luckow 1993). Pods are **sessile,** linear or **falcate, suberete** to **dorsi-ventrally flattened,** with a coriaceous or rarely **chartaceous** pericarp (Luckow 1993). Figure 2.1 shows the different parts of a *Desmanthus* plant.

Figure 2.1 Different parts of a *Desmanthus* plant: A) Leaves, B) Bud, C) Flower, and D) Pods

2.2 Genotypes studied

The experiments of this thesis were carried out by using existing *in situ* collections of forage legumes, and by the introduction of new genotypes into different sites; 6 *Desmanthus virgatus*, 3 *Desmanthus leptophyllus*, 1 *Desmanthus pernambucanus*, 1 *Desmanthus bicornutus*, 1 *Desmanthus pubescens*, and 1 *Desmanthus* spp. genotypes were used in the present study (Table 2.1). Decisions about number and selection of genotypes to be used in the trials were taken according to seed availability, previous information about the genotypes, and the amount of available resources for the research program (personnel, time, physical space, and funds). Tables 2.1 to 2.4 show the general characterization, collection data, morphological characteristics, and anatomical characteristics of the *Desmanthus* genotypes used in the study. Genetic classification and other information about genotypes in the first three columns in Table 2.1 were obtained from AusPGRIS (2004) data bank. Genotype common names in the last column of Table 2.1 are from Luckow (1993) and Burt (1993).

The selected genotypes originated from a wide diversity of climates and altitudes existing between the latitudes 18˚ N in Mexico and 7˚ S in Brazil, and the longitudes 35˚ W in Brazil and 100˚ W in Mexico (Table 2.2). Elevation of collection sites varied from 700 m above the sea in Mexico to 50 m in the Virgin Islands, and annual rainfall varied from 1250 mm in Virgin Islands to 500 mm, also in the Virgin Islands (Table 2.2).

Genotype selection was also made with respect to a large variety of phenological plant characteristics. The intent was not to evaluate the behaviour of different species which have in somehow high similarity in the phenological variation of their components and form the "*Desmanthus virgatus* complex" (Luckow 1993), but work with a number of *Desmanthus* genotypes sufficient to cover the range of plant forms existing in the genus. Shrubs and herbaceous plants, 20 cm to 200 cm tall, having erect, ascending, decumbent, procumbent, prostrate, and scrambling growth habits were selected (Table 2.3). Genotypes were also differentiated by other phenological characteristics such as days to flowering (70-110 days) (Table 2.3), and by stem consistence (pithy and woody), stem color (green and brown), stem diameter (3 - 15 cm), number of branches under 5 cm (3 -22), number of pairs of pinnae (3 - 32), number of pairs of pinnules (11 - 22) (Table 2.4).

Table 2.1 General characterization of the *Desmanthus* genotypes used in the study. Adapted from AusPGRIS (2004), Luckow (1993) and Burt (1993).

Scientific name	**Registration code#**	**Preferred name**	**Common/ Commercial name**
Desmanthus.virgatus ("L.") Willd.	AusTRCF 67643	CPI* 67643	Guajillo
Desmanthus.virgatus ("L.") Willd.	AusTRCF 78372	CPI 78372	Guajillo
Desmanthus.virgatus ("L.") Willd.	AusTRCF 78373	CPI 78373	Guajillo, cv. Marc
Desmanthus.virgatus ("L.") Willd.	AusTFCF 78382	CPI 78382	Guajillo
Desmanthus.virgatus ("L.") Willd.	AusTRCF 79653	CPI 79653	Guajillo
Desmanthus.virgatus ("L.") Willd.	AusTRCF 83563	CPI 83563	Guajillo
Desmanthus pubescens ("L.") Willd.	AusTRCF 92803	CPI 92803	Guahillo, cv. Uman
Desmanthus leptophyllus ("L.") Willd.	AusTRCF 38351	CPI 38351	Guajillo
Desmanthus leptophyllus ("L.") Willd.	AusTRCF 37143	CPI 37143	Guajillo
Desmanthus leptophyllus ("L.") Willd.	AusTRCF 321107	TQ 88**	Guajillo
Desmanthus pernambucanus ("L.") Thellung	AusTRCF 40071	CPI 40071	Jureminha, anil
Desmanthus spp. ("L.")	Unknown	Unknown	Alligator Creek
Desmanthus bicornutus S. Watson	AusTRCF 91162	CPI 91162	Malvilla de laguna, guaje de ratón

*CPI - Commonwealth Plant Introduction number
**TQ – Townsville, Queensland Introduction number; CSIRO Davies Laboratory, Townsville, QDL(1995).
Code of registration in AusPGRIS (2004)

Table 2.2 Data of collection of the *Desmanthus* genotypes used in the study. Adapted from AusPGRIS (2004)

Genotype (Preferred name)	Data of Collection				
	Collector	**Origin**	**Site of collection**	**Elevation m above sea level**	**Annual rainfall mm**
D. virgatus CPI 67643	R.L. Burt	Virgin Islands	5 km, W Zacapa, 15^0 Lat. N, $89^0$3' Long. W.	185	500
D. virgatus CPI 78372	R. Reid	Argentina (State: Salta, Region: Southern South America)	15 km S. W. of Embarcacion, Salta, $23^0$33' Lat. S, $64^0$25'Long. W	400	680
D. virgatus CPI 78373	R.Reid	Argentina (State: Salta, Region: Southern South America)	42 km S.W. of Oran on Road to Esteban, $23^0$25' Lat. S, $64^0$08' Long. W	300	650
D. virgatus CPI 78382	R. Reid	Argentina (State: Salta, Region: Southern South America)	15 km S.E of N.S. de Jujuy on road to Perico, $24^0$2' Lat. S, $65^0$67' Long. W	700	700
D. virgatus CPI 79653	R. Reid	Virgin Islands	42 km S.E. of Guantanamo on road to Baracoa, $21^0$98 Lat. S, $74^0$92' Long. W	5	600
D. virgatus CPI 83563	A. Kretschmer	Costa Rica (Region: Mesoamerica)	Taboga Expt. Stn., Guanacaste, 1038^0 Lat. N, $84^0$35' Long. W	Not available	Not available
D. pubescens CPI 92803 cv. Uman	J.R. Lazier	Virgin Islands	Km 40, Uman Union RD, S of Large Quarry, top of Next, $18^0$15' Lat. N, $88^0$68' Long. W	50	1250
D. leptophyllus CPI 37143	W.T. Atkinson	Mexico (State: Veracruz, Region: North and Central Mexico)	56 km Vera Cruz to México City via Puebla rd., 18^0 77' Lat. N, $96^0$52' Long. W	150	Not available
D. leptophyllus CPI 38351	W.T. Atkinson	Venezuela (State: Portuguesa, Region: Northern South America)	12 km from Acarigua to Barinas, $9^0$47' Lat N, $69^0$3' Long. W	250	Not available
D. leptophyllus TQ 88	Not available	Not available	Not available	Not available	Not available
D. pernambucanus CPI 40071	R.J. Williams	Brazil	8 km S. Vertentes, Western Pernambuco, $7^0$92' Lat. S, $35^0$93' Long. W	Not available	Not available
*Desmanthus.*sp Alligator creek	R.L. Burt	Not available	Not available	30	1100
D. bicornutus CPI 91162	R. Reid	Mexico (State: Guerrero, Region: North and Central Mexico)	87 km W. Teloloapan, road to Ciudad Altamiro, Gue. $18^0$4' Lat. N, $100^0$25' Long. W	340	800

Table 2.3 Plant morphological characters of the *Desmanthus* genotypes used in the study. Adapted from R. L. Burt (unpublished data), Luckow (1993), and observed by the author in plants from the JCU plant collection.

Genotype (Preferred name)	**Shape**	**Height (cm)**	**Growth habit**	**Days to flower**	**Weight of 1000 seeds (g) #**
D. virgatus CPI 67643	Herbaceous*	20-40*	Decumbent*	80*	Not available
D. virgatus CPI 78372	Herbaceous*	20-40*	Erect-ascending*	80*	4.6
D. virgatus CPI 78373	Herbaceous*	20-40*	Erect-ascending*	80*	Not available
D. virgatus CPI 78382	Herbaceous*	20-40*	Procumbent*	80*	2.2
D. virgatus CPI 79653	Herbaceous*	10-20*	Prostrate*	70*	4.5
D. virgatus CPI 83563	Herbaceous*	30-50*	Erect-ascending*	110*	Not available
D. pubescens CPI 92803 cv. Uman	Shrub**	50 to more than 100**	Erect**	110**	4.6
D. leptophyllus CPI 37143	Herbaceous*	30-50*	Procumbent*	80*	4.0
D. leptophyllus CPI 38351	Shrub*	50-80*	Ascending*	70*	4.9
D. leptophyllus TQ 88	Shrub*	50 to more than 100*	Ascending/ Scrambling*	Not available	5.2
D. pernambucanus CPI 40071	Shrub**	50 to more than 100**	Erect**	96*	3.6
Desmanthus spp. Alligator creek	Herbaceous*	30-50*	Procumbent*	Not available	4.3
D. bicornutus CPI 91162	Shrub**	150 to more than 200**	Erect**	Not available	5.4

* Described by Burt (unpublished data)
** Described by Luckow (1993)
# Measured by the author in seeds from the JCU plant collection

Table 2.4 Plant anatomical characteristics of the *Desmanthus* genotypes used in the study. Adapted from R. L. Burt (unpublished data)

Genotype (Preferred name)	Stem characteristics			Mean number of branches under 5 cm	Mean number of pairs of pinnae	Mean number of pairs of pinnules
	Consistency	Diameter mm	Color			
D. virgatus CPI 67643	Pithy	7	Green	17	4	17
D. virgatus CPI 78372	Pithy	3	Green	7	4	11
D. virgatus CPI 78373	Pithy	3	Green	7	4	11
D. virgatus CPI 78382	Pithy	3	Green	7	4	11
D. virgatus CPI 79653	Pithy	2	Green	22	3	15
D. virgatus CPI 83563	Pithy	7	Green	17	4	17
D .pubescens CPI 92803 cv. Uman	Woody	12	Gray	17	7	22
D. leptophyllus CPI 38351	Pithy	12	Brown	3	7	20
D. leptophyllus CPI 37143	Pithy	7	Green	17	4	17
D. leptophyllus TQ 88	Woody	Not available	Green brown	Not available	Not available	Not available
D. pernambucanus CPI 40071	Woody	12	Green brown	4	7	22
Desmanthus spp. Alligator creek	Pithy	Not available	Green brown	Not available	Not available	Not available
D. bicornutus CPI 91162	Pithy	15	Green	10	32	15

2.3 The study area

The following sections of this chapter aim to give general and more detailed information about the area where the trials of the present study were carried out.

2.3.1 Location

All the field trials in the present study were conducted at the James Cook University Douglas Campus, in areas under control of the School of Biomedical and Tropical Veterinary Sciences. Laboratory trials were conducted in the laboratories of the School of Tropical Biology of James Cook University and at the Davies Laboratory of CSIRO in Townsville. The trial with housed sheep was conducted at the CSIRO Lansdown Station at Woodstock, some 50 km south of Townsville.

The Douglas Campus of James Cook University is located 20 m above the sea level, at 19°19'41'' South and 146°45'38'' East and lies to the southeast of the city of Townsville, in northern Queensland . Figure 2.2 shows an aerial view of the Douglas Campus. The School of Biomedical and Tropical Veterinary Sciences is located at the northeastern corner of Fig. 2.2. A more detailed map of the studied sites is shown in Fig. 2.3. Trials described in sections 3.1 and 3.3 of this thesis were conducted at site 1 of Fig. 2.3, trials of chapter 4 at sites 2 and 3, and the trials described in chapters 5 and 6, at site 4.

2.3.2 Climate of the area

Townville has a climate characterized by a hot wet summer from November to March, and a dry and mild winter season from April to September. Townsville receives a mean annual rainfall of 1129 mm (Australian Government 2005), (Fig. 2.4), and the average monthly rainfall of Townsville and the actual monthly rainfall occurring during the experimental period (1992 – 1994) is shown in Fig. 2.5. Total annual rainfall throughout the experimental period was about a half of the long term average annual rainfall (Fig. 2.5 and Tabs. A.1.1 to A.1.5*), showing the severity of the drought during which the research was undertaken.

* See Appendix

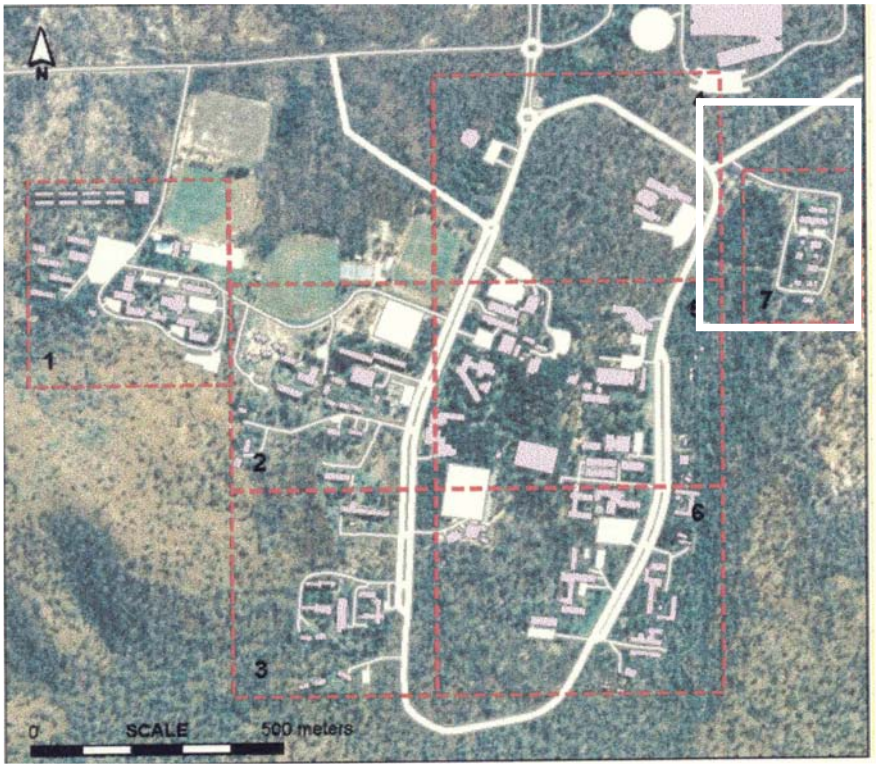

Figure 2.2 Aerial view of James Cook University Douglas Campus. The box shows the location of Fig. 2.3. Adapted from JCU location maps.

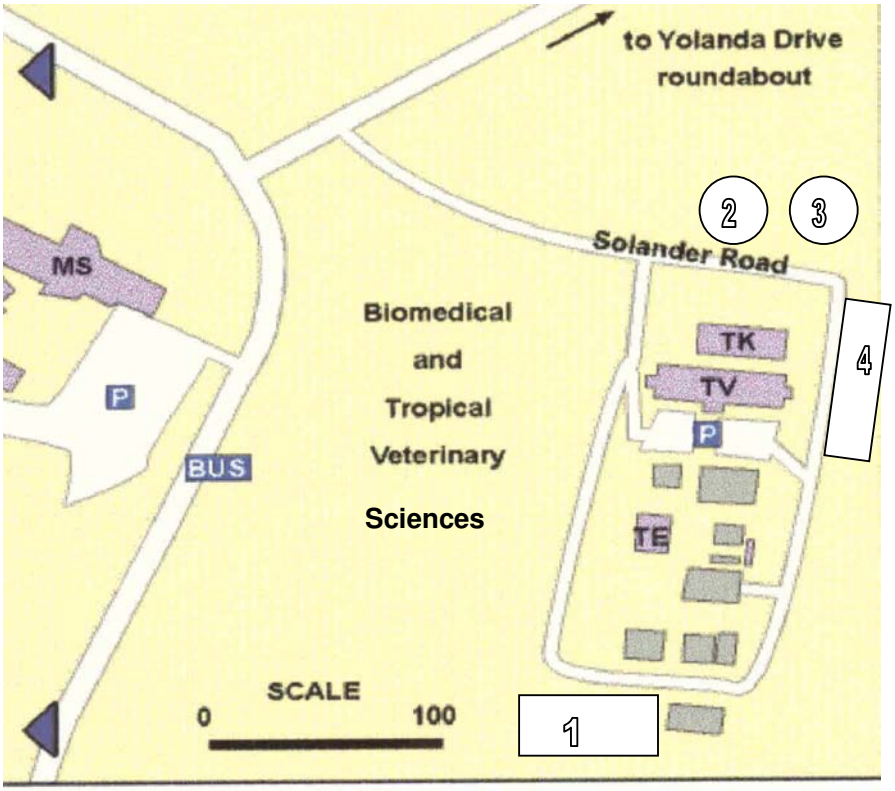

Figure 2.3 Experimental sites (1 to 4) at School of Biomedical and Tropical Veterinary Sciences. TK, TV, and TE are all part of the Veterinary teaching complex. MS is part of the Medicine complex. Adapted from JCU location maps.

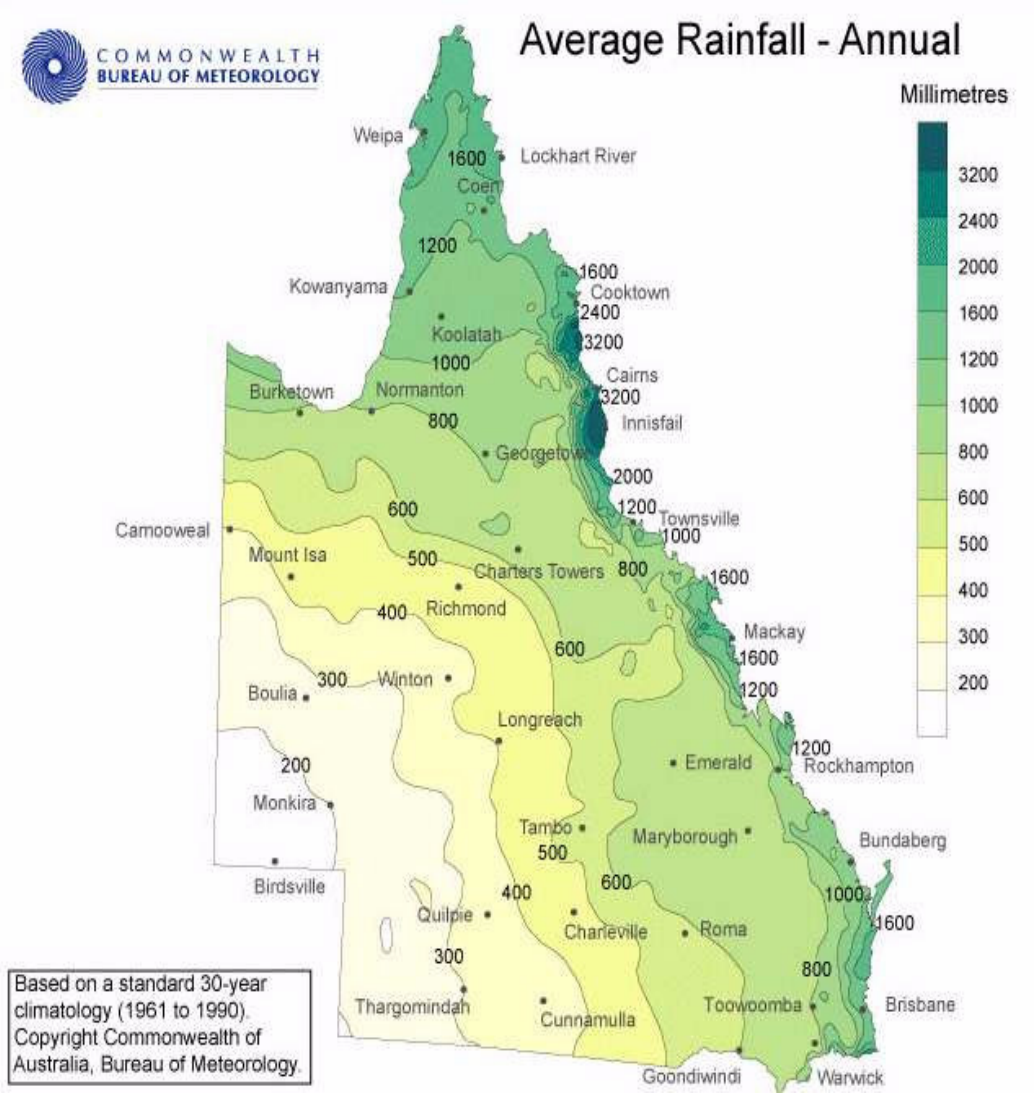

Figure 2.4 Queensland long term rainfall map.

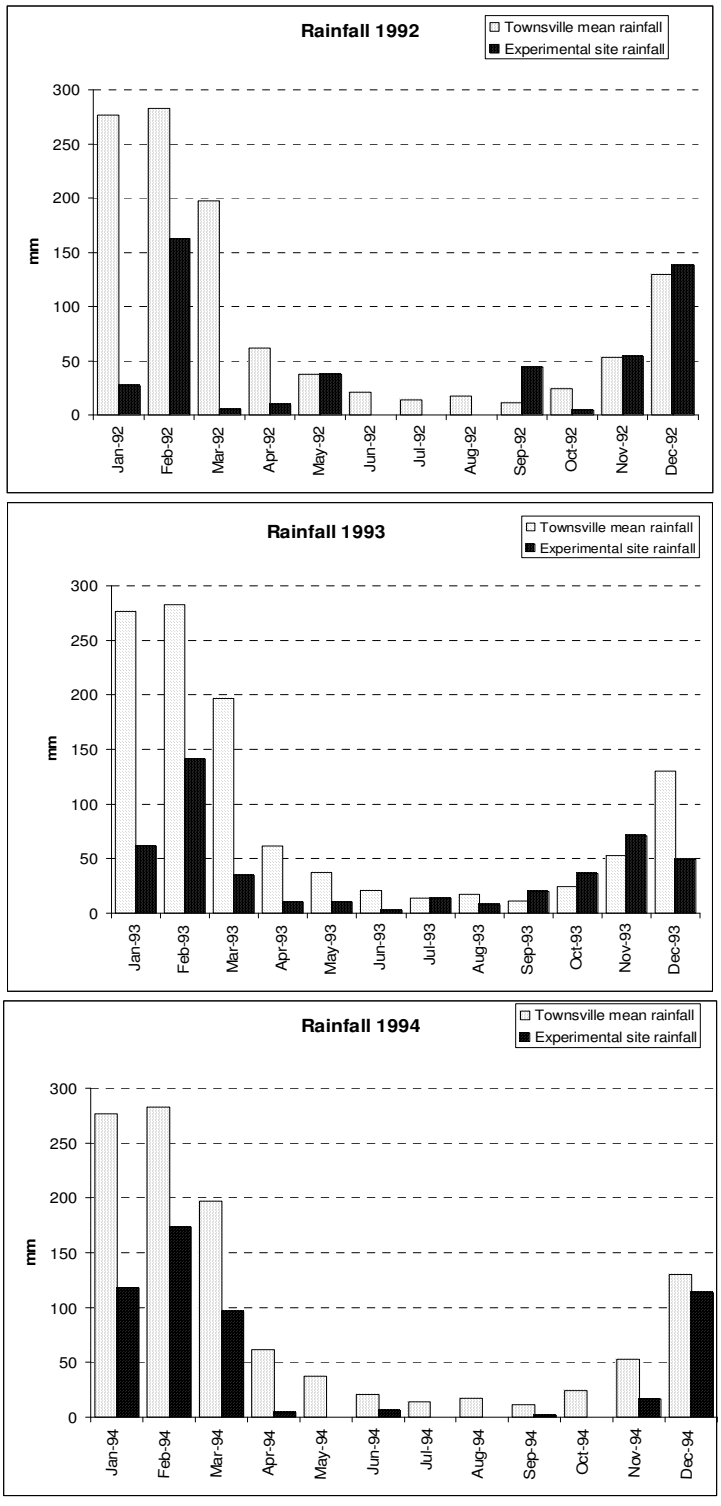

Figure 2.5 Townsville 100 year average monthly rainfall and experimental site monthly rainfall at the years of 1992, 1993, and 1994.

2.3.3 Vegetation of the study area

2.3.3.1 Nature of vegetation at JCU Douglas Campus

Natural vegetation of JCU Douglas Campus is typically an open eucalypt woodland community with a grassy understorey (Queenslad Government 2005).

2.3.3.3 Vegetation of the experimental sites

- *Site 1 (Fig. 2.3)*

Most of the original trees of this site had been removed. Only a few sparse ironbark (*Eucalyptus crebra*) trees were present in the area when the trials were carried out. The ground cover was also removed when a forage legume collection was established by Suparto (1990) that occupied most of the area, which included some aviaries and possum sheds.

- *Sites 2 and 3 (Fig. 2.3)*

Sites 2 and 3 are contiguous in an open woodland eucalypt savannas. The arboreal vegetation is predominantly of narrow-leaved red ironbark (*Eucalyptus creba*), but red blood wood (*E. erythrophloia*) and poplar gum (*E. platyphylla*) are also present. Chinee apple (*Ziziphus mauritiana*) is the most common shrub, and Townsville wattle (*Acacia leptostachya*) occurs in low density. Black spear grass (*Heteropogon contortus*) and kangaroo grass (*Themeda triandra*) are the most common grasses in the herbaceous layer. Other less frequently occurring grasses were also identified in the area including: sabi grass (*Urochloa mosambicensis*), giant spear grass (*Heteropogon triticeus*), forest blue grass (*Bothriochloa bladhii*), *Dicanthium* spp., silky browntop (*Eulalia aurea*), woodland lovegrass (*Eragrostis sororia*), *Eragrostis* spp., pigeon grass (*Setaria apiculata*), brown sorghum (*Sorghum nitidum*), scented top grass (*Capilepidium spicigerum*), purpletop rhodes grass (*Chloris inflata*), feathertop Rhodes grass (*Chloris virgata*), cockatoo grass (*Alloteropsis semialata*), guinea grass (*Panicum maximum*), green panic (*Panicum maximum* var. *trichoglume*), and red Natal grass (*Melinis repens*). The common forbs are hairy

cassia (*Cassia absus*), *Cajanus scarabaeoides*, *Galactia* spp., and rhyncosia (*Rhynchosia* spp.). Other forbs, shrub stylo (*Stylosanthes scabra* cv. Seca), Verano (*S. hamata* cv. Verano), gambia pea (*Crotalaria goreensis*), yellow rattlepod (*Crotalaria mitchellii*), *Crotalaria calycina*, *Crotalaria montana*, indigo (*Indigofera* spp.), *Alysicarpus ovalifolius*, mung bean (*Vigna radiata* var. *sublobata*), native bean *(Vigna lanceolata*), *Tephrosia* spp., *Zornia muelleriana*, and *Flemingia parviflora* were identified as being of sparse occurrence.

- *Site 4 (Fig. 2.3)*

This is a wire mesh fenced area inside a sown pasture 10 years old. The trees had been cleared at the time the pasture was established. The herbaceous vegetation in the studied paddock was primarily composed of sabi grass (*Urochloa mosambicensis*) and Verano (*Stylosanthes hamata* cv. Verano) with a much smaller contribution from native or naturalised grasses: spear grass (*Heteropogon contortus*), giant spear grass (*Heteropogom triticeus*), kangaroo grass (*Themeda triandra*), Indian couch (*Bothriochloa pertusa*), red Natal grass (*Melinis repens*), and the forbs hairy cassia (*Cassia absus*), *Cajanus scaribaeoides*, *Galactia* spp., rhyncosia (*Rhynchosia* spp.), and small patches of snakeweed (*Stachytarpheta* spp.).

2.3.3.3 Seasonality of growth

Townsville area has a strongly seasonal rainfall distribution with approximately 70% of the annual rainfall occurring in the January-March period (Table A.1.1). The variability of rainfall from the annual average was calculated by Murtha (1982) as exceeding 30%. Such strong seasonality of rain results in strong seasonality of plant growth. Figs 2.6 and 2.7 show the contrasting vegetation growth in the experimental area in a wet and a dry season respectively. Poor plant growth during dry season was accentuated by the impact of drought periods such as the occurred during the three year experimental period.

Figure 2.6 General view of the sites in the wet season in February 1993

Figure 2.7 General view of the sites in the dry season in August 1993

An important ecological indicator of the severity of the drought stress in the area is illustrated in Fig. 2.8, where a 2 years old Chinee apple (*Ziziphus mauritiana*), know as a strongly drought resistant plant, was killed by the severity of the drought.

Figure 2.8 August 1993, plant of Chinee apple (*Ziziphus mauritiana*) killed by the drought on under-canopy site

2.3.4 Soils of the study area

General soil type

The soil of the area is part of the Healy soil association (Fig 2.9) (Murtha 1982) that is characterised by having a strongly bleached sandy loam A horizon, showing an abrupt change at 20-40 cm to a strongly alkaline mottled brownish grey and yellowish brown heavy clay B horizon (Gardiner 1992).

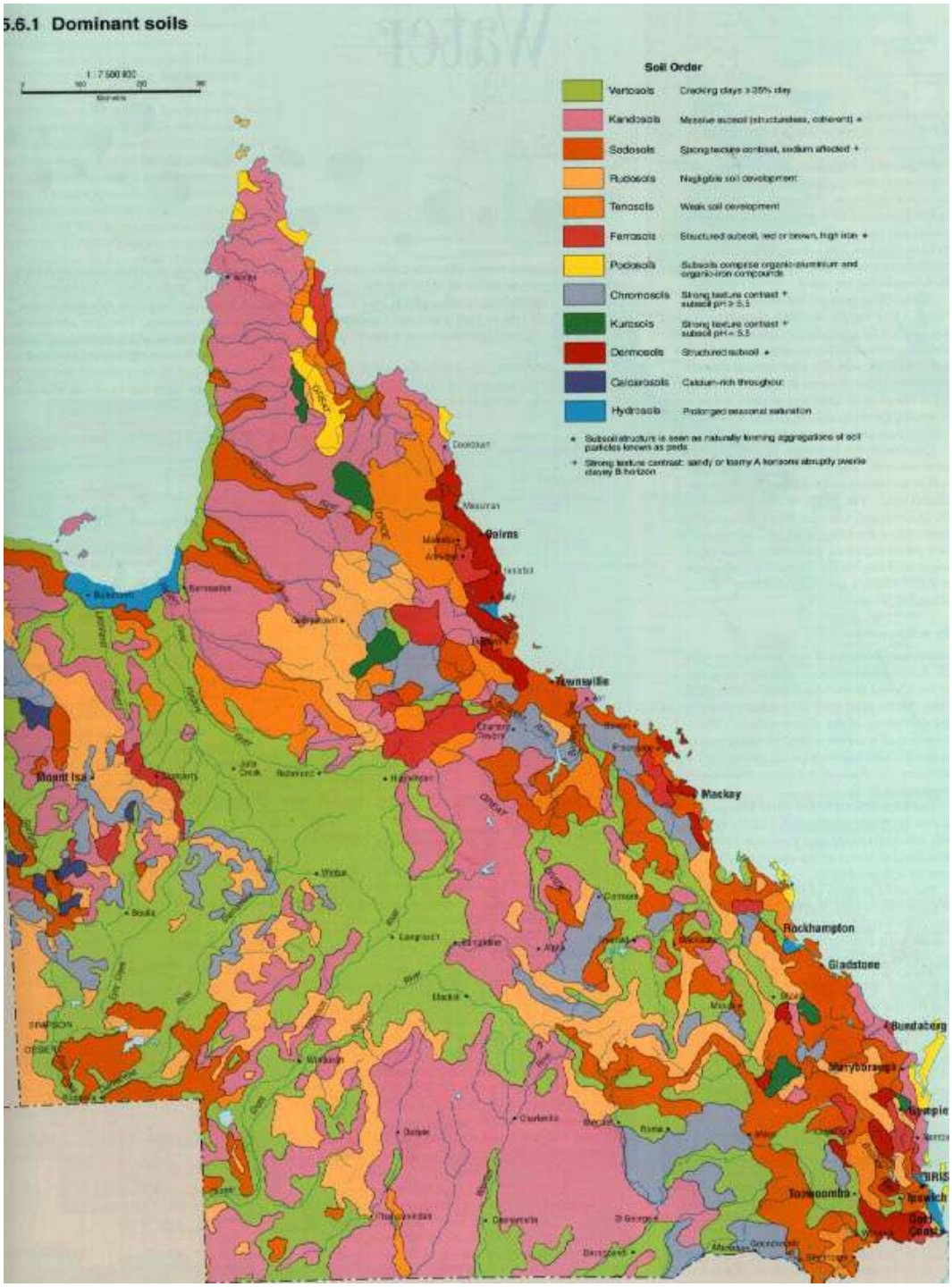

Figure 2.9 Queensland soil map. ASRIS (2005).

Chapter 3
BREAKING THE DORMANCY OF *DESMANTHUS* SEEDS

3.1 Viability of seeds in soil seed banks

Seeds stored in soil seed banks for long periods of time are constantly exposed to environmental and biotic constraints which may provoke loss of their viability, or their ability to germinate. Excessive soil heating and long periods of waterlogging are the major environmental constraints, while insect, bacterial, and fungal attacks are the biotic ones.

A seed bank evaluation of *Desmanthus* genotypes was conducted in October 1992, in an experimental plot at the Townsville campus of James Cook University, containing genotypes of an experimental collection of thirty tropical herbaceous pasture legumes (Fig. 3.1), planted in March 1989 (Suparto 1990).

3.1.1 Materials and methods

3.1.1.1 The study area

The location of the experimental site is shown as site 1 of Fig. 2.3. The area was equipped with a "T-tape" watering system not used since 1990. No burning event occurred in this area from the time of planting (1989) until after the seed bank survey which was carried out 4 years later.

3.1.1.2 Genotype selection

Because of the severe drought experienced at the site over the four year period prior to the seed bank survey, many of the original, sown plants had died. The genotypes selected for study were those which, in Blocks 1 and 2 (Fig. 3.1), showed a minimum of 2 plants per genotype. Nine of the twelve genotypes originally sown met this requirement. Block 3 (Fig. 3.1) was preserved for use in a fire study, discussed in section 3.2 of this thesis.

BLOCK III

Desmanthus bicornutus CPI 91162
Desmanthus spp. Alligator Creek
Desmanthus leptophyllus CPI 38351
Desmanthus virgatus CPI 83563
Desmanthus virgatus CPI 78382
Stylosanthes humilis Khon Kaen
Aeschynomene americana Glenn
Desmanthus virgatus CPI 37143
Desmodium heterocarpon
Desmanthus pubescens CPI 92803
Centrosema *pubescens*
Desmanthus virgatus CPI 78373
Clitoria ternatea
Desmodium heterophyllum
S. aff. *scabra CPI 70522*
Stylosanthes hamata Verano
Arachis pusilla
Chamaecrista rotundifolia Wynn
Stylosanthes hamata local
Desmanthus virgatus CPI 78372
Calopogonium mucunoides
Desmanthus virgatus CPI 79653
Macroptilium atropurpureum
Centrosema pascuorum
Vigna trilobata
Desmanthus leptophyllus TO 88
Desmodium ovalifolium
Stylosanthes scabra Seca
Desmanthus virgatus CPI 67643
Stylosanthes guianensis Graham

← N

BLOCK I

Stylosanthes hamata Verano
Desmodium ovalifolium
Stylosanthes hamata local
Desmanthus virgatus CPI 37143
Aeschynomene americana Glenn
Stylosanthes guianensis Graham
Desmanthus virgatus CPI 79653
Centrosema pubescens
Stylosanthes humilis Khon Kaen
Desmanthus virgatus CPI 67643
Chamaecrista rotundifolia Wynn
Desmanthus virgatus CPI 78372
Desmanthus leptophyllus CPI 38351
Vigna trilobata
Desmodium heterophyllum
Arachis pusilla
Desmodium heterocarpom
Centrosema pascuorum
Desmanthus virgatus CPI 78382
Desmanthus virgatus CPI 83563
Desmanthus bicornutus CPI 91162
Desmanthus virgatus CPI 78373
Macroptilium atropurpureum
Desmanthus pubescens CPI 92803
Clitoria ternatea
Stylosanthes scabra Seca
Stylosanthes aff. *scabra* CPI 70522
Desmanthus sp. Alligator Creek
Desmanthus leptophyllus TQ 88
Calopogonium mucunoides

BLOCK II

Stylosanthes hamata local
Desmanthus sp. Alligator Creek
Stylosanthes scabra Seca
Desmanthus virgatus CPI 37143
Desmanthus virgatus CPI 79653
Desmanthus pubescens CPI 92803
Chamaecrista rotundifolia Wynn
Desmanthus leptophyllus TQ 88
Desmanthus leptophyllus CPI 38351
Arachis pusilla
Calopogonium mucunoides
Desmanthus virgatus CPI 78373
Desmanthus virgatus CPI 67643
Stylosanthes humilis Khon Kaen
Stylosanthes hamata Verano
Desmanthus bicornutus CPI 91162
Centrosema pascuorum
Vigna trilobata
Clitoria ternatea
Desmodium ovalifolium
Desmodium heterocarpom
Desmanthus virgatus CPI 78372
Desmanthus virgatus CPI 83563
Desmodium heterophyllum
Stylosanthes guianensis Graham
Centrosema pubescens
Stylosanthes aff. *scabra* CPI 70522
Desmanthus virgatus CPI 78382
Macroptilium atropurpureum
Aeschynomene americana Glenn

Figure 3.1 Experimental layout of 30 herbaceous forage legumes at the School of Biomedical and Tropical Veterinary Sciences, established by Suparto (1990). Lanes containing *Desmanthus* genotypes are shaded. Plots sized 8 m x 2.5 m, composing blocks of 75 m x 8 m. Plots were contiguous with a row of plants in the middle, which were spaced 50 cm from each other in the rows.

3.1.1.3 Selected genotypes and their previous seedling dynamics in the area

Selected genotypes, plant survival, and seedling recruitment of *D. virgatus* accessions, recorded in the area prior to the seed bank survey are shown in Table 3.1. The high numbers of surviving plants and seedling recruitment recorded in May 1992 compared with earlier observations is probably due to more frequent irrigation after the T-tape was turned on 2 months earlier, which resulted in the regrowth of some plants previously thought to be dead. There were clear and consistent differences between genotypes (Table 3.1). Some, such as CPI 38351, survived well and seedling recruitment was high; others had a low survival level with few seedlings. There was no clear relationship between plant survival and seedling recruitment; the Alligator Creek genotype had high values for both, whilst CPI 37143 had high values for survival only.

3.1.1.4 Seed recovery from the soil seed bank

On 4 October 1993, a 1 x 1 m iron quadrat was placed on the soil surface surrounding each of the selected *Desmanthus* plants. All of the surface litter and soil to a depth of 5 cm inside the quadrat was collected and stored in labeled plastic bags for later examination. Layers deeper than 5 cm were left untouched so as to avoid damage to the root systems of the plants. Jones and Bunch (1977) showed that 85 - 95% of the seeds in a seven-year-old mixed pasture of Siratro/*Setaria anceps* was located in the uppermost 5 cm layer and recommended this depth as a standard in seed recovery from soil seed banks.

Each soil sample was sieved through a 1 cm automatic shaking sieve for separation of rocks, stems, thick roots, and leaves. Seeds were separated from the soil remaining after the initial sieving by the method described by Jones and Bunch (1977) with only slight modifications. The steps were as follows:

1) Each soil sample was first soaked in water and then washed through a set of three round sieves with mesh sizes of 2.0 mm, 1.5 mm, and 1.0 mm. The material retained in the top coarse sieve was discarded and that from the middle and bottom sieves was thinly spread on fine mesh and air dried.

Table 3.1 Plant survival and seedling recruitment of *Desmanthus* genotypes in three blocks at the experimental site.

		June.91					February.92					May.92				
		Plant Survival*				Seedling Recruitment**	Plant Survival †				Seedling Recruitment*	Plant Survival*				Seedling Recruitment*
Accession	**Name**	**Bl. 1**	**Bl.2**	**Bl. 3**	**Mean**	**Mean**	**Bl. 1**	**Bl. 2**	**Bl. 3**	**Mean**	**Mean**	**Bl. 1**	**Bl. 2**	**Bl. 3**	**Mean**	**Mean**
D. virgatus AusTRCF 67643	CPI 67643	1.0	0.0	4.0	1.6	0	1.0	0.5	1.0	0.8	Not scored	3.0	2.0	7.0	4.0	12
D. virgatus AusTRCF 78372	CPI 78372	3.0	4.0	11.0	6.0	0	0.5	0.5	2.0	1.0	Not scored	7.0	14.0	17.0	12.7	18
D.virgatus AusTRCF78373	CPI 78373	0.0	1.0	3.0	1.3	0	0.0	0.0	1.0	0.3	Not scored	1.0	0.0	7.0	2.7	6
D. virgatus AusTRCF 78382	CPI 78382	0.0	4.0	11.0	5.0	1	0.0	1.0	1.0	0.6	0	1.0	10.0	8.0	6.3	19
D. virgatus AusTRCF 83653	*CPI 83563*	0.0	1.0	5.0	2.0	0	0.0	0.5	2.0	0.8	Not scored	1.0	9.0	19.0	9.7	24
D. leptophyllus AusTRCF 321107	TQ88	1.0	2.0	9.0	4.0	0	1.5	1.5	2.0	1.6	Not scored	9.0	11.0	12.0	10.7	36
D. leptophyllus AusTRFC 37143	CPI 37143	12.0	1.0	30.0	14.3	0	2.0	1.5	0.5	1.3	Not scored	27.0	10.0	19.0	18.7	8
D. leptophyllus AusTFCR 38351	CPI 38351	3.0	3.0	9.0	5.0	0	1.5	2.0	2.5	2.0	23.3	10.0	17.0	22.0	16.3	29
Desmanthus spp.	Alligator Creek	0.0	9.0	9.0	6.0	0	1.5	2.0	2.0	1.8	Not scored	17.0	22.0	23.0	20.7	68

* Number of plants per row, originally planted with 32 spaced plants

** Score of presence (1) or absence (0), per row, originally planted with 32 spaced plants

† Score of abundance: absence (0), low (1), medium (2), high (3) per row, originally planted

2) After drying, the material was lightly rubbed by hand for separation of the particles and passed twice through a small seed cleaning aspirator ("Bates Laboratory Aspirator" from H.T. McGill, 548, N. Milby St. Houston, Texas, USA). During the first passage of the sieved samples, the aspirator was regulated to lift the light particles of soil and organic material, and deposit them in the upper container. In the second pass, the aspirator was set to blow the seeds of *Desmanthus* and other material of similar density to the upper container. In both instances the material remaining was examined for the presence of *Desmanthus* seed before being discarded.

3) Seeds of *Desmanthus* were manually separated from the residue on a glass plate using an illuminated magnifying lamp, counted, and stored in paper seed bags. Considering the large distance between rows in the field (2.5 m), there was a very low probability of seeds of one genotype to be contaminated by seeds from neighboring genotypes. Despite this, recovered seeds were compared with the original seeds of the surveyed genotype, and the seeds that did not match were discarded.

4) Seeds of the nine genotypes of *Desmanthus* were tested, under laboratory conditions for viability by the Tetrazolium test (AOSA 1970). Approximately 30% of the seeds were longitudinally cut and soaked for three hours in a 2, 3, 5-triphenyl-tetrazolium chloride (TTC) 0.5% solution, and checked for presence of red-stained embryo or cotyledons (Copeland 1976).

3.1.1.5 Statistical analysis

Data on the number of recovered seeds were statistically analysed in a completely randomised design with nine factors (genotypes) with a different number of replications, using an ANOVA analysis of variance in *Statistic version 4* software computer program. Means were compared by LSD test with a 0.05 confidence interval.

3.1.2 Results

Persistent seed banks were found to exist under all of the studied genotypes. They were significantly different ($p < 0.05$) in the numbers of seeds/m^2 recovered from a layer of 5 cm depth under individual plant canopies (Table A.2.1). The highest numbers of seeds

were found in the genotypes of the group in which CPI reference number started with 78 and collected in Argentina (Table 3.2), indicating that they have adapted well to their new environment. The highest seed bank values were recorded for CPIs 78373 and 78382 (more than 1200 seeds/m^2). Alligator Creek, TQ 88 and CPI 38351 had seed banks of intermediate size (600 to 800 seed/m^2); while CPIs 37143, 67643 and 83563 had relatively small seed banks (less than 500 seed/m^2). Significant differences between means were tested by the LSD pairwise comparison method for different numbers of replications (Table 3.2).

Table 3.2 Soil seed bank of *Desmanthus* genotypes. Means refer to number of seeds/m^2 in the soil to a depth of 0 - 5 cm

Genotype	**Seed/m^2**
CPI 78382	1303.10 a
CPI 78373	1227.50 ab
CPI 78372	818.25 abc
TQ 88	747.75 bcd
CPI 38351	640.75 cd
Alligator Creek	605.98 cd
CPI 83653	491.65 cd
CPI 67643	329.50 cd
CPI 37143	280.98 d
Mean	716.16

Note: means followed by the same low case letter are not significantly different at LSD< 0.05

The sizes of the seed banks suggest that most of seeds falling on the soil surface do not germinate, but are recruited to the seed bank for several seasons. A comparison of the data in Table 3.2 with those for May 1992 (Table 3.1) is of interest. Although the introductions from Argentina (CPI numbers 78372, 78373, and 78382), had large seed banks, they had few surviving plants. By contrast, some of the other genotypes, such as, Alligator Creek, TQ 88 and CPI 38351, had small seed banks, but high seedling recruitment (Table 3.2).

Seeds recovered from the soil seed banks showed high viability in all genotypes as indicated by the Tetrazolium test (Table 3.3). Seeds were clearly still viable after being buried in the soil for up to 4 years. This must be attributed to the effectiveness of the seed-coat and sealed strophioles in preventing water entry and invasion by soil insects and saprophytes.

Table 3.3 Seed viability % of nine genotypes of *Desmanthus* by theTetrazolium test.

Genotype	Viability %
CPI 83653	96
CPI 37143	94
CPI 38351	94
CPI 78372	92
CPI 78373	92
CPI 78382	90
Alligator Creek	86
TQ 88	82
CPI 67643	80
Mean	89.6

3.2 Effects of heat on seed-coat structure and germination of genotypes of the *Desmanthus* complex

Seed-coat dormancy or hardseededness is one of the most important features influencing the establishment and regeneration of herbaceous tropical legumes in pastures. While seed-coats protect seeds from cellular rupture and from fungal and insect attacks, and allow pasture legumes to produce a seed bank reserve, they also may prevent germination in seeds of some species, causing problems of plant establishment and regeneration. Problems of seed-coat dormancy in seeds may be overcome before planting by a specific treatment to remove hardseededness. On the other hand, the release of seed-coat dormancy in seeds from soil seed banks that will allow new seedling recruitment, and assure consequent plant persistence in the pasture community, can not be obtained by artificial methods. These dormancy issues are dependent on the type of seed-coat dormancy of the species (Morrisson *et al.* 1992), and on the occurrence of environmental factors, wherein natural soil heating is the most important. The relationships between soil heating and softening of seeds located in the soil seed-bank are well established for some commercial cultivars of tropical forage legume species. The softening of seeds of *Stylosanthes humilis*, *S. hamata, S. scabra,* and *S. viscosa* in soil seed-banks at Katherine, Townsville, and Narayen, in Australia, was related to the number of days during which the soil temperatures rose above 50-55 ºC (Mott *et al.* 1981). The existence of a relationship between soil heat and softening of

seeds for the majority of tropical forage legumes does not imply, however, the existence of a general model for all species, cultivars, or genotypes.

Ripe seeds of genotypes of *Desmanthus* stored in soil seed-banks have a relatively high fraction of dormant seeds. Except for the observations of Borrows and Porter (1993) on the formation of soil seed bank and seedling recruitment of *D. virgatus* CPI 78382 at Gayndah, Queensland, Australia, patterns of natural or enforced after-ripening on seeds of *Desmanthus* have not been established so far. Such information may be very helpful to support farmers in their choices of a specific cultivar according the environmental conditions prevailing at specific sites.

In the present study, patterns of seed softening were established in response to a range of heat intensities in 9 genotypes of *Desmanthus,* and were related to groups of genotypes, according their origins. Strophiole permeability and germination were the parameters considered in the study. It was assumed that a permeable strophiole is the only way for water to enter intact seeds of *Desmanthus,* and water entry is significantly correlated with germination. Considering such as statements it would be very useful to determine this character to evaluate the potential of a specific genotype to release soft seeds.

3.2.1 Material and methods

Seeds recovered from the soil seed banks of the 9 genotypes of *Desmanthus* listed in Table 3.1, were exposed to a range of temperatures under laboratory conditions and changes in strophiolar structure, hardseededness, and germination responses were observed.

3.2.1.1 Heat treatments

On 7 May 1994, seeds from different seed banks under the same genotype in Blocks 1 and 2 of Fig. 3.1 were bulked into one lot of seed for each genotype. The seeds were mixed with a dry sandy loam soil and distributed over 18 Petri dishes (60 seeds/Petri dish). Three Petri dishes for each genotype were exposed to the following six temperature treatments, in a three shelved drying oven: Control (room temperature = 30

ºC); 40 ºC; 60 ºC; 80 ºC; 100 ºC and 120 ºC. The shelves in the oven composed the experimental blocks and had one Petri dish/genotype/shelf. The oven was turned off when it reached the set treatment temperature and left to cool down to room temperature. Time taken to cool down ranged from 12 to 15 minutes. Seeds were then separated from the soil by sieving through a 1.18 mm sieve.

3.2.1.2 Germination, hard seed and imbibed seed

On 15 May 1994, 50 seeds for each combination of genotype x temperature x block, from section 3.2.1.1, were placed in Petri dishes of 10 cm diameter half filled with vermiculite and with two filter paper sheets on the top of the vermiculite. These were placed in a constant temperature room at 28 ºC with 24 hours of fluorescent lighting per day and the filter paper wetted twice daily with distilled water. Petri dishes were arranged in a randomised block design of 9 genotypes, 6 heat treatments, and 3 blocks (total of 162 dishes).

Germination (recognised by the emergence of the radicle and its extension to the epicotyl) was recorded daily over a 21 day period but no germination occurred after the 14th day. Non-germinated seeds (water-imbibed or remaining hard) were recorded at the end of germination trial.

Some seeds took up water and became imbibed during the washing and sieving process described above (section 3.1.1.4). These seeds were removed and tested separately for germination without any previous heat treatment. Their germination percentages, calculated from the total number of recovered seeds in the sample, were then added to germination percentage of the control treatment.

3.2.1.3 Strophiolar structure

Changes in the strophiolar structure (normal, raised or swolen, or cracked) were registered by using an illuminated magnifier lamp (100 times amplified) on the surface of heat-treated seeds (section 3.2.1.1) not submitted to the germination test, and in the non-germinated seeds from 3.2.1.2.

3.2.1.4 Statistical analysis

A randomised block design with two factors (genotypes and temperatures), and three replications (Petri dishes) was used for the data analysis. Significant differences within main factors and interactions were analyzed using the ANOVA model in the Statistic version 4 software computer program. Means were compared by LSD ($p < 0.05$).

3.2.2 **Results**

The ANOVA procedure detected statistically significant differences ($p < 0.05$) among genotypes, heat treatments, and their interactions for percentage of raised strophioles and germination (Tables 2.A.2 and 2.A.3).

3.2.2.1 Raised or swollen strophiole

Comparisons of means for different genotypes under different temperature treatments and interactions by LSD ($p < 0.05$) are presented in Table 3.4. Higher temperatures were correlated with an increase in the percentage of seeds with raised strophioles ($r^2 = 0.84$). There were, however, two patterns of response. In the first, as shown by genotypes CPI 67643, CPI 78373, and CPI 78382, strophiolar structures were not significantly affected by temperatures below 80 ºC, and genotype CPI 78372 was little affected by temperatures less than 100 ºC. In contrast, significant changes in the strophiolar structure of the remaining genotypes occurred when temperature rose to as little as 60 ºC.

By combining the data on the size of seed banks (Table 3.2) with those for changes in strophiolar structure (Table 3.4 an Figs. 3.2 to 3.4), it is clear that the subtropical Argentinean accessions (CPI 78372, CPI 78373, and CPI 78382) produced the largest seed banks and had the lowest percentage of raised strophioles at temperatures up to 100 ºC. On the other hand, accessions with the smallest seed banks, CPI 37143, CPI 67643, and CPI 83653, from tropical Central America, CPI 38351 from northern Venezuela, and Alligator Creek whose origin is unknown, had the highest percentage of their strophioles raised at temperatures as low as 60 ºC .

Table 3.4 Percentage of raised seed strophioles of nine *Desmanthus* genotypes in response to heat treatments (Control, 40 ºC, 60 ºC, 80 ºC, 100 ºC, and 120 ºC). Means of three replications.

Accession No.(CPI)	Heat Treatments ºC						
	Control	40	60	80	100	120	Mean
CPI 37143	0.0 D c	0.0 D c	50.0 C abc	83.3 AB abc	80.0 B abc	100.0 A a	52.2 b
CPI 38351	8.7 C abc	3.7 C bc	33.3 B bc	93.3 A ab	92.7 A ab	96.3 A a	54.6 b
CPI 67643	9.7 C ab	20.0 BC a	28.3 BC cd	100.0 A a	100.0 A bc	100.0 A a	51.9 b
CPI 78372	0.0 C c	3.3 C bc	3.0 C e	58.0 B cd	70.0 B abc	100.0 A a	39.1 c
CPI 78373	3.0 B bc	7.0 B bc	7.0 B de	36.7 B d	36.7 B c	100.0 A a	31.7 c
CPI 78382	4.0 C bc	4.0 C bc	10.0 C de	65.0 B bcd	60.0 B abc	100.0 A a	40.5 c
CPI 83563	15.3 B a	13.3 B ab	40.0 B bc	73.3 A abc	76.7 A abc	93.3 A a	52.0 b
All.Cr.	4.0 C bc	6.0 C bc	54.0 B ab	93.0 A ab	100.0A a	100.0 A a	59.5 ab
TQ 88	8.0 C abc	13.3 C ab	70.7 B a	100.0 A a	100.0A a	100.0 A a	65.3 a
Mean	5.8 D	7.8 D	32.9 C	78.1 B	82.2 B	98.8 A	

Means followed by the same CAPITAL letter in rows, or by the same lower case letter in columns, are not significantly different (LSD, $p < 0.05$).

3.2.2.2 Germination

Table 3.5 contains the means of the two factors (genotype and temperature), and means of their interactions, compared by the LSD ($p < 0.05$). In contrast to what was found for raised strophioles, there was only a weak relationship between temperature and seed germination ($r^2 = 0.24$). Such lack of significance was caused by a reduction in germination of seeds of many genotypes exposed to temperatures higher than 80 ºC (Table 3.5). Seed germination of individual genotypes in response to heat has to be interpreted firstly with reference to the lowest temperature necessary to promote germination; and secondly with regard to the highest temperature to which seeds can be exposed without reduction in viability.

Germination of all genotypes started to be promoted by temperatures of 60 ºC, had a maximum at 80 ºC, and was reduced again by higher temperatures (Figs. 3.2 – 3.4). In these figures, room temperature was taken as 20 ºC, for ease of preparation of the graphics.

The genotypes could be divided into three groups in their germination responses to heat: one group is "highly temperature specific" for germination (Fig. 3.2), and consisted of CPI 78373 and CPI 78382, where germination was promoted by a very narrow range of temperatures around 80 ºC. In this group CPI 78373 had percentages of germination greater than 10% only at a temperature of 80 ºC.

Another group is "intermediate temperature specific" for germination (Fig. 3.3) and comprises CPI 37143, CPI 67643, CPI 78372, and CPI 83563, where germination was promoted by an intermediate range of temperature of 60 - 80 ºC, or 80 - 100 ºC. The last group, "indifferent to temperature" in its germination (Fig. 3.4), is composed of the genotypes Alligator Creek, TQ 88 and CPI 38351, where germination was not correlated with increasing temperatures over the temperature range of 60-100 ºC.

Two different groups of plants showed reductions of germination at temperatures higher than 80 ºC. One group consists of CPI 38351, CPI 78372, CPI 78382, CPI 83563, and the genotype Alligator Creek, in which seeds were exposed to temperatures as high as 100 ºC without reduction in germination.

Table 3.5 Percentage of germination in seeds of nine *Desmanthus* genotypes in response to heat treatments (Control, 40 ºC, 60 ºC, 80 ºC, 100 ºC, and 120 ºC). Means of three replications.

Genotype (CPI)	Temperatures ºC						
	Control	40	60	80	100	120	Mean
CPI 37143	0.8 D d	5.8 D c	40.0 BC b	75.0 A a	45.3 B ab	14.0 CD ab	30.2 abc
CPI 38351	8.0 C bc	4.0 C c	40.7 B b	61.3 A ab	62.3 A a	1.3 C b	29.7 bc
CPI 67643	10.0 C b	13.3 C abc	41.7 B b	66.7 A ab	18.7 C ab	10.0 C ab	26.7 bc
CPI 78372	6.7 B bc	4.0 B c	15.3 B c	50.0 A ab	51.3 A ab	8.7 B ab	22.7 cd
CPI 78373	8.0 A bc	8.0 A bc	4.0 A c	32.0 A b	7.3 A b	2.7 A b	10.3 d
CPI 78382	4.0 B cd	4.7 B c	5.3 B c	68.0 A ab	18.7 B ab	33.3 B a	22.3 cd
CPI 83563	17.5 B a	22.5 B a	20.0 B c	80.0 A a	55.3 A ab	26.7 B ab	37.0 ab
Alligator Creek	6.7 B bc	4.0 B c	53.3 A ab	64.0 A ab	60.0 A a	3.3 B b	31.9 ab
TQ 88	16.0 B a	18.7 B ab	72.0 A a	79.3 A a	43.3 A ab	9.3 B ab	39.8 a
Mean	8.6 C	9.4 C	32.5B	64.0 A	40.3 B	12.2 C	

Means followed by the same CAPITAL letter in rows, or by the same lower case letter in columns, are not significantly different (LSD, $p < 0.05$).

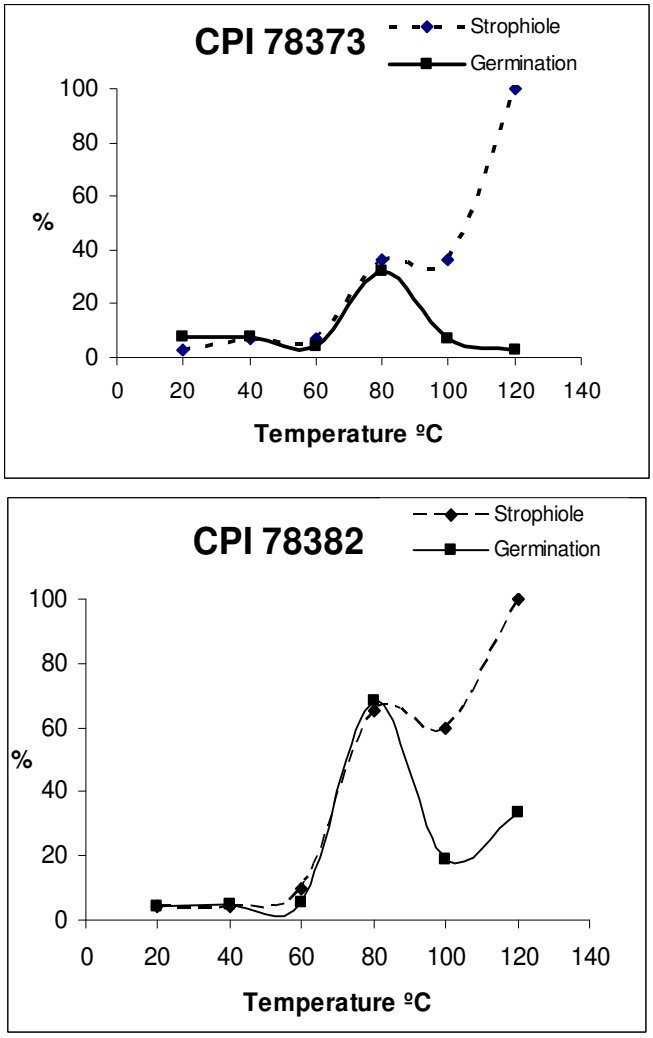

Figure 3.2 Effect of heat on the percentage of strophiole permeability (-----Strophiole) and the percentage of germination (—Germination) in seeds of *Desmanthus* genotypes in the group with "highly specific temperature for germination".

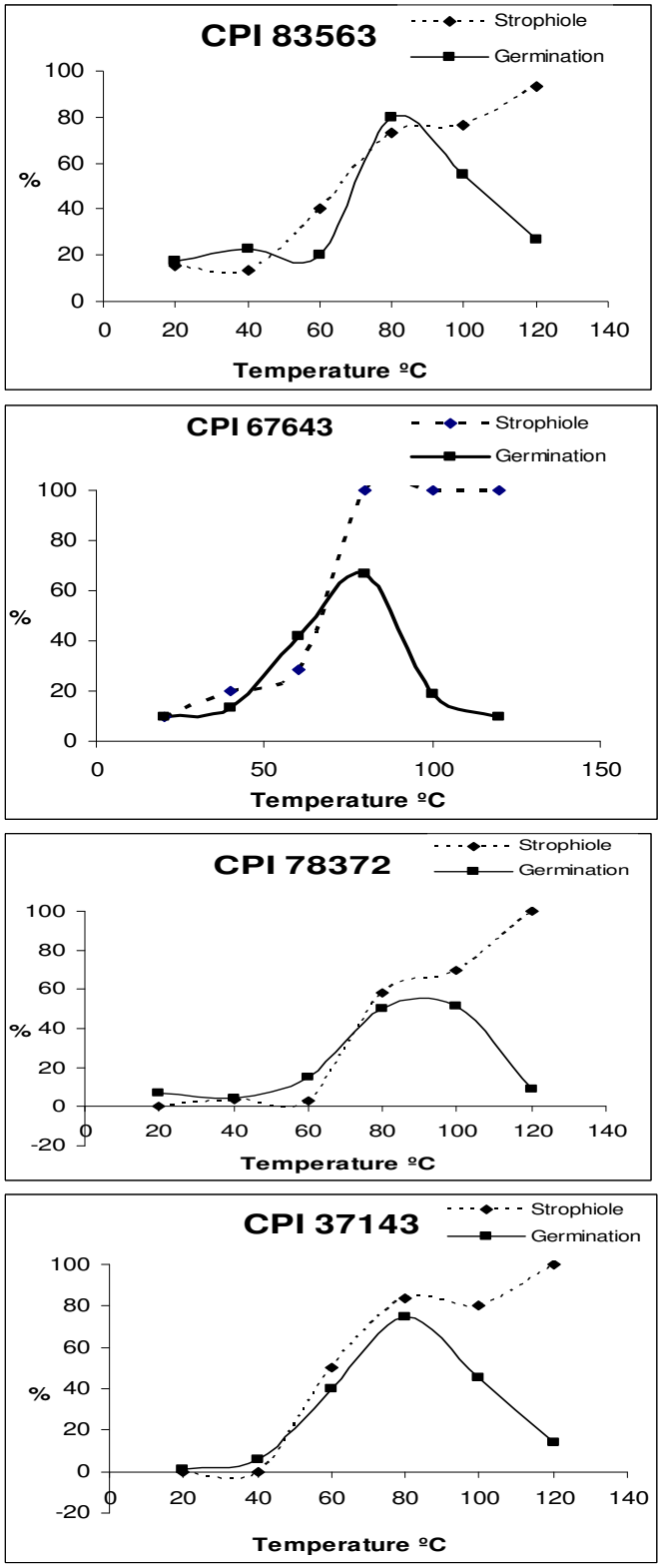

Figure 3.3 Effect of heat on the percentage of strophiole permeability (-----Strophiole) and the percentage of and germination (—Germination) in seeds of *Desmanthus* genotypes in the group with "intermediate specific temperature for germination".

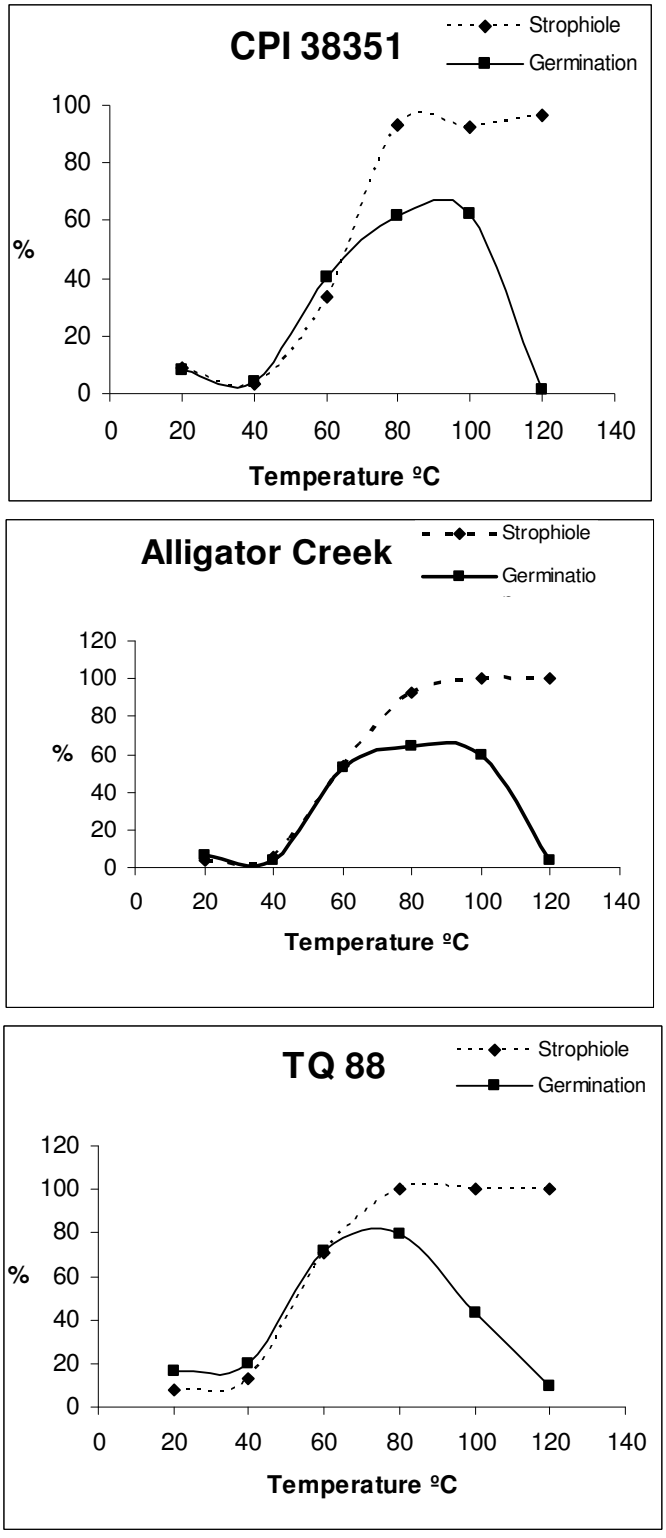

Figure 3.4 Effect of heat on the percentage of strophiole permeability (----Strophiole) and on the percentage of germination (—Germination) in seeds of *Desmanthus* genotypes in the group with "indifferent to temperature for germination".

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Seeds of CPI 78382 and CPI 83563, showed an outstanding resistance to temperature, recording respectively 33.3% and 26.7% of germination at 120 ºC. The other group consists of all the remaining genotypes showed a significant reduction in germination percentage at all temperatures above 80 ºC.

The failure of a seed to germinate, either with or without heat treatment, can be attributed to one of three causes: the seed was dead before treatment; it was killed during the treatment; or it remained dormant despite the treatment. As the number of viable seeds used in the experiment was known (from the Tetrazolium test), as was the number of germinated and hard seeds, it was possible to calculate the number of seeds killed by the heat treatment.

The analysis of variance detected significant difference in the percentage of **residual hardseededness** for the main treatments and their interactions ($p < 0.01$; Table A.2.4). Means of hard seeds for the factors temperature, genotype, and their interactions were compared by LSD ($p < 0.05$) and are presented in Table 3.6.

3.2.2.3 Hardseededness

The correlation coefficient of percentage of seeds remaining hard, for the average of all genotypes, and temperature showed a negative relationship ($r^2 = -0.82$; Table 3.6). Two groups of genotypes with similar trends could be detected. One group comprising CPI 78372, CPI 78373, CPI 78382, and CPI 83563, in which temperatures lower than 80 ºC did not reduce the percentage of seed remaining hard. In other group, comprising the remaining genotypes, the percentage of hard seeds was reduced at temperatures higher than 60 ºC. CPI 78373 and CPI 78382 still had very high percentages of hard seeds after exposure to 100 ºC. Microscopic examination of seed exposed to 120 ºC of heat showed that any seed which failed to germinate was soft and dead.

Table 3.6 Percentage of seeds which remained hard after the germination test of nine *Desmanthus* genotypes submitted to heat treatments. Means of three replications.

Accession Number	Temperature ºC						
	Control	40	60	80	100	120	Mean
CPI 37143	99.2 A a	94.2 A a	59.2 B bc	20.0 C bc	10.8 BC bc	0 C	47.2 b
CPI 78372	93.3 A ab	95.3 A a	83.3 A a	45.3 B ab	33.3 B abc	0 C	58.4 a
CPI 78373	92.0 A ab	92.0 A a	94.7 A a	68.0 A a	64.0 A a	0 B	68.4 a
CPI 78382	93.3 A ab	95.3 A a	92.7 A a	50.0 B ab	30.0 BC abc	0 C	60.2 a
Alligator Creek	90.7 A b	94.7 A a	39.3 B cd	31.0 B abc	6.0 CD c	0 D	43.8 b
TQ 88	78.7 A c	81.3 A ab	22.0 B d	1.3 C c	2.0 C c	0 C	30.9 c
CPI 83563	77.5 A c	75.0 A b	74.2 A ab	26.7 B abc	1.0 BC bc	0 C	43.9 b
CPI 67643	81.7 A c	75.0 A b	48.3 B c	20.0 C bc	16.7 C bc	0 C	40.3 bc
CPI 38351	89.3 A b	93.3 A a	50.0 B c	0.7 C c	3.3 C c	0 C	39.4 bc
Mean	87.6	88.4	62.6	29.2	18.6	0	r^2*= - 0.82

Means followed by the same CAPITAL letter in rows, or by the same lower case letter in columns, are not significantly different (LSD p< 0.05).

* Coefficient of correlation of percentage of seeds remained hard, for the average of all genotypes, and temperature.

3.3 Effect of fire on soil heating and seed-coat dormancy

Although high solar temperatures occur in the dry season, they may not be sufficient to break down dormancy of many hard-seeded populations stored in soil seed banks (Probert 1992). As was shown in the previous section, some of the *Desmanthus* genotypes may need soil heating higher than 60 ºC of temperature to promote seed germination.

Such temperatures are not common under normal conditions (Mott *et al*. 1981). However, under a brief exposure to higher temperatures, as a result of fire, seed-coat break down may be more rapid (Probert 1992).

The critical determinants of whether or not hard seed break down may occur are the specific plant population, the position of the seeds within the soil profile, and the intensity and duration of the fire (Floyd 1966; 1976; Walker *et al.* 1981; Keeley 1987; Probert 1992; Bradstock and Auld 1995). Intensity and duration of fire depend on the amount and the quality of the burnt material.

In the following section, the effects of fire on soil heating, and on seedling recruitment as a result of the softening of seeds in soil seed banks in genotypes of *Desmanthus,* are presented and discussed.

3.3.1 Effect of fire on soil heating

3.3.1.1 Materials and methods

Temperature variations in different layers of soil during experimental grassland fires were evaluated in an area on the Douglas Campus of James Cook University, away from the plant collection at site 4 of Fig. 2.3. The short grasses and forbs at the study site were mowed on 20 June 1993 and the cut vegetation left on the soil surface to dry. One shallow pit was dug to 3 cm depth in each one of three points selected at random in the mowed area, and three thermocouples were installed at depths of 3 cm, 1 cm, and on the soil surface. The thermocouples were attached to a data-logger (LI - 1000 manufactured by LI - COR, Inc., 4421, Superior Street, Lincoln, Nebraska, USA).

Layers between each thermocouple sensor were filled with the loose soil previously removed from the pit. Dry plant material from the previous mowing was arranged on the soil surface of the three points to provide fire fuel equivalent to approximately 3-4 t/ha of dry herbage. This material did not have uniform composition across the points, having a higher proportion of forbs than grasses in points 1 and 3. Three separate fires were lit at 30 minute intervals, to allow time to reset the thermocouples for each subsequent fire. After ignition, the fires took an average of 10 minutes to complete burning the dry plant material. Soil temperatures at 3 depths were recorded every 15 seconds for a 14 minute period following ignition.

3.3.1.2 Results

The patterns of temperatures at 0, 1.0 and 3.0 cm depths in the soil during the three separate fires are shown in Figs. 3.5 and 3.6. There were similar trends of heating and cooling in all of the fires, but there were differences in the maximum temperatures obtained (Figs. 3.5 and 3.6). No changes in the temperatures of the soil layers were recorded during the first two minutes after each ignition. At the soil surface, temperature then rose abruptly after the second minute, reaching a peak of around 300 °C in fires 1 and 3, and about 120 °C in fire 2, during the 3rd to 4th minute. This peak was maintained for around one minute and then, the heat started to dissipate at a rate of about 50 °C/minute, stabilising at 40-50 °C for another 3-4 minutes. At 1.0 cm depth in the soil, the temperatures reached a maximum of 70-80 °C around the sixth minute and maintained this level for 4-5 minutes. Fire had little effect at soil depths of 3.0 cm, with a maximum increase of 5 °C noted in fire 3.

3.3.2 Effect of fire on seedling recruitment

3.3.2.1 Materials and methods

On 10 July 1993, an experimental fire was lit on part of block 3 of the plant collection at James Cook University (Fig. 3.1). Plants of 6 *Desmanthus* genotypes, located of the northern end of that block were exposed to the fire*: D. bicornutus* AustTRCF 91162 (CPI 91162), *D.*spp. Alligator Creek. *D. leptophyllus* AustTRCF 38351 (CPI 38351), *D. leptophyllus* AustTRFC 37143 (CPI 37143), *D. virgatus* AustTRFC 83563 (CPI 83563)

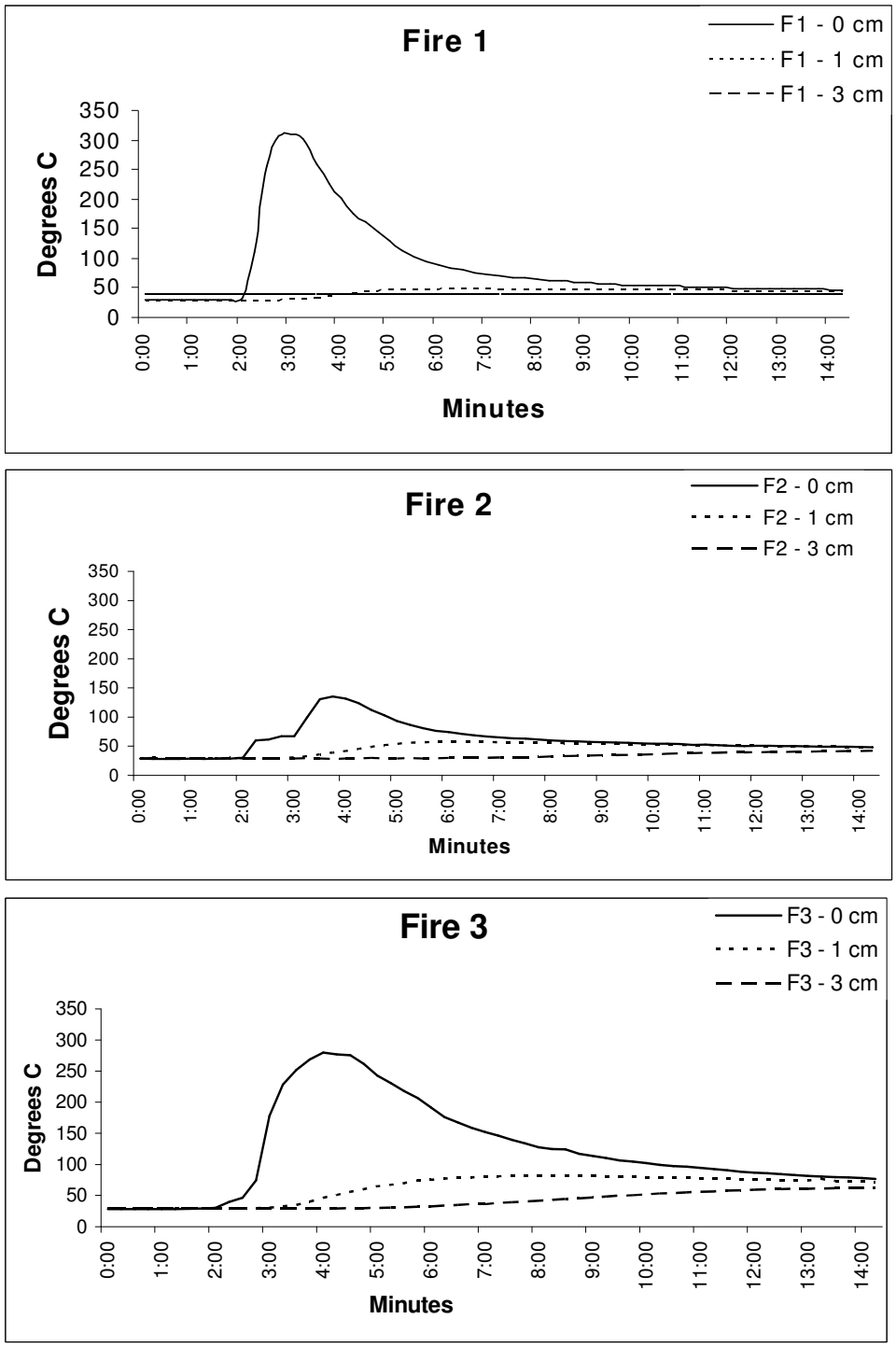


Figure 3.5 Soil temperatures during three experimental grassland fires. F1, F2, F3 = Fire 1, Fire 2, and Fire 3 respectively. 0 cm, 1 cm, and 3 cm = Temperatures variation during fire at 0, 1, and 3 cm, respectively, below the soil surface.

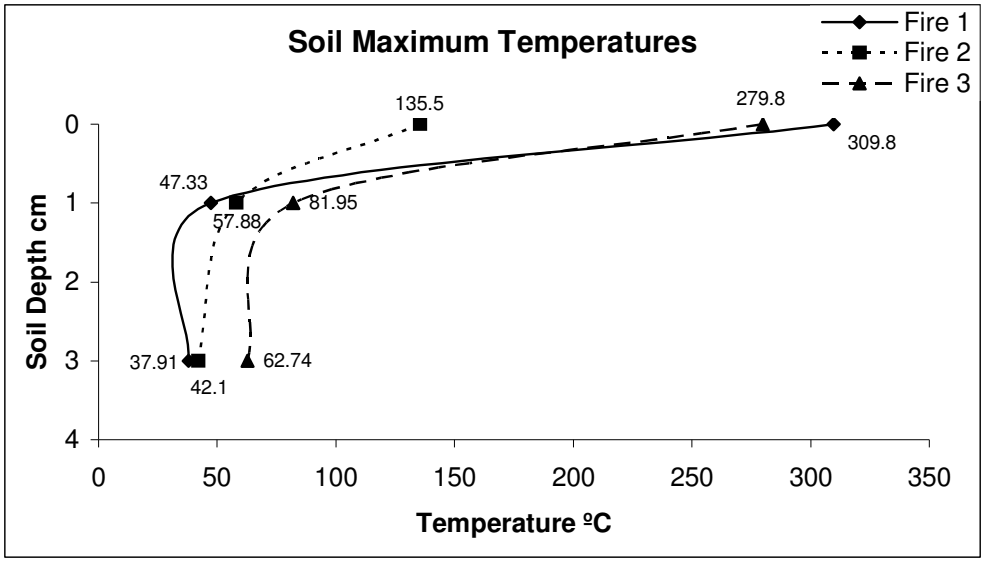


Figure 3.6 Maximum temperatures observed at different depths in the soil under three grassland experimental fires at James Cook University. 10 July 1993.

and *D. virgatus* AustTRCF 78382 (CPI 78382). Two months prior to the fire, irrigation had been discontinued in this part of the block to ensure a good supply of combustible material.

Irrigation was restored immediately after the fire and, 21 days after the fire event, the number of recruited seedlings/genotype/row was recorded in a 1 m wide strip, down the row axis.

3.3.2.2 Results

An estimation of the number of recruited seedlings from each parent plant after the fire was obtained by dividing the number of seedlings/row by the number of surviving parent plants in the row.

The number of seedlings present in the field increased markedly after the fire (Fig. 3.7), compared with previous records of natural recruitment (Table 3.1). The magnitude of this increase was three-and four-fold higher in the genotypes Alligator Creek and CPI 38351. Other genotypes in the burned area had not produced a single seedling before

fire, but showed a profuse recruitment after the fire, especially *D. virgatus* CPI 83563 from which 386 seedlings were noticed in a single, 8 m long row. *Desmanthus bicornutus* CPI 91162, on the other hand, still showed no seedling recruitment after the fire. Indeed this genotype had only one surviving plant in the row and it was never seen to flower.

There were large differences in post-fire seedling recruitment between genotypes (Fig. 3.7), and these differences seem to be related to the initial plant survival. Seedling recruitment from each of the initial surviving plants was generally similar between genotypes (Table 3.1 and Fig. 3.7). The sole exception was CPI 83563 (Fig. 3.7). This genotype had a much higher post-fire recruitment rate/parent plant than any other, which could be an adaptation to the occurrence of fire in its environment of origin.

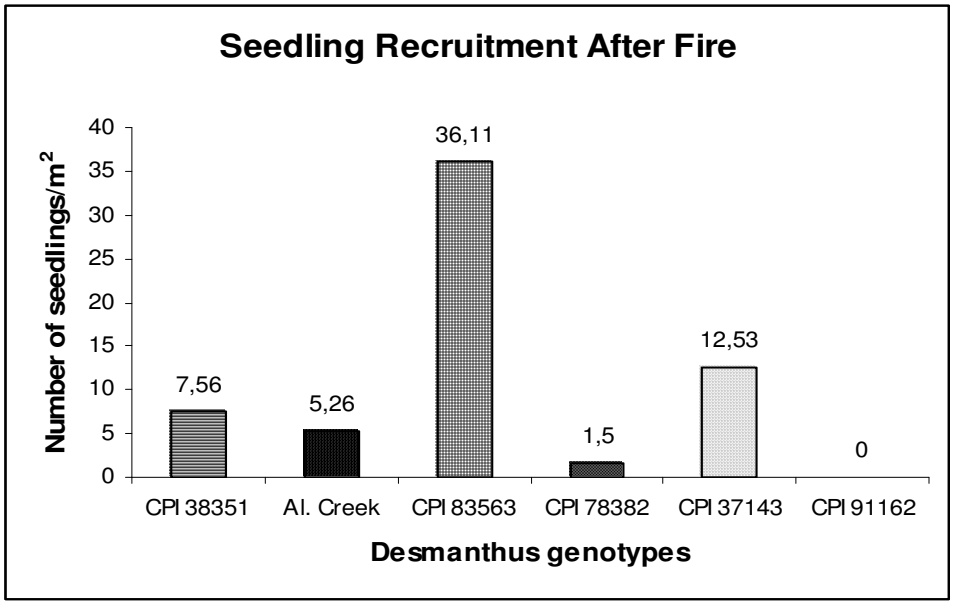


Figure 3.7 Seedling recruitment in genotypes of *Desmanthus* after a grassland fire. Seedling/m^2 = mean number of seedlings per m^2 of plot under surviving plants

3.4 Discussion

3.4.1 Soil seed banks

All the *Desmanthus* genotypes produced persistent seed banks under the conditions prevailing in the experimental site. Under drought conditions the seed bank size varied

between genotypes (Table 3.2). As the parent plants had all been cultivated on the same soil and under the same climatic and management conditions, these differences must be attributed to different rates of seed production and/or seedling recruitment between the genotypes and not to different management conditions as was found by Ward and Jennings (1990) and Tremont (1994). Certain species of the genus *Desmanthus* are regarded as prolific seed producers (Piper 1986; Piper and Towne 1988; Piper *et al.* 1988; Adjei and Pitman 1993). Nevertheless, the present study highlighted the variability between genotypes in the size of the seed bank that they produced in the soil.

An average of more than 700 seeds/m^2 recovered from soil in the present study was around five times higher than the 150 seeds/m^2 for the average of grazed and ungrazed paddocks reported for *D. virgatus* CPI 78382 by Borrows and Porter (1993). In the present study, 1303 seeds/m^2 of CPI 78382 were recovered from soil (Table 3.2), which is three times more than the maximum of 450 seeds/m^2 observed by Borrows and Porter (1993) in grazed paddocks at Bryan Pasture Research Station, Narayan, south Queensland, on a moderately self-mulching and cracking brown clay soil. Such differences may be expected, given the differences in the conditions of the environment and of the parent plants. The capacity of *D. virgatus* CPI 78373 cv. Marc to form large seed banks (1227,5 seed/m^2) shown in this study was also recognized by Gardiner *et al*. (2004), in plots 10 - 14 years old, after planting at Blackall and Isisford, in the semi-arid, black clay soil of Queensland's Mitchell Grass Bioregion. Contrasting with the relatively low plant survival and seedling recruitment observed in the present study in May 1992 (Table 3.1) in CPI 78373 cv. Marc, it had the highest survival rate at Blackall with mean of 9 plants/m^2 in 2003, showing its better adaptation to the black clay soil conditions of Mitchell Grass Bioregion than to the duplex soil of Coastal region (Gardiner *et al*. 2004).

3.4.2 Effects of heat on seed-coat structure and seed germination

Although the age of the recovered seeds could not be estimated in the present study, their distribution in soil from the surface throughout a 5 cm deep layer, confirms that they are a mixture of seeds having ages varying from some months to a maximum of four years determined by the period when *Desmanthus* genotypes were introduced to

the study site. Their high viability suggests that four years storage in the soil did not reduce their germination potential.

The potential role of the strophiole as the main site of water uptake in seeds of the genus *Desmanthus* was stressed by Hopkinson (1993b). As in seeds of many other species of the Mimosaceae (for instance, *Albizia lebbeck,* Dell 1980*),* the water-permeable strophioles in *Desmanthus* were raised and gold in colour on smooth brown seeds (Hopkinson 1993b). Legume seeds deposited in soil generally have a very high percentage of impermeable strophioles and will not germinate under normal conditions (Hopkinson 1993a; Hopkinson and English 2004). Increasing the soil temperature by intense solar radiation during summer, or by occasional burning of the aboveground vegetation, may promote strophiole permeability and so promote high seed germination in the next wet season (Quinlivan 1965; 1966; 1971a; Gill 1981; Lodge *et al.* 1990; Murdoch and Ellis 1992).

The two ends of the horseshoe-shaped pleurogram of *Desmanthus* lie near the pointed end of the seed that encloses the radicle, adjacent to which on the main ridge lays the hilum and strophiole (Fig. 3.8). Impermeable strophioles appear near the narrow, white, longitudinal strip of the vascular strand (Fig. 3.9). Raised and permeable strophioles form a small golden lump on the seed surface (Figs. 3.10 and 3.11); such seed structures changes are known to occur naturally and to be promoted by heat treatments (Hopkinson 1993b). When exposed to high temperatures, the strophiole may show cracks in the epidermis or may even have a lacerated epidermis. The photographs of Figs. 3.8 to 3.11 were taken by J. H. A. Rangel in 16 October 1994 using a YASHICA Microscopy Connect Camera of the Photography Laboratory of the School of Tropical Biology, JCU, Townsville.

Seed polymorphism in terms of the temperature required to break seed-coat dormancy has been reported in many legume genera (Floyd 1976; Taylor and Ewing 1992); but there are no reported data for the genus *Desmanthus*. The results presented here on raised strophioles, germination rates, and remaining hard seeds as a function of a range of applied temperatures show that *Desmanthus* genotypes vary widely in their responses to heat (Tables 3.4 – 3.6, and Figs. 3.2 – 3.4).

In genotypes belonging to the “highly temperature specific group”, seed strophioles did not become raised and germination was not increased until temperatures reached 80 ºC. On the basis of these results, seeds of such genotypes might or might not have their seed-coat dormancy removed by normal summer heat as suggested by Quinlivan (1965, 1966, 1971a), Lodge *et al.* (1990), and Murdoch and Ellis (1992).

Dormancy would not also be affected, during a grass fire, in seeds below 3 cm in the soil (Pieterse and Cairns 1986). It is also clear that temperatures higher than 80 ºC did not further stimulate germination in any of the genotypes and, indeed, were harmful for the majority of them. Seeds of genotypes in the "intermediate temperature specific group" and "indifferent to temperature group" were more likely to show raised strophioles and to germinate in response to summer heat or grassfires if not buried in the soil below 3 cm. Seeds of some genotypes needed temperatures higher than 100 ºC to have 100% of the strophioles raised. These temperatures however, promoted very high seed mortality and a poor correlation between germination and raised strophiole was obtained (Fig. 3.12). In general, raised strophiole and germination success in seeds of *Desmanthus*, were highly correlated with a range of temperatures from room temperature up to 60 ºC. Above this temperature the heat started to depress germination (Fig. 3.12).

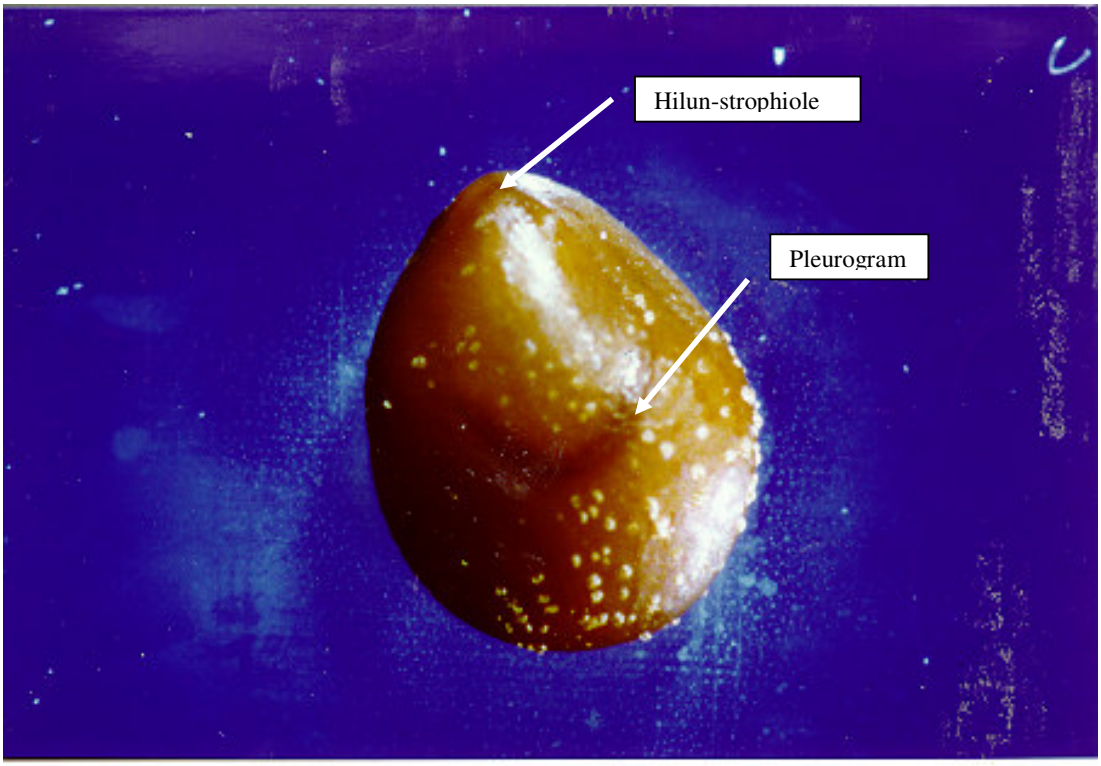


Figure 3.8 Seed of *D. virgatus (*x 30) showing the pleurogram and hilum-strophiole structures. Seed length = 2.1 x 1.7 mm.

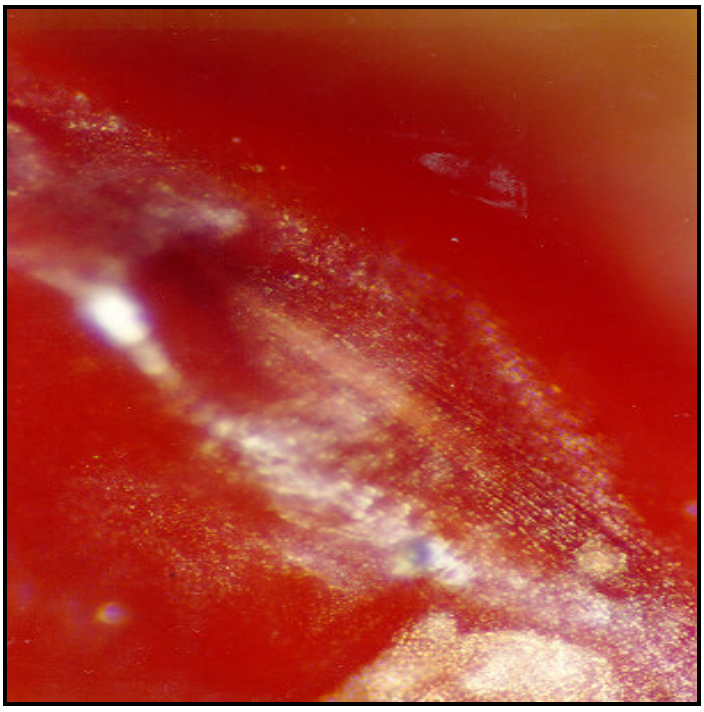


Figure 3.9 Impermeable flat-striped strophiole structure in seed of *D. virgatus* not submitted to heat treatment (x 100). Length of strophiole = 0.45 x 0.25 mm.

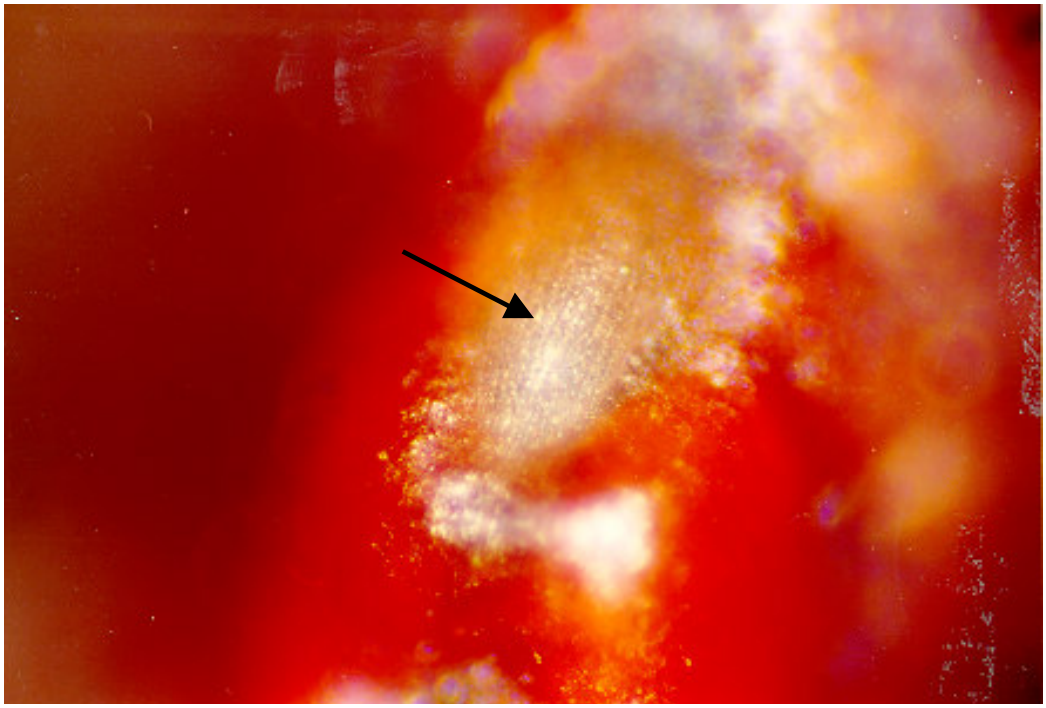


Figure 3.10 Raised strophiole in seed of *D. virgatus* submitted to a temperature of 80 ºC. Frontal view (x 100). Length of strophiole = 0.45 x 0.25 mm.

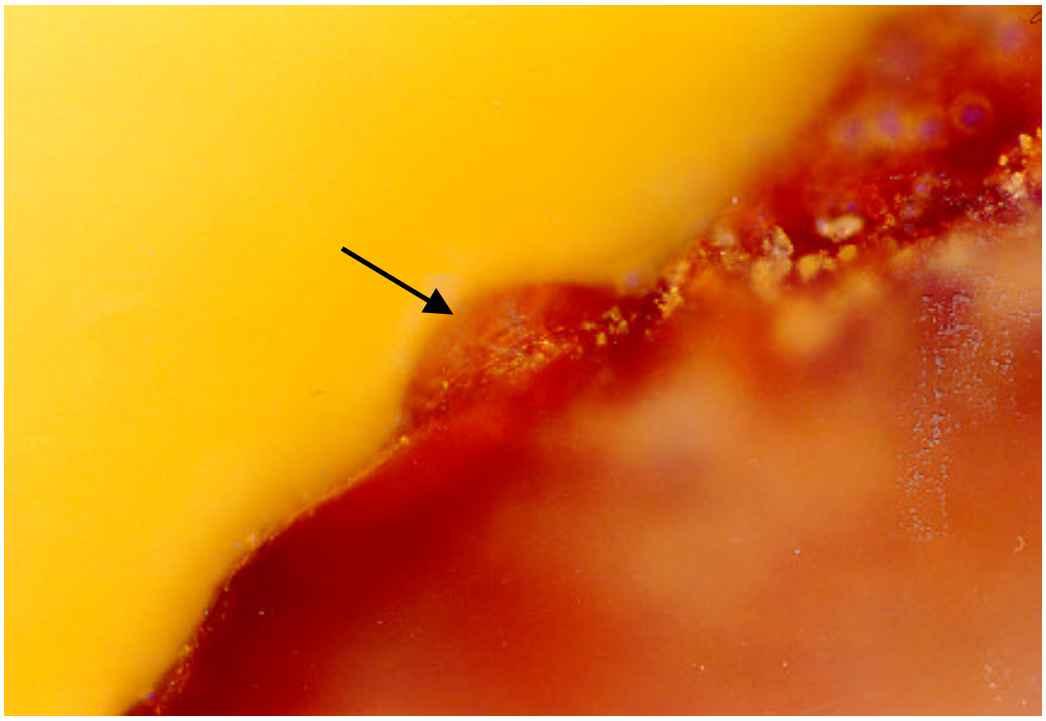


Figure 3.11 Raised strophiole in seed of *D. virgathus* submitted to a temperature of 80 ºC. Lateral view (x 100). Height of strophiole = 0.15 mm.

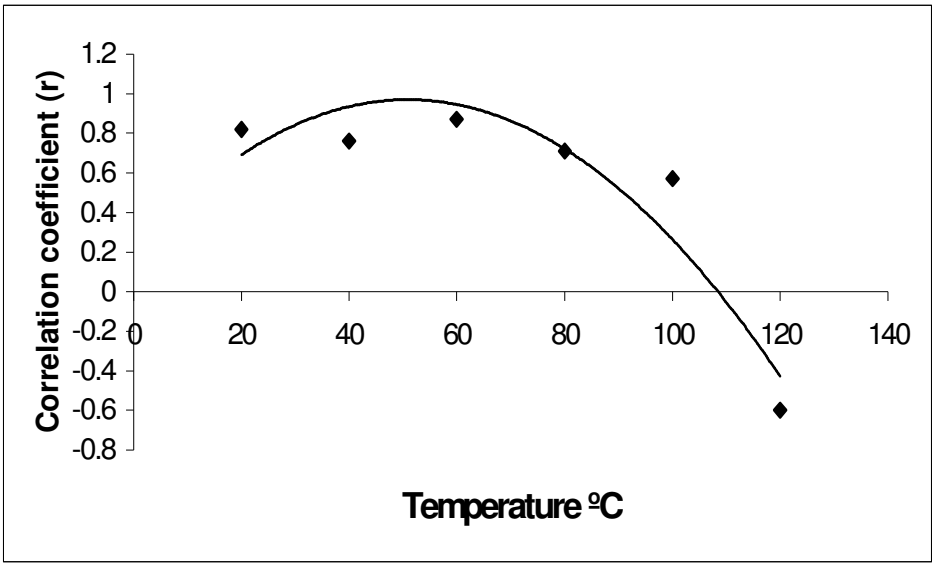


Figure 3.12 Effect of heat on the coefficients of correlation between germination and raised strophiole in seeds of *Desmanthus* genotypes. The curve represents the mean of the nine genotypes tested at each temperature. Data originated from Tables 3.4 and 3.5.

High seed production associated with dependence of seeds on elevated temperatures to release their seed-coat dormancies can be a very important strategy used by plants such as *D. virgatus* CPI 78373 cv. Marc, to survive in dry tropical regions subjected to periodical droughts. Such plants will form large seed banks in soil and more intensive seedling recruitments will occur only after the occurrence of certain events such as a drought period. Seed-coat dormancy in seeds in soil seed bank will be broken by the intense heat in the dry period and intensive seedling recruitment will certainly occurs in the next wet season assuring the establishment of a new stand of plants. Such a strategy can probably explain the greatest number of seeds in soil seed bank and the highest rate of plant survival found by Gardiner *et al.* (2004) in *Desmanthus virgatus* CPI 78373 cv. Marc when compared to 35 other genotypes of *Desmanthus,* in plots 14 years after planting at Blackall, in the semi-arid, black clay soil of Queensland's Mitchell Grass Bioregion.

The percentage of hard seed at the end of the germination test (Table 3.6) was inversely related to the percentage of raised strophioles observed in the seed samples (Table 3.4). Some genotypes showed a high percentage of hard seeds at 100 ºC at the end of the germination test (Table 3.5): this was also inversely related to percentage of raised strophioles. No hard seed was found at 120 ºC. At this temperature all seeds had

permeable strophioles, but the majority of the seeds was killed by the intensity of the heat and did not germinate.

Genotypes with similar behaviour to CPI 78382 and CPI 83563 would appear to be particularly suitable for agronomic use in areas subjected to occasional burning in view the ability of their seeds to resist high temperatures without serious losses in viability. For regions not subject to occasional burning, and where soil temperatures are not expected to reach levels much higher than 60 ºC, genotypes having similar ecological behaviour to CPI 78382 and CPI 83563 are not recommended. Resistance to seed softening by boiling water was reported by Loch and Harvey (1992) in seeds of *D. virgatus* CPI 78382. Therefore the genotypes CPIs 37143, 38351, 67643, 78372, TQ 88 and Alligator Creek would probably be more suitable for such conditions. Reasonable number of seeds of these genotypes would be expected to become soft and germinable in the soil-seed bank after a normal summer wet season in tropical and sub-tropical regions. Field confirmation of these conclusions is awaited.

3.4.3 Effects of fire on seedling recruitment from the soil seed-bank

The experimental area at James Cook University was free of domestic animals and only lightly grazed by wallabies or other native animals. Accumulated litter and live vegetation insulated the soil from direct heating by the sun. In such situations, as found by Borrows and Porter (1993), soil temperatures did not reach the level of 60 ºC necessary to break seed-coat dormancy of some genotypes, and seedling recruitment was very low even under large seed banks.

Seeds deposited or incorporated into the upper soil layer may be protected from natural factors (heat, microorganisms, and humidity) which normally break seed-coat dormancy. Fire may however be effective in breaking such dormancy (Gill 1981; Probert 1992) and this appeared to be the case with some of the genotypes of the present study. The "fire recruitment syndrome" is difficult to validate at the present time; the genotypes do not have a known history of use in Australian pastures and too little is known about fire in the environments from which they originated. However, in the light of the results obtained in the present study, the responses of some accessions to high temperatures may indicate their inclusion in the category of plants having a “fire

recruitment syndrome": *Desmanthus virgatus* CPI 83563 with a small number of seeds in the soil seed-bank (Table 3.5), had the highest seedling recruitment after the burning (Fig. 3.5); the accession CPI 78373 of *D. virgatus* (not included in the trial of seedling recruitment after fire), had a seed-coat dormancy of 64% of the seeds submitted to temperatures as high as 100 ºC (Table 3.6).

3.4.4 Effects of fire on soil temperatures

The soil temperatures observed in the present study during the fire were intermediate to those obtained by Shea *et al*. (1979), and Gibson *et al*. (1990) for high and low intensity fires in Florida. The soil at the experimental plots proved to be a very good heat insulator and the temperatures of the fires declined very rapidly with depth (Fig. 3.4). The trends of temperature transmission in soil obtained here were typical of dry soil exposed to short durations of burning in grass fires (Bradstock and Auld 1995) and hyparian habitats at Charters Towers, Queensland, Australia (Babawi and Campbell 2002).

Soil surface temperatures of between 150 and 310 ºC in the three fires in the present trial were high enough to kill all seeds situated on, or close to the surface (Gill 1981), but at just 1.0 cm depth in the soil they reached no higher than 80 ºC. Between 1.0 and 3.0 cm, temperatures were slightly lower and the field evidence, together with that found for other legume species, suggests that the effect of fire temperatures on seed dormancy would vary with the concerned genotypes (Floyd 1966, 1976; Piterse and Cairns 1986; Keeley 1987; Munoz and Fuentes 1989; Probert 1992; Taylor and Ewin 1992).

Chapter 4

EFFECTS OF SHADE ON EARLY GROWTH OF *Desmanthus*

4.1 Introduction

Natural Australian eucalyptus woodlands are similar in many ways to the savannas of Africa in that tree density and the tree canopy have strong influences on the understorey stratum, composed mostly of native grasses, and on the stability of the soil-plant-animal system. It is well known in the literature that herbage yields decrease with increasing numbers of trees of popular box (*Eucalyptus populnea*) (Walker *et al.* 1972), narrow leaf ironbark (*Eucalyptus creba*) (Gillard 1979; Walker *et al.* 1986), flooded gum (*Eucalyptus grandis*) (Cameron *et al.* 1989), and *Eucalyptus* spp. (Scanlan and Burrows 1990). The probable reason for the decrease in grass production in the dry tropics is the intense competition between trees and grasses for soil water and nutrients. Scanlan and Burrows (1990) focussed on tree clearing effects found that decreases in tree density as a consequence of clearing resulted in increases of the density of grasses and legumes, in eight different *Eucalyptus* communities around Rockhampton, central Queensland. In the subtropics such competition is more likely to be for water; however, only a few workers have documented the changes in botanical composition under such conditions (McCown *et al*. 1974; McIvor 1981; Mott and Tothill 1984; Scanlan and Burrows 1990). Many papers have reported a great increase in grass biomass compared with forbs following tree removal (Miller *et al.* 1980; Ball *et al*. 1981; Clary and Jameson 1981). The legumes, when mentioned, are generally not identified by species, genera, or even growth habit (e.g. erect vs. prostrate legumes), but are frequently reported simply as “legumes", making it hard for readers to go in a clear idea which legumes took advantage from tree removal.

Comparing the results obtained from the Australian eucalypt woodlands with those obtained for grasses under non-legume tree canopies in the wet tropics (Stur 1991; Stur and Shelton 1991; Chong *et al*. 1991; Wong 1991), in the African savannas (Belski *et al.* 1989; 1993; Smit and Swart 1994; Wasonga *et al*. 2003), or under artificial shade and watered conditions (Ludlow *et al*. 1974; Wong and Wilson 1980;

Eriksen and Whitney 1981; Wong *et al.* 1985a), it can be argued that the decrease in the yield of grasses with the increase in tree density is probably due to low tolerance of the studied grasses to low light conditions, rather than to water or nutritional stresses. In most of the works above cited, water and available nutrients under tree canopies were present in greater amounts than in sites between the canopies.

The studies of Pressland (1986) at two sites in the **mulga lands** of south-eastern Queensland revealed that disturbance through thinning of the mulga overstorey encouraged the establishment of other woody shrubs, and had a greater impact on land degradation than did inappropriate stock management strategies. Tree and shrub clearance over the north-eastern **Caatinga** (thorn scrub region) of Brazil led to a high proportion of inedible annual herbs; less use by goats and sheep of the stem fraction of the herbaceous vegetation; and loss of leaf litter from woody plants representing more than 90% of available forage throughout the dry season on uncleared Caatinga (Malechek 1984; Kirmse *et al.* 1986). Hardesty (1986) proposed a selective and seasonal cutting strategy for the Caatinga environment to ensure the resprouting of important tree and shrub forage sources for browsing goats.

The effect of individual trees of *Eucalyptus* spp. on the under-canopy micro-environment and on the establishment of introduced pasture legumes has not been well documented in the literature. This is probably related to the success of the stylo legumes thriving well in association with native and introduced grasses in open or sparsely shaded habitats of the semiarid tropics. Most of the grass species used in the system are well adapted to the open environment; however, under-canopy sites might have higher levels of water and nutrients than between trees, giving shade tolerant pasture legume species a better chance to initiate nuclei of colonisation in savanna landscapes. Connor (1983) considered the agroforestry systems of tropical regions as a pool of suboptimal levels of environmental resources. He suggested that successful plant adaptation for such sub-optimal conditions depends on local characteristics of those pools, and on the relative ability of the cohabiting species to gain access to them and to interact for use or conservation.

A new grazing pasture system for the world's arid and semiarid regions, based on the use of species well adapted to the under tree canopy micro-environment in

combination with species adapted to open conditions with more light, would reduce the necessity for tree clearing in such regions. Such an approach would also suit to the present conservationist view of modern society (see, for instance, "Queensland Country Life" 1995a; b). This has been accomplished in some subtropical forests of the United States where the introduction of a shade tolerant ecotype of the herbaceous legume *Trifolium subterraneum* in mixtures with the grass *Paspalum notatum* supports the combined production of timber and cattle (Johnson and Davis 1983; Lewis and Pearson 1987).

The experiment reported in this chapter was carried out to test the following hypotheses:

- Some plants of the genus *Desmanthus* have developed shade tolerance during their life-history adaptations. There are some unpublished field observations by R. L. Burt and C. P. Gardiner (personal communications) suggesting *Desmanthus* plants may grow better in shade habitats than under full sun;
- Better soil conditions (in terms of water holding capacity, organic matter status, and mineral contents) exist under the canopy of *Eucalyptus* species than outside the canopy;
- The better soil conditions under the canopy may offer a preferential niche for *Desmanthus* seedling survival and development in tropical, semiarid savannas.

An experiment was designed to allow comparison of the development of population of different accessions of *Desmanthus* sown from seed in under-and between-canopy environments.

4.2 Materials and methods

4.2.1 Location and site condition

The experiment reported in this section was carried out on an area of the campus of CSIRO Davies Laboratory at Townsville, located between Discovery Drive and Solander Drive, and the fence marking the CSIRO and the James Cook University boundary (sites 2 and 3 of Fig. 2.3).

The soils, vegetation and climate of the experimental site were described above (sections 2.3.2, 2.3.3, and 2.3.4).

A fire occurred in the area in November 1991 and burned all the above-ground herbaceous vegetation. Thus the area was almost free of established herbaceous competitors at the time the experiment commenced.

4.2.2 Experiment layout

The study plots consisted of two, 113 m^2 circular plots (6 m radii) with one plot centred on a trunk of an adult ironbark (*Eucalyptus creba*) tree, designated as the "under-canopy" environment, and the other in the open area between tree canopies, designated as the "between-canopies" environment (Fig. 4.1). Each plot was divided into four subplots ("quadrants") according the cardinal orientation of, NW, SW, SE, and NE. Seven 6 m long radii were drawn inside each subplot, starting 1 m away from the trunk of the tree on the under-canopy environment, and 1 m from the centre on the between-canopies environment.

Seven genotypes of *Desmanthus* were allocated at random to the seven radii in each subplot (Fig. 4.1).

Figures 4.2 and 4.3 show the environmental conditions of plots under-and between-canopies respectively, at the time of seed sowing. Clearly, tree density was not uniform across the site. Understorey vegetation began to recover after the pre-experiment burn in response to rains in previous months. A denser herbage cover developed over the soil in the under-canopy environment than that between the canopies.

4.2.3 Seed sowing

Before sowing, the seeds were scarified with concentrated sulphuric acid for ten minutes (Ossiya 1993), and then were tested for germination on filter paper (Table 4.1). All genotypes showed good germinability (59-87%, Table 4.1). Rows 1 cm deep were dug along each radius of the experimental plots.

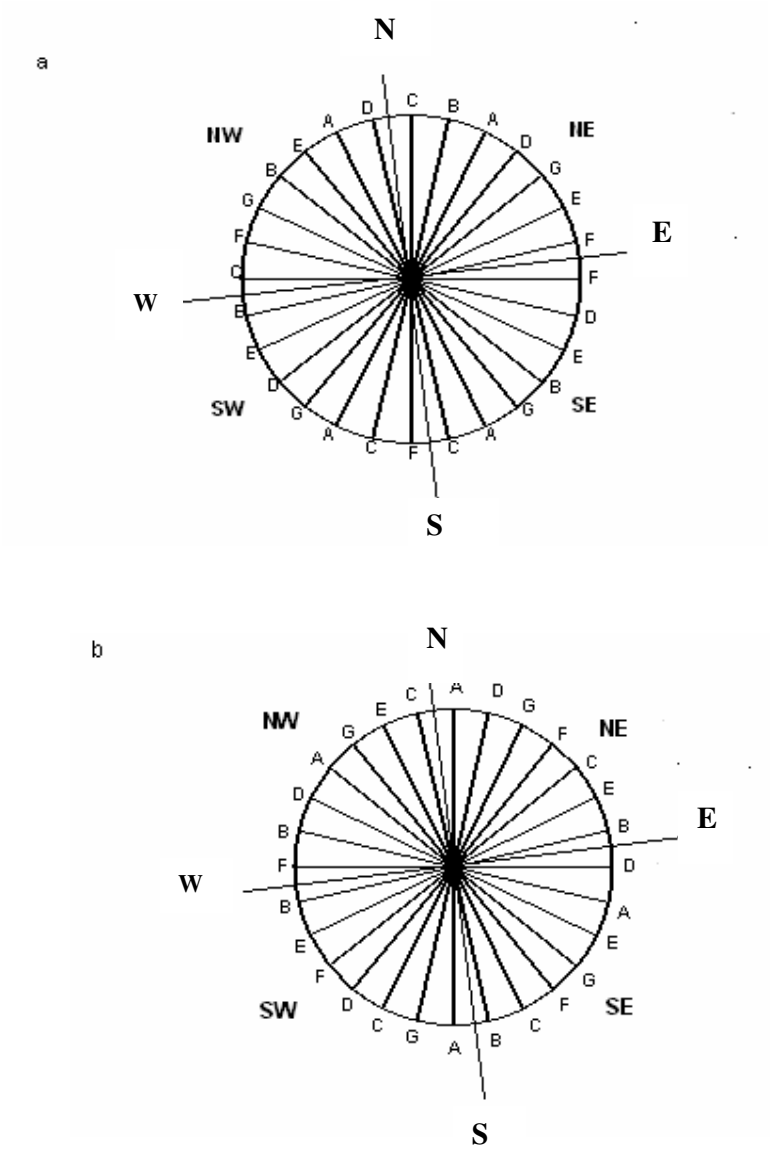


Figure 4.1 Genotype distribution on the plots. a) Under-canopy; b) Between-canopies.
SW - South-West; NW - North-West; NE - North-East; SE - South-East.

A – *D. leptophyllus* CPI 38351
B – *D. virgatus* CPI 78382
C – *D. virgatus* CPI 79653
D – *D. pubescens* CPI 92803
E – *D. leptophyllus* TQ 88
F – *D. bicornutus* CPI 91162
G – *D. pernambucanus* CPI 40071

Figure 4.2 Under-canopy environment in March 1992

Figure 4.3 Between-canopies environment in March 1992

On 21 February 1992, 25 points 25 cm apart from each other were marked along each row and seeds were sown at a density of four seeds/point; rows were 25 cm apart in the innermost point of the rows and 150 cm apart in the outermost point. The seeds were obtained from a seed collection of *Desmanthus* genotypes stored on the CSIRO Davies Laboratory in Townsville. Because of a shortage of seed, the genotype *Desmanthus virgatus* TQ 88 was tested only in the under-canopy environment.

4.2.4 Plant propagation

Because of absence of rain after sowing, the plots were watered with 30 mm/week, from 10 March 1992 to 21 April 1992, by sprinkling, using a hose connected to a tap on the JCU campus close to the site, to ensure germination and establishment. No further irrigation was applied to the area from 21 April 1992 until December 1995; rainfall during the experimental period is shown in Fig. 2.5.

Table 4.1 Genotypes of *Desmanthus* used in the trial and germination %. Means of 3 x 100 seeds scarified by sulphuric acid.

Accession	Name	Germination %
D. virgatus AusTRCF 78382	CPI 78382	68.0
D. virgatus AusTRCF 79653	CPI 79653	66.7
D. pubescens AusTRCF 92803	CPI 92803	76.7
D. bicornutus AustTRCF 91162	CPI 91162	87.3
D. leptophyllus AusTRCF 321107	TQ 88	78.4
D. leptophyllus AusTFCR 38351	CPI 38351	75.3
D. pernambucanus AusTRCF 40071	CPI 40071	59.1
Mean		73.1

Three weeks after sowing the emerged seedlings were thinned or transplanted to give, where possible, a final density of two plants per point and 48 plants per row.

4.2.5 Environmental data collected

4.2.5.1 Tree density

The tree density was measured by both population and tree basal area techniques (Scanlan and Burrows 1990), inside a sample area of 50 x 50 m, at the study site, and extrapolated to abundance per hectare. Shrubs (< 2 m high) were not included in the determination. The tree population was expressed as total number of trees per hectare and basal area as the total area of tree stems per hectare. The basal area was determined by recording the stem circumference of every tree inside the sample area, at 30 cm above ground.

4.2.5.2 Soil

Topsoil samples (0 to 20 cm deep) were collected in the study site under six randomly chosen ironbark trees for the under-canopy environment, and at six randomly chosen sites in the open area for the between-canopies environment. The samples were bulked per environment, thoroughly mixed and one sub-sample representing each environment taken for analysis. Analyses were conducted for pH of 1:5 soil/water suspension (Rayment and Higginson 1992); organic matter (OM) by ignition (Hesse 1971); organic carbon (C), (Walkley and Black cited by Rayment and Higginson 1992); total nitrogen (N) by semimicro Kjeldahl digestion, determined colorimetrically (Baethgen and Alley 1989) at 655 nm using an atomic absorption spectrophotometer; phosphorus (P) by the bicarbonate-extractable phosphate method (a modified Olsen method as described by Rayment and Higgison, 1992) and determined colorimetrically at 880 nm in atomic absorption spectrophotometer.

4.2.5.3 Light interception

Absolute and relative photosynthetic photon fluxes (PFF µmol/m^2/s and %, respectively) reaching the soil, in both the open site and under the ironbark tree canopy of the shaded environment, were monitored by light sensors (LI-190SA, LI-COR Inc., Lincoln, Nebraska). One light sensor in the open environment and three under the tree canopies at 60° (Position 3), 170º (Position 1) and 260° (Position 2)

from magnetic North, were monitored from 8:00 a.m. to 6:00 p.m. on 11 September 1994. The sensors were connected to a data-logger and the light measured as absolute photosynthetic photon flux density (PFF). Measurements were made on a sunny day considered to be typical for the dry season. Measurements of PFF were taken every 15 seconds, and a data logger (LI-1000, LI-COR Inc., Lincoln Nebraska) recorded the average value over 30 minute intervals. No observations were made in the wet season. Individual records from the open site, and the mean of the values from the three sensors under tree canopies, defined the patterns of light intensity in this environment.

4.2.6 Plant observations

The total number of expanded leaves/plant, plant height (length of the main stem from ground level to the point of insertion of the last expanded leaf), and percentage of survival (percentage of live plants per datum point, in relation to the stand in the same place one week after thinning; section 4.2.4), were first recorded two weeks after the thinning or transplanting date (Table 4.2). Records were more frequent during the initial phase of seedling establishment. More infrequent records were taken as the experiment progressed to evaluate the drought tolerance of the genotypes. When two surviving plants were found at the same datum point in the row, the averages of measurements of their numbers of leaves and heights were used.

4.2.7 Experimental design and statistical analysis

A block design was used for the statistical analysis of numbers of leaves, plant heights and plant survival for each environment. Blocks were represented by the four geographic positions (quadrants). Seven genotypes in the under-canopy environment and six genotypes for the between-canopies, randomised inside each quadrant, constituted the treatments.

Table 4.2 Dates of data recording of field observations. Time in weeks from thinning of seedlings, and rainfall occurred during each recording interval.

Date	Weeks	No. of leaves	Height	Survival	Rainfall** mm
25 March 92	3	Yes	yes	yes	195
08 April 92	4	Yes	yes	yes	4
06 May 92	8	Yes	yes	yes	5.5
03 June 92	12	Yes	yes	yes	37.5
01 July 92	16	Yes	yes	yes	0
26 August 92	24	Yes	yes	yes	0
16 December 92	40	Yes	yes	yes	167
24 March 93	54	Yes*	Yes*	yes*	306
08 June 94	118	No	No	yes*	613.4
15 March 95	158	No	No	yes*	420.5

yes – Recorded at this date; no – Not recorded at this date
yes* - Recorded at this date only for the under-canopy environment
** - Rainfall in the period preceding the observation date

The general ANOVA procedure of "Statistix 4.1" was used in the analysis of variance for numbers of leaves and plant heights recorded in March 1993 (54 weeks). Means were compared by LSD Comparisons test at the 95% significance level. The importance of the general behaviour of each individual during the experimental period was studied using the statistical analysis of survivorship curves as recommended by Muenchow (1986) and Pyke and Thompson (1986). In this specific case, the Kaplan-Meier cumulative survival curves procedure of the "SPSS 6.1" statistics computer program was used. Equality of survival distributions for genotypes in quadrants was compared by Log-Rank, Breslow, and Tarone-Ware tests in "SPSS 6.1". The ANOVA procedure was also used to detect differences in percentage of survival between genotypes and quadrants at June 1994, for under-canopy sites, and in December 1992 for between-canopy sites. No attempt was made to compare statistically the data for between sites, as they were not replicated.

4.3 Results

4.3.1 Soil

The chemical characteristics of soils collected under and between-canopies are shown in Table 4.3. As the value of each variable in Table 4.3 represents the mean of the values obtained from 6 localities under-canopies or between-canopies, and not only from the experimental sites, it is valid to compare soil characteristics between the environments.

Table 4.3 Soil chemical composition under and between tree canopies (means of 6 localities under-canopies and between-canopies).

Variable	Under-Canopies	Between-Canopies
pH in H_2O	7.28	6.71
Organic Matter (ignition) %	4.40	2.74
Organic Carbon %	1.62	0.86
Total Nitrogen %	0.49	0.11
C/N ratio	3.3	7.8
Total Phosphorus g/kg	160.25	143.34
Available Phosphorus ppm	270	100

The higher average content of organic matter observed in soils from under the tree canopies was related to higher concentrations of carbon, nitrogen, and phosphorus in that environment compared with the soils collected outside the tree canopies. Such a finding agrees with that reported by Virginia (1986), Belsky *et al.* (1989, 1993a; b, 1994), and Smith and Stuart (1994) for tropical African savannas. The average carbon content in soil under the canopies was almost twice that found in the between-canopies sites. The average nitrogen content was also higher under the canopies than in the between-canopies environment. There was a lower C:N ratio in soil between-canopies than under-canopies indicating that the organic matter in that soil is strongly mineralised. The general results of the analyses suggested that the soils are likely to be more fertile under the canopies than between the tree canopies.

4.3.2 Tree density

Ironbark (*Eucalyptus creba*) with 212 trees/ha and a basal area of 8.71 m^2/ha was the most common tree on the experimental site, representing close to 77% of the total number of trees and of the total basal area (Table 4.4). *Eucalyptus erythrophloia* (red bloodwood) and *Melaleuca* spp. (paper bark) with 56 and 6 trees respectively, also contributed to the tree canopy stratum. The total tree basal area of the site was greater than 11 m^2/ha. A *Eucalyptus* sp. tree basal area of 10 m^2/ha was found by Scanlan and Burrows (1990) to be capable of reducing the herbaceous standing crop in central Queensland by 35% and 80% of the yield in full sun for high and low production potential sites, respectively. Such a reduction was mostly represented by the decline in production of the grass component in the shaded environment. Forbs were a minor component and were not affected or enhanced by the shade, so they contributed little to changes of the total herbaceous yield between microenvironments.

Table 4.4 Tree density at the experimental site at James Cook University. Values in parentheses represent the percent contribution of each species to the total.

Species	Number of trees/ha	Basal area (m^2/ha)
Ironbark *(Eucalyptus creba)*	212 (77.4%)	8.71 (76.9%)
Red blood wood (*Eucalyptus erythrophloia*)	56 (20.4%)	2.57 (22.7%)
Paper bark (*Melaleuca* spp.)	6 (2.2%)	0.05 (0.4%)
Total	274 (100%)	11.33 (100%)

4.3.3 Light irradiancy

The pattern of light irradiancy obtained for under-and between-canopies environments (Table 4.5 and Figs 4.4 and 4.5) may not necessarily represent a standard behaviour for sunny and shaded habitats in open tropical woodland. However, it gives a good indication of light irradiancy in the specific conditions of the experimental site during the dry season. Variation in sun declination during the

season would cause some difference in the daily pattern of light recorded by each sensor under the tree canopy, but it is unlikely to alter seriously the means of the three sensors, or the differences between the instrumented microenvironments.

Interception of incident light by tree canopies during the day ranged from 80% at 8:00 a.m. to 0% at 4:00 p.m., with a mean of 30% for all records (Table 4.5). This reduction in light irradiancy was slightly smaller than that observed by Belsky *et al.* (1993a; b) under the canopies of *Acacia tortilis* and *Adansonia digitata* in African savannas. Both species have denser crowns than the trees of the present study site. The canopy light interception, averaged over a whole day, showed a synodal trend, being intense during the morning, null at noon, and then again intense in the afternoon (Fig. 4.4).

More effective light interception was registered by the sensors located under the canopy and close to South-East/South-West orientations [170º (Position 1) and 260º (Position 2) from magnetic North] than by the one located close to North-East orientation [60º (Position 3) from magnetic North] (Table 4.5 and Fig. 4.5). Light interception at 60º was very low, or even null from 11:00 a.m. to 4:00 p.m. A scant leaf canopy in the tree's north-west quadrant was probably responsible for this. Some afternoon shading of the between-canopies site by surrounding tree canopies may explain the lower values of light irradiancy observed in the between-canopies site compared with the under-canopy site. Only for short periods were the levels of shade observed under the canopy greater than the value of 85% of light interception (Fig. 4.4), which is regarded as critical for the development of tropical legumes (Ludlow *et al.* 1974; Ludlow 1980; Stur 1991; Wong 1991).

Expressive changes were observed in the thickness of the herbaceous vegetation of the sites with the transition from the dry to the wet season. The scarce and brown herbaceous layer during the dry season was replaced by a dense and green ground cover in the wet season, in both the under-and the between-canopies sites (Figs. 2.6 and 2.7). However, in the arboreal layer mostly composed by Eucalyptus species which kept most of their leaves over the dry season, minor changes were observed in the thickness of the tree canopies.

Table 4.5 Photosynthetic photon flux (PPF, μmol/$m^{2/}$s) reaching soil surface at one position between canopy and three positions under-canopy from 8:00 a.m. to 6:00 p.m. on 11 September 1994, a typical sunny day of the site.

Time	Under Position 1	Under Position 2	Under Position 3	Mean under	Between	Ratio under/between %
8:00	51.71	120.6	179.2	117.17	574.6	20.39
8:30	662.1	984	651.4	765.83	1082	70.78
9:00	526.5	1337	879	914.17	1310	69.78
9:30	361.1	771.1	472.2	534.80	1492	35.84
10:00	1259	822.4	780.6	954.00	1663	57.37
10:30	1813	1136	1545	1498.00	1735	86.34
11:00	1943	1385	1984	1770.66	1911	92.66
11:30	1172	1455	1873	1500.00	1885	79.58
12:00	2016	1981	2031	2009.33	1972	99.65
12:30	1992	977.3	2054	1674.43	1977	84.70
13:00	1159	321.8	2015	1165.27	1951	59.73
13:30	646.3	1056	1930	1210.77	1888	64.13
14:00	1795	299.8	1787	1293.93	1774	72.94
14:30	1070	318.9	1585	991.30	1600	61.96
15:00	658.6	301.8	1385	781.80	1425	54.86
15:30	403	921.3	1190	838.10	1255	66.78
16:00	707.6	951.7	814.1	824.47	600.8	137.23
16:30	587.2	671.4	557.1	605.23	572.7	105.68
17:00	249	267.1	261.6	259.23	380.3	58.17
17:30	192.9	170.6	203.8	189.10	239.5	78.96
18:00	76.17	66.45	69.32	70.65	73.1	96.64
Mean	879.50	742.15	1102.68	908.11	1249.55	72

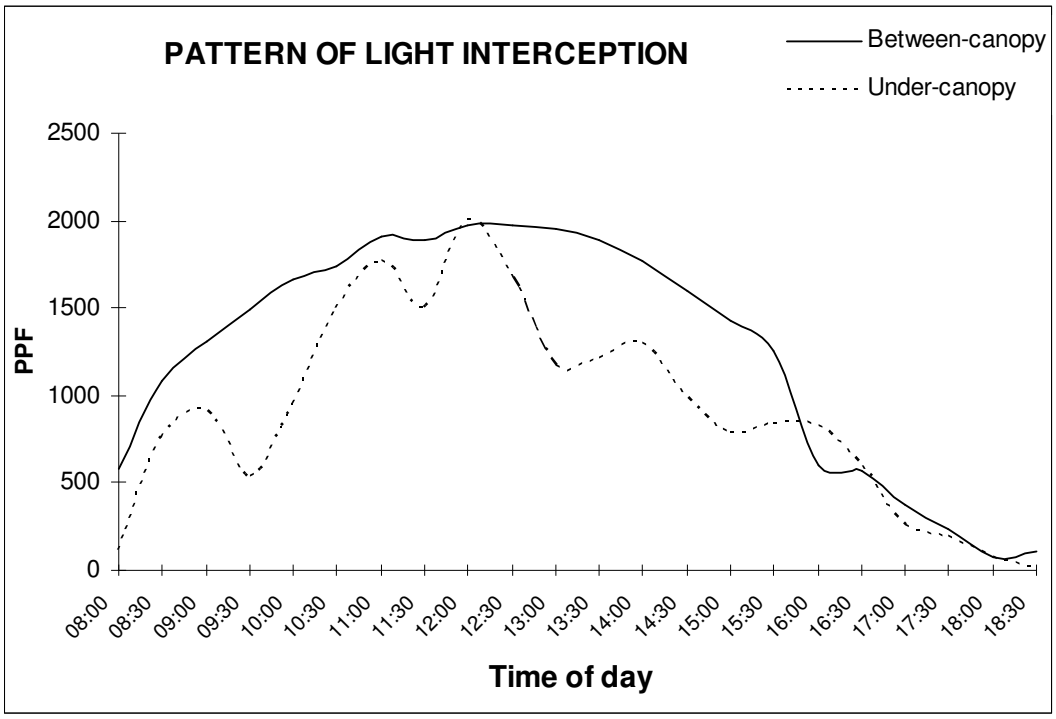


Figure 4.4 Mean of photosynthetic photon flux (PPF, µmol/m^2/s) reaching the soil surface registered from 8:00 a.m. to 6:30 p.m., for the under-canopy and between-canopies sites on 11 September 1994.

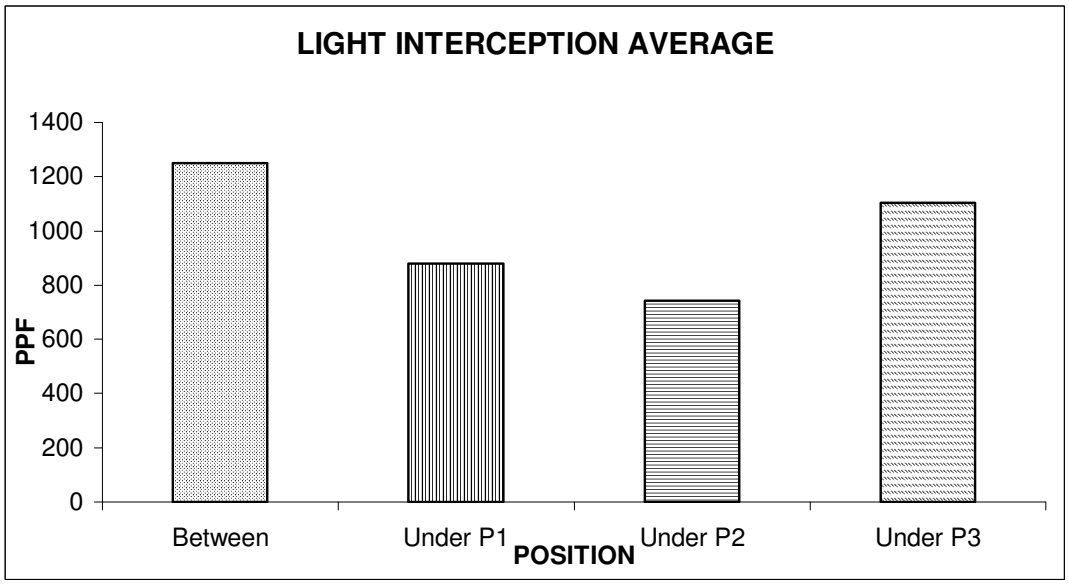


Figure 4.5 Photosynthetic photon flux (PPF, µmol/m^2/s) reaching soil surface at one point between-canopy and three points under-canopy, averaged for twenty two observations times on 11 September 1994.

4.3.4 Legume evaluation

4.3.4.1 Numbers of leaves per plant

The total number of leaves/plant observed in December 1992 (wet season) was significantly ($p < 0.05$) affected by genotypes, but not by quadrant in both sites (Table A.2.5 and A.2.6). *Desmanthus leptophyllus* TQ 88 in the under-canopy site and *D. virgatus* CPI 79653 in the between-canopies site, with 13 and 5 leaves/plant respectively, had the highest leaf numbers (Table 4.6 and 4.7). *D. virgatus* CPI 79653 also showed good initial growth in the under-canopy site with 7 leaves/plant. All genotypes, with exception of *D. pubescens* CPI 92803; had higher numbers of leaves when growing under the canopy compared with the between-canopies location. The accessions of *D. bicornutus* CPI 91162 and *D. virgatus* CPI 78382 were the most plastic in response to shading (Tables 4.6 and 4.7; Fig. 4.6).

The more favourable physical and chemical conditions of the under-canopy soil (Table 4.3) gave seedlings in the under-canopy site better access to water and nutrients than in the between-canopies site.

The monthly records of leaves/plant at the under and between-canopies sites plotted against rainfall distribution in 1992 are shown in Fig. 4.6. The under-canopy plants responded more rapidly and more efficiently to rainfall events by producing many more leaves than the plants between-canopies. This might reflect the better condition of water storage, water retention, nutrient availability, and less plant transpiration at the under-canopy site. The year 1992 was a drought year when monthly rainfall from March to October was less than 50 mm. This is a common occurrence in tropical savannas where evapotranspiration for the majority of the months is much higher than precipitation. Most of the early leaves of the seedlings had senesced with the onset of the dry season. Despite the early loss of leaves, compensatory regrowth occurred in the majority of genotypes in the under-canopy site (Fig. 4.6). *D. leptophyllus* TQ 88 and *D. virgatus* CPI 79653, and *D. bicornutus* CPI 91162 showed the best leaf recovery under the canopy after the dry season; leaf recovery on the between-canopies site was weak and was barely discernible for *D. virgatus* CPI 79653 (Fig. 4.6 b).

Table 4.6 LSD pairwise comparisons of mean number of leaves/plant of legumes growing under-canopy by genotype at December 1992.

Genotype	Mean number of leaves/plant	Total number of leaves
D. leptophyllus TQ 88	13.25 a	416
D. virgatus CPI 79653	7.26 ab	387
D. bicornutus CPI 91162	6.37 ab	350
D. leptophyllus CPI 38351	4.04 ab	120
D. virgatus CPI 78382	2.50 ab	33
D. pernambucanus CPI 40071	1.37 ab	68
D. pubescens 92803	0.67 b	31

Means followed by the same letter in the column are not significantly different by LSD ($p < 0.05$).

Table 4.7 LSD pairwise comparisons of mean number of leaves of legumes growing between-canopies by genotype at December 1992.

Genotype	Mean number of leaves/plant	Total number of leaves
D. virgatus CPI 79653	5.08 a	35
D. pernambucanus CPI 40071	2.88 ab	26
D. bicornutus CPI 91162	2.32 ab	28
D. leptophyllus CPI 38351	1.70 b	52
D. pubescens CPI 92803	1.25 b	28
D. virgatus CPI 78382	0.75 b	19

Means followed by the same letter in the column are not significantly different by LSD ($p < 0.05$).

4.3.4.2 Stem length

Stem length was measured as the vertical or horizontal length of the main stem. Significant effects of genotype and quadrant on stem length were noticed in the under-canopy environment at December 1992 (Tables A.2.7 and A.2.8). Comparisons of mean stem length of genotypes by LSD ($p < 0.05$) are presented in Tables 4.8 and 4.9. Differences in stem lengths of genotypes in the between-canopies locations were large but not statistically significant (Table 4.9).

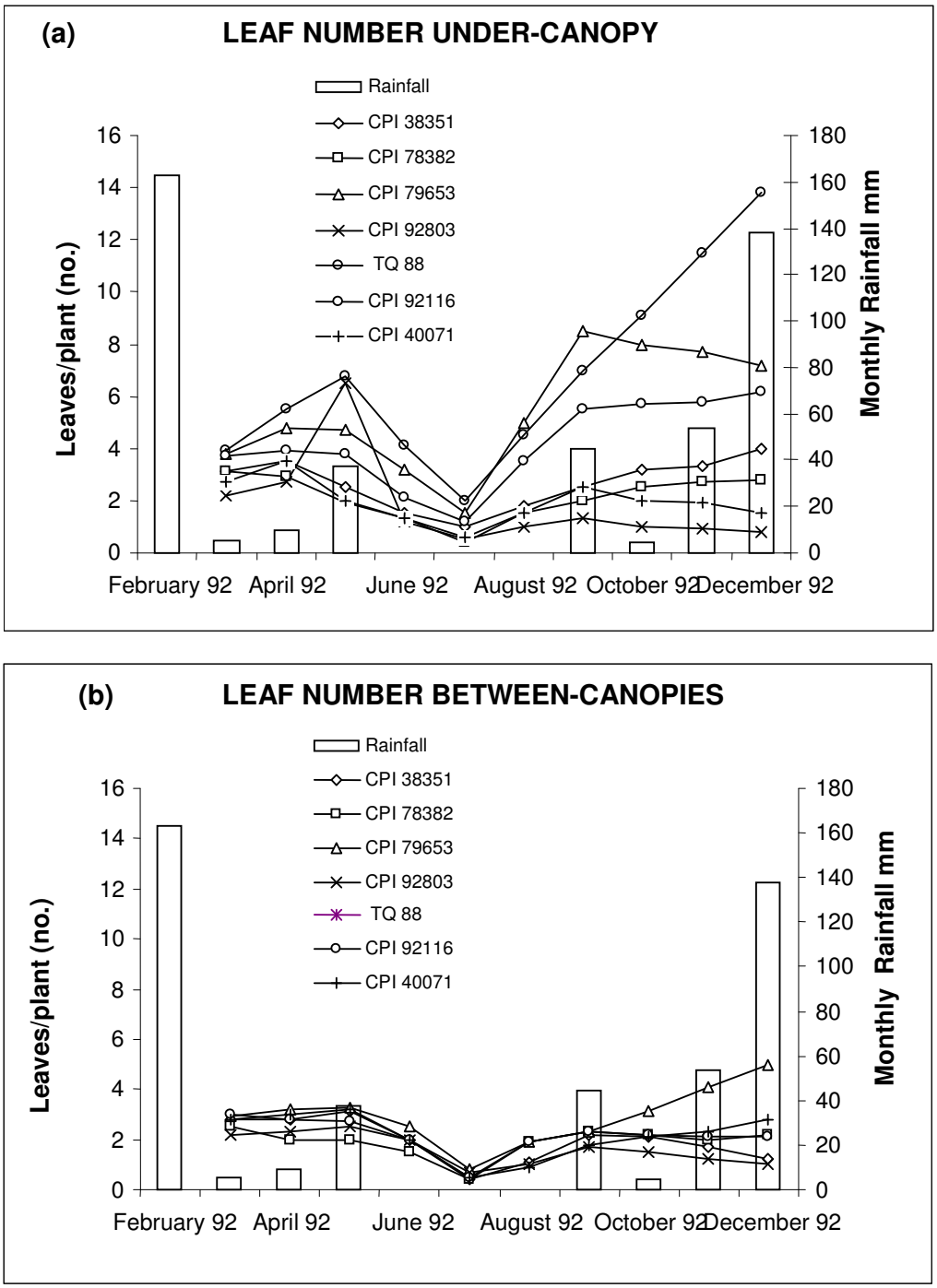


Figure 4.6 Number of leaves per plant in genotypes of *Desmanthus* from February to December 1992 in two microenvironments, and actual monthly rainfall.
(a) Legumes grown under the tree canopy
(b) Legumes grown between the tree canopies
CPI 38351 – *D. leptophyllus* CPI 38351; CPI 78382 – *D. virgatus* CPI 78382;
CPI 79653 – *D. virgatus* CPI 79653; CPI 92803 – *D. pubescens* CPI 92803
TQ 88 – *D. leptophyllus* CPI 321107; CPI 92116 – *D. bicornutus* CPI 92116;
CPI 40071 – *D. pernambucanus* CPI 40071.

Table 4.8 LSD pairwise comparisons of mean lengths of legume plants at under-canopy sites by genotype at December 1992.

Genotype	Mean length (mm)	Growth habit
D. leptophyllus TQ 88	144.91 a	Ascending-scrambling
D. bicornutus CPI 91162	88.24 ab	Erect
D. leptophyllus CPI 38351	61.67 ab	Ascending
D. virgatus CPI 79653	41.18 b	Prostrate
D. virgatus CPI 78382	37.25 b	Erect-ascending
D. pernambucanus CPI 40071	31.88 b	Erect
D. pubescens CPI 92803	12.38 b	Erect

Means followed by the same letter in the column are not significantly different by LSD ($p < 0.05$).

Genotypes that were the tallest under the canopy were not the tallest in the between-canopies sites. *D. bicornutus* CPI 91162 and *D. virgatus* CPI 78382 growing at under-canopy sites increased their lengths by 306% and 181% respectively, compared with their lengths at between-canopies sites. On the other hand, *D. pubescens* CPI 92803 and *D. pernambucanus* CPI 40071 had decreased lengths and number of leaves when growing in under-canopy sites compared with between-canopies sites. Such a differentiated behaviour of genotypes when growing in the under and or between-canopies environments suggest differences in **"plant plasticity"**. In Table 4.8, a faster vertical growth was observed at under-canopy sites in the genotypes with erect ascending growing habit (i.e. *D. leptophyllus* TQ 88 and CPI 38351, and *D. bicornutus* CPI 91162). The genotypes with erect ascending and procumbent growth habits, *D. pernambucanus* CPI 40071 and *D. pubecens* CPI 92803 respectively, showed smaller stem lengths. Inability to adapt to the local shade and soil conditions may explain the poor growth performance of these two genotypes.

One of these, *D. pubescens* CPI 92803 (cv. Uman), performed excellently in full sun at the Burdekin Agriculture College, Clare, some 100 km south of Townsville, grown as a field crop on a cultivated and fertilised sandy clay soil. *D. pernambucanus* CPI 40071 is reported to produce high yields in the Indonesian wet tropics (Gardiner and Burt 1995).

Table 4.9 LSD pairwise comparisons of mean lengths of legume plants at between-canopies sites by genotype at December 1992.

Genotype	Mean length (mm)	Growth habit
D. pernambucanus CPI 40071	45.75 a	Erect
D. leptophyllus CPI 38351	38.00 a	Ascending
D. pubescens CPI 92803	29.50 a	Erect
D. virgatus CPI 79653	23.00 a	Prostrate
D. bicornutus CPI 91162	21.75 a	Erect
D. virgatus CPI 78382	13.25 a	Erect-ascending

Means followed by the same letter in the column are not significantly different by LSD ($p < 0.05$).

Higher exposure to the afternoon sun in the north-west and north-east quadrants as noted above (section 4.3.3), seems to have reduced plant stem length in these positions (Table 4.10). Plants in the south-west quadrant, where a more dense leaf canopy was observed, were four times taller than those in the northeast quadrant (Table 4.10). Soil water availability seems to have been the main factor governing the seedling growth in the different sites, but this possibility could not be tested in the present study. Nor was it possible to determine if different amounts of rain reached the soil from different sites within the tree canopy as predicted by Stuart-Hill *et al.* (1987) and Belsky *et al.* (1989).

Table 4.10 LSD pairwise comparisons of mean lengths of legume plants at under-canopy sites by quadrant at December 1992.

Quadrant	Mean length (mm)
South-west	101.47 a
South-east	63.18 ab
North-west	48.24 bc
North-east	25.69 c

Means followed by the same letter in the column are not significantly different by LSD ($p < 0.05$).

Continuous increases in plant length from March to December 1992 at the under-canopy site were very clear for *D. leptophyllus* TQ88 and *D. bicornutus* CPI 91162, but less clear for *D. leptophyllus* CPI 38351 (Fig. 4.7). Other genotypes in this environment did not produce significant increases in length during the study period,

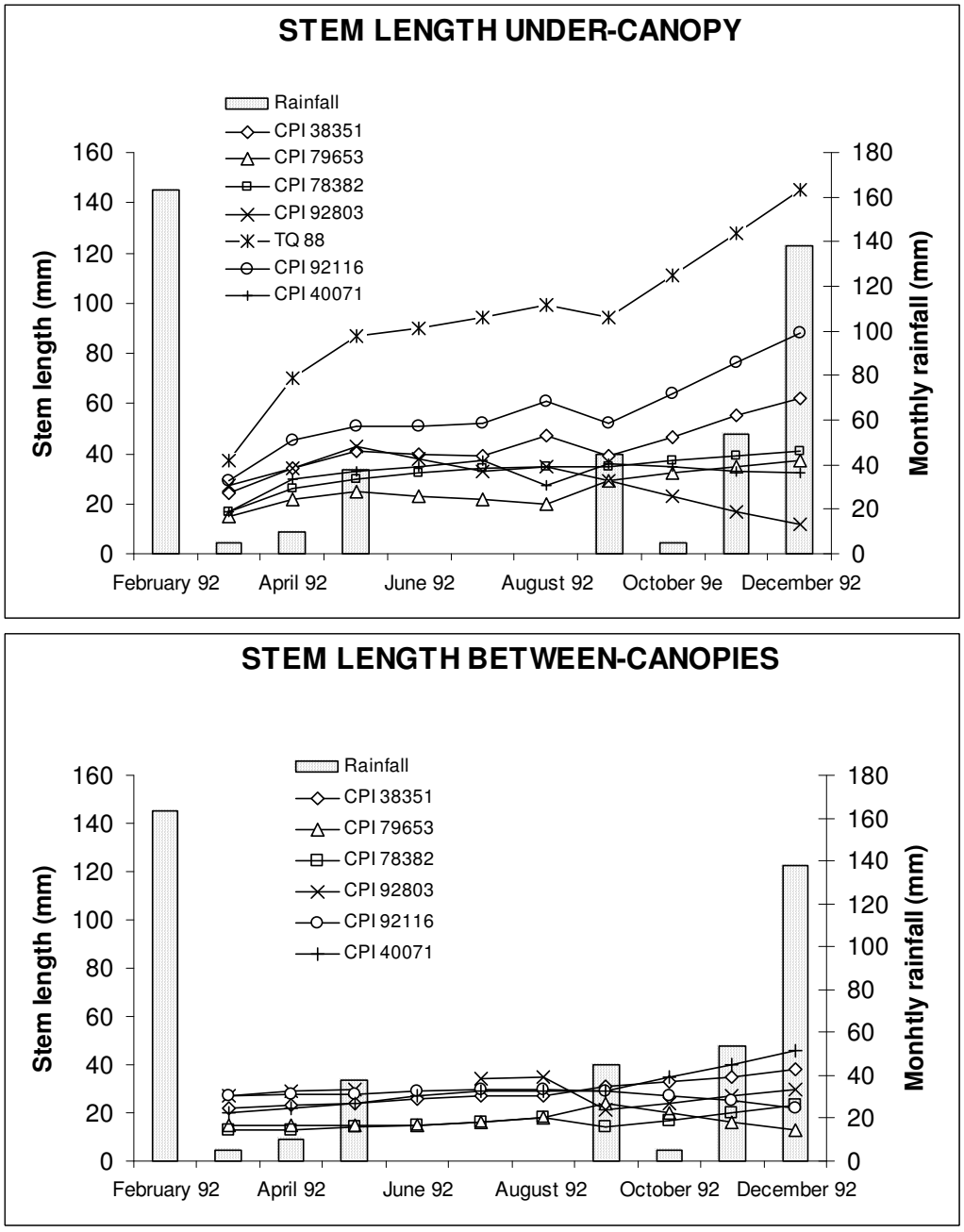


Figure 4.7 Stem length in genotypes of *Desmanthus* from February to December 1992 in two sites, under and between-canopies, plotted against rainfall in the same period.

(a) Legumes grown under the tree canopy
(b) Legume grown between the tree canopies

CPI 38351 – *D. leptophyllus* CPI 38351; CPI 78382 – *D. virgatus* CPI 78382; CPI 79653 – *D. virgatus* CPI 79653; CPI 92803 – *D. pubescens* CPI 92803; TQ 88 – *D. leptophyllus* CPI 321107; CPI 92116 – *D. bicornutus* CPI 92116; CPI 40071 – *D. pernambucanus* CPI 40071

with *D. pubescens* CPI 92803 even showing a reduction in length. This was probably due to losses of the plant tops by dehydration as was observed for some of the other genotypes during the prolonged drought.

Figure 4.7 also shows a slight increase of length on the between-canopies sites, for *D. pernambucanus* CPI 40071 and *D. leptophyllus* CPI 38351 at the end of the study period in response to their more efficient use of water from several light rainfall events.

4.3.4.3 Plant survival

4.3.4.3.1 Under-canopy environment

Differences in relative survival of legumes under the tree canopy at June 1994 were highly significant between genotypes ($p < 0.01$), and between quadrants ($p < 0.05$) (Table A.2.9). Compared to the other genotypes, *D. leptophyllus* TQ 88 and *D. bicornutus* CPI 91162 showed a significant and outstanding site adaptation with 30 and 17% respectively of the original seedlings surviving after three years of drought (Table 4.11). The severity of the drought in the year 1993 was so intense that even plants of Chinee apple (*Ziziphus mauritiana*), an aggressive and drought-tolerant woody weed common in the study area, died from desiccation (Section 2.2.3.3; Fig. 2.8). The surviving seedlings of *D. leptophyllus* TQ 88 and *D. bicornutus* CPI 91162 survived the drought, and developed into vigorous plants (Fig. 4.8), set seed in the 1995 wet season, and are likely to function as a future nucleus of dispersion.

D. virgatus CPI 79653, despite low survival (6%), also formed healthy prostrate plants with good soil cover and profuse seed production. *D. leptophyllus* CPI 38351, *D. virgatus* CPI 78382, and *D. pernambucanus* CPI 40071, had similar relative survival rates with less than 5% of the original plants remaining at the end of the trial (Table 4.11 and Fig. 4.8). *D. pubescens* CPI 92803 had completely disappeared from the experimental place by June 1994. In terms of response in plant growth (section 4.3.4.2), the northeast quadrant was the harshest, with the lowest level of plant survival (Table 4.12).

Table 4.11 LSD pairwise comparisons of mean numbers of plants of surviving genotypes at under-canopy site in June 1994.

Genotype	Mean
D. leptophyllus TQ 88	29.75 a
D. bicornutus CPI 91162	16.75 b
D. virgatus CPI 79653	6.00 c
D. leptophyllus CPI 38351	3.00 c
D. pernambucanus CPI 40071	2.00 c
D. virgatus CPI 78382	0.75 c
D. pubescens CPI 92803	0.00 c

Means followed by the same letter in the column are not significantly different by LSD ($p < 0.05$).

Table 4.12 LSD pairwise comparisons of means of relative plant survival by quadrant at under-canopy site in June 1994.

Quadrant	Number of surviving plants
South-east	12.14 a
South-west	9.71 ab
North-west	6.86 ab
North-east	4.57 b

Means followed by the same letter in the column are not significantly different by LSD ($p< 0.05$).

Analysis of variance based on a given point of time is not a valid method for comparing survival in different treatments during the whole experimental period. Similarly, a regression analysis is not suitable to address this problem because the variation in the number of individuals from one to another record can lead to false estimation of the tendencies. Therefore the Kaplan-Meyer survival analysis, as suggested by Muenchow (1986) and Pyke and Thompson (1986), was used to predict plant survivorship in experiments maintained over a period of time.

Predicted values were calculated from the probability of death events in the population based on the previous occurrence of such events on the sampled individuals (Kaplan-Meier Survival Table, SPSS Advanced Statistic 1994). To build up the Kaplan-Meir table, individuals (plants) were monitored at each sampling date.

Figure 4.8 Vigorous plants of *Desmanthus bicornutus* CPI 911628 and *D. leptophyllus* TQ88 at under-canopy site in March 1995

Each dead plant on a sampling date was nominated as an event and a value 0 attributed to it; each surviving plant was nominated as "censored" and attributed the value 1. The value of the probability of surviving at each sampling date was calculated by the quotient of the total of "censored" cases on that date by the total of censored cases in the previous sampling date. Cumulative probability of surviving at each sampling date was calculated by multiplying the probability of surviving on that date by the probability of surviving on the previous sampling date. Cumulative survival curves for each genotype were drawn from the points of cumulative survival obtained at each date sampling (Kaplan-Meyer survival curve, SPSS Advanced Statistic 1994). In this way, "periods of hazards" for the plant population could be detected from the curves. The predicted survival distribution of genotypes showed significant differences ($p < 0.01$), by Log Rank, Breslow and Tarone -Were test statistics for equality, in the entire four quadrants of the under-canopy environment (Table A.2.10).

Figure 4.9 shows the values of proportional survival plotted for different points on the Kaplan Mayer survival curve. They do not correspond to the actual values

obtained by normal calculation, but to an estimation of life expectancy of a plant at that moment. These results give a more realistic image of initial plant adaptability to the environment. A better seedling adaptation of *D. leptophyllus* TQ 88 and *D. bicornutus* CPI 91162 to local conditions compared with the other genotypes became evident early in the 1992 dry season. These genotypes had an undetermined life expectancy, even for the harsh North-East and North-West quadrants where a short life expectancy was likely because of the low protection from solar radiation. *D. virgatus* CPI 78382 and *D. pubescens* CPI 92803 had short life expectancies in three of the four quadrants, confirming their low site adaptation. Despite the predicted undetermined life expectancy of *D. pubescens* CPI 92803 in the South-East quadrant, it is not possible to obtain a true projection of life expectancy, as this genotype actually disappeared from all the quadrants by June 1994 (Fig. 4.9).

The survival analysis as used here is a relatively recent approach in the field of plant and animal ecology and has the potential for assessing success of agriculturally important plants under different environmental conditions.

4.3.4.3.2 Between- canopies environment

Seedling survival in the between-canopies environment by December 1992 was not significantly affected either by genotype or by quadrant (Table A.2.11). Survival at that date was generally low and varied from 7.5% in *D. virgatus* 38351 to 1.7% in *D. virgatus* 78382 (Table 4.13). In the next sampling date (in the next wet season of March 1993), all seedlings had disappeared from the stand and the experimental activities ceased for the between-canopy site. For the period in which the plants were alive (March 1992 to December 1992), however, genotypes showed significant relative expectancy of survival in three of the four quadrants (Table A.2.11). Seedlings showed stable survival from March to June 1992 and had sharp downward trends from this date, reaching the zero line on April 1993 (Fig. 4.10). No survival expectancy was suggested beyond this date for any of the genotypes. It seems likely that the main reason for the seedling deaths was the severe drought experienced from June 1992.

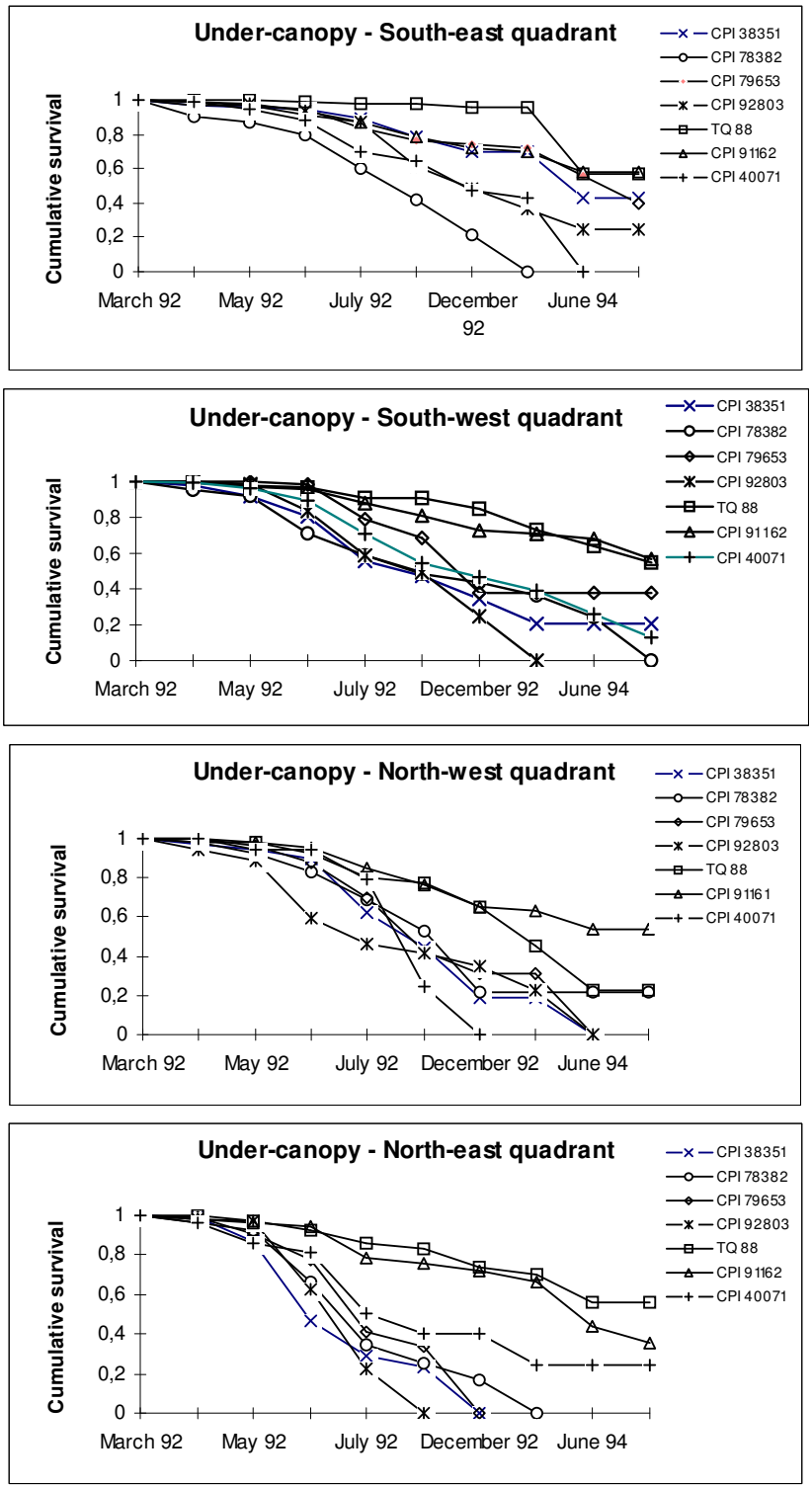


Figure 4.9 Kaplan-Meier cumulative survival curves of seven genotypes of *Desmanthus* at four locations in the under-canopy environment.
CPI 38351 – *D. leptophyllus* CPI 38351; CPI 78382 – *D. virgatus* CPI 78382; CPI 79653 – *D. virgatus* CPI 79653; CPI 92803 – *D. pubescens* CPI 92803; TQ 88 – *D. leptophyllus* CPI 321107; CPI 92116 – *D. bicornutus* CPI 92116; CPI 40071 – *D. pernambucanus* CPI 40071.

Table 4.13 LSD pairwise comparisons of means of relative plant survival by genotype at between-canopies sites in December 1992.

Genotype	Mean	Homogenous Groups
D. virgatus CPI 38351	7.50	a
D. pernambucanus CPI 40071	6.25	a
D. virgatus CPI 79653	5.00	a
D. bicornutus CPI 91162	3.75	a
D. pubescens CPI 92803	2.00	a
D. virgatus CPI 78382	1.75	a

Means followed by the same letter in the column are not significantly different by LSD 0.05.

4.4 Discussion

As shown in Fig. 4.3, the canopy of *Eucalyptus* spp. reduced solar irradiance at the ground surface by 28% for an average dry season day (from 08:00 to 18:00h) compared with solar irradiance on a nearby between-canopies site. This reduction was close to the 35% found by Wilson and Ludlow (1991) under the canopies of five year old trees of *Eucalyptus grandis* and *E. deglupta* in southeast Queensland. In the present study, only early in the morning and late in the afternoon were light levels under the canopy lower than the 200 μmoles/m^2/s suggested by Wilson and Ludlow (1991), or lower than 10-15% of the midday illuminance at unshaded sites (Ludlow *et al.* 1974), which are thought likely to limit seriously the photosynthetic efficiency of tropical legumes. Under these conditions, soil water contents and soil fertility were probably the next most important factors influencing the different behaviours presented by plants in the under and between-canopies environments.

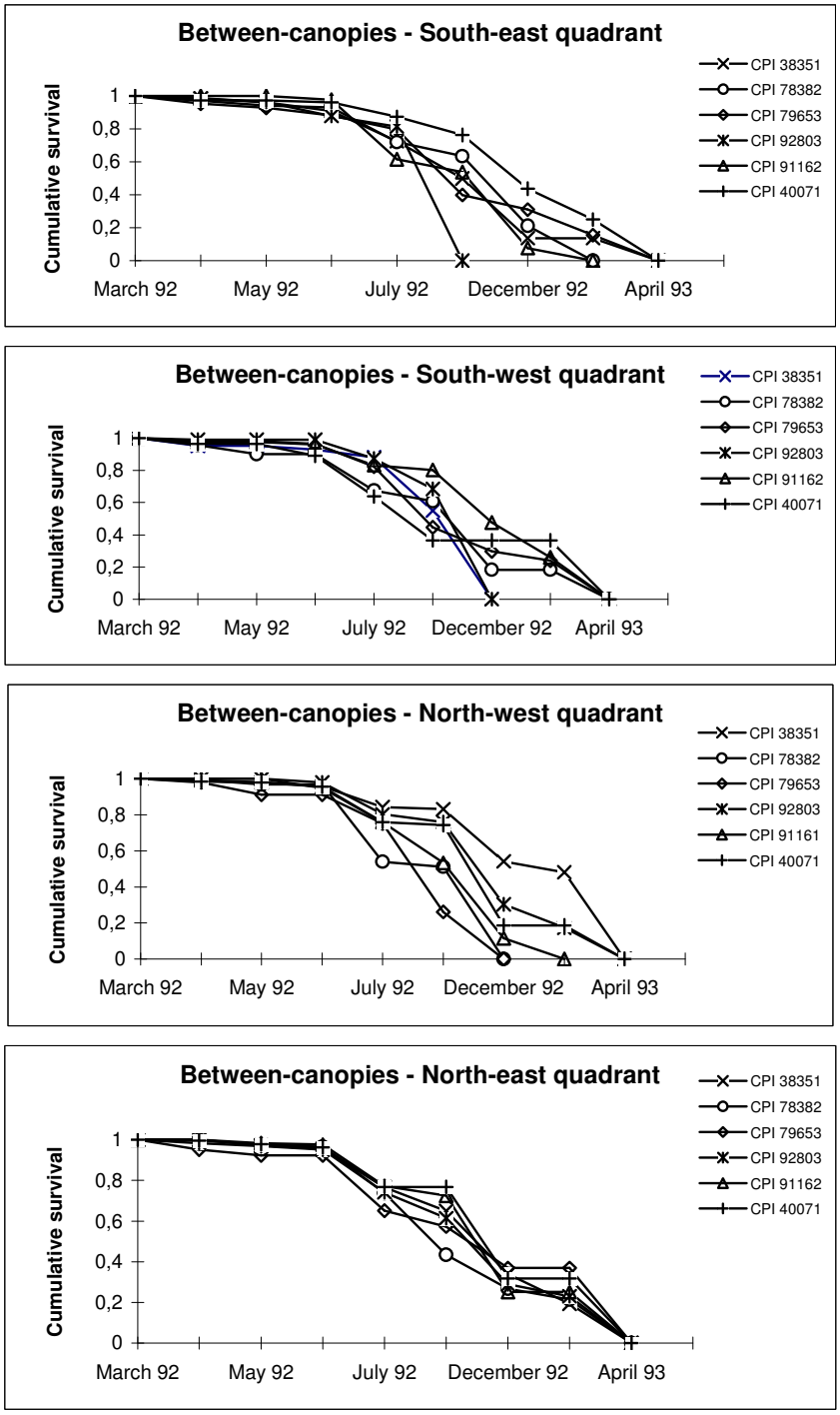


Figure 4.10 Kaplan-Meier cumulative survival curves of seven genotypes of *Desmanthus* at four locations in the between-canopies environment

CPI 38351 – *D. leptophyllus* CPI 38351; CPI 78382 – *D. virgatus* CPI 78382; CPI 79653 – *D. virgatus* CPI 79653; CPI 92803 – *D. pubescens* CPI 92803; CPI 92116 – *D. bicornutus* CPI 92116; CPI 40071 – *D. pernambucanus* CPI 40071.

In spite of the role of overhead tree crowns as suggested by Amundson *et al.* (1995) in intercepting and reducing the amount of rain reaching the under-canopy ground area, and possible competition for this water by the tree, the herbaceous legumes in the present study generally produced more leaves, grew higher, and persisted better under trees than between trees (Figs 4.6, 4.7, 4.9, and 4.10). These data are in accord with the higher herbaceous productivity often found under tree crowns than in surrounding tropical and subtropical grasslands (Radwanski and Wickens 1967; Sandford *et al.* 1982; Stuart-Hill *et al*. 1987; Belski *et al.* 1989; 1993 a; b; Charreau and Vidal 1996; Wasonga *et al*. 2003 in African savannas; Singh and Lal 1985 in Asia; Ebersohn and Lucas 1965 in tropical Australia; Tiedemann and Klemmedson 1977, Holland 1980 in North America; and Kellman 1979; Ovalle and Avendaño 1987 in South America).

A hypothesis that trees improve the fertility of below-canopy soils by adding nutrients to nutrient-deficient soils is supported by many authors (Radwanski and Wickens 1967; Garcia-Moya and McKell 1970; Kellman 1979; Escudero *et al.* 1985; Durr and Rangel 2000). According to Amundson *et al.* (1995), the nutrients reach the soil in leaf litter, bark, twigs, branches and stems from the trees, and are supplemented by decomposed roots in the soil mineral compounds. The nutrients are mostly recycled from deep soil layers to the soil surface and are taken up by tree roots (Prinsley, 1991). A consistently higher concentration of soil nutrients, especially N, P, K, and Ca, in below-canopy soil than in adjacent open grassland has been reported by Charrau and Vidal (1966), Joffre *et al.* (1988), Young (1989), Vetaas (1992). The higher productivity of plants in the under-canopy soils, when compared with plants on the soils between and beyond the tree crowns (Tiedmann and Klemmedson 1973; Georgiadis 1989; Callaway *et al.* 1991), support such a hypothesis. Soil nutrient-enrichment under tree crowns is strongly evident in the present study. The soil analyses (section 4.3.1), represent the results of six sites under and six between tree canopies within the local savanna. Despite some small differences among locations, there was a consistent trend for more fertile soils to occur under the canopies rather than between-canopies.

It was not possible to measure soil moisture contents at the sampling sites. It is expected, however, that levels of soil moisture under the canopy would be higher

than those between-canopies (see for instance Belsky *et al.* 1989; 1993a; b; 1994, Wilson and Ludlow 1991). Various authors (Maranga 1984; Lailhacar-Kind 1986; Smith *et al.* 1987; Ovalle and Avendano 1987; Belsky *et al.* 1989; 1993a; b; 1994; Ammundson *et al.* 1995) have attributed high soil water contents to the combined effects of reduced evapotranspiration and an improvement in the soil's water holding capacity, water infiltration, aeration, and bulk density, under the trees following the input of organic matter from leaves, bark, twigs, and branches. In the present study, despite the absence of direct measurements of soil water content, the symptoms of water stress in plants were more evident in the between-canopies sites than in the under-canopy sites: wilting, and dropping of leaves by plants in the between-canopies sites during the dry season was more evident than in under-canopy sites (Fig. 4.5). On the other hand, the number of new leaves emerging in the subsequent wet season was much higher in plants from under-canopy sites than between-canopies sites. By December 1992, the plants growing under the tree canopy had twice as many leaves, and were 50% bigger, than the plants growing between trees (Figs 4.5 and 4.6). Water content in the soils of under-canopy sites however, may be reduced when roots from the tree are present in shallow layers of soil, absorbing water, and competing with the herbaceous plants (Walker *et al.* 1972; Tiedmann and Klemedson 1977; Kellman 1979; Knoop and Walker 1985; Castellanos *et al.* 1991; Smith and Stuart 1994). Peláez *et al.* (1994) stressed that the pattern of water use by herbaceous and shrub plants in shrublands may be influenced by the strategy adopted to counteract the water stresses of species within each life form. Shrubs and trees tolerant of water stress tend to exploit water in the upper soil layers while shrubs and trees that avoid water stress develop long roots to exploit the moisture held in the deeper layers of the soil. In the present study *Eucalyptus* spp. seem to have an effective water stress avoidance strategy and their roots are not frequently found in the upper layer of the soil; little competition with herbaceous plants might therefore be expected.

There were interesting differences in growth of legume genotypes under and between the tree canopies. The most persistent legume under the tree canopy was *D. leptophyllus* TQ 88; this is an erect, ascending shrub. It was not sown in the open area between the trees because of the shortage of seeds, and so its performance in such an environment could not be determined. The next most persistent legumes

were *D. bicornutus* CPI 91162 and *D. virgatus* CPI 79643. These accessions had higher survival rates under the canopy than between the canopies. Together with *D. virgatus* CPI 78382, they produced many more leaves and grew higher under shaded conditions than in open areas (Figs. 4.6 and 4.7); *D. virgatus* CPI 78382, however, had the lowest productivity of the tested genotypes under all conditions, and died out in both environments. In contrast *D. pubescens* CPI 92803, which died under trees, and D. *pernambucanus* CPI 40071 were much shorter and produced fewer leaves under the canopy than in the open (Fig. 4.6). Further studies are needed to clarify the reason for these genotype differences, but large differences in plant form, seedling habit, and root characteristics are well known (Burt 1993).

The traditional system of tree clearing to improve pasture productivity has been dominant worldwide since the early stages of grazing activities. This practice, however, is leading to serious problems of land degradation in tropical regions (Isichei and Muoghalu 1992; Cameron *et al.* 1989). Silvipastoral practices, the deliberate integration of trees and shrubs in livestock production systems, are a recent paradigm. The practice is distinguished by an underlying assumption of the potential beneficial effects to ecosystem stability and sustainability resulting from the addition or maintenance of trees in tropical grasslands (Young 1989; Cannell *et al.* 1996). Nevertheless, studies of the interrelationships between tropical forage legumes and trees in silvipastoral or **agroforestry systems** for the dry areas of the tropics are very scarce. The analysis of the data obtained from some of the *Desmanthus* genotypes studied here (especially *D. leptophyllus* TQ 88, *D. virgatus* CPI 79643, and *D. bicornutus* CPI 91162) reveal the potential of these genotypes to form effective herbaceous layers of **silvipastoral systems** in the dry tropics.

Chapter 5

SEEDLING BEHAVIOUR OF TWO *DESMANTHUS* GENOTYPES UNDER DIFFERENT SOIL, LIGHT INTENSITY, AND WATERING FREQUENCY TREATMENTS

5.1 Introduction

In the previous chapter it was shown that the different environments under and between tree canopies affected the establishment, growth, and survival of seedlings of the legume *Desmanthus*; some genotypes preferred the more shaded environment and others did not. Differences in performance were possibly related to differences in soil moisture and soil nutrient levels. The study described in the present chapter has evaluated the effects of shading, soil nutrients, and water stress in a pot experiment carried out in the field. Germination, initial growth, and early shoot and root yields of two *Desmanthus* genotypes were studied. *D. leptophyllus* TQ 88 and *D. virgatus* CPI 78382 were chosen as illustrating genotypes with good and poor performance under shaded environments, respectively.

5.2 Materials and methods

The experiment was carried out at site 4 of Fig. 2.3 whose the vegetation and soil characteristics have been discussed above (Sections 2.3.1 and 2.3.4).

5.2.1 Preliminary tests

5.2.1.1 Soil density and daily water balance in soil

Soil bulk density was obtained by weighing two 105 ºC dried samples of 50 cm^3 of soil, collected from specified locations in the field using Kopeck rings (Kiehl 1979).

Six black plastic pots were filled with 2 kg of sun-dried and homogenised soil that had been passed through a 2 mm sieve and had been collected from under ironbark tree canopies, and six other pots with soil from between the tree canopies. The

characteristics of the soils are given in section 4.3.1. On 30 August 1994 the pots were watered to saturation with tap water, and left to drain under shade. On 01 September 1994, when no more water was draining, pot weights were recorded using a 5 kg bench scale. The latter records were taken to express the pot weight at soil field capacity and were later used in the main experiment. On the same day, one set of three pots with soil at field capacity for each type of soil was placed under full sunlight condition and one other under 50% of incident sunlight, in a glass house. Pots were weighed daily from day 1 (01 September 1994) to day 14 (14 September 1994). Daily soil water percentage in relation to soil volume for each pot was obtained by the follow equations:

Wu% = (Sf - Sd)/Sd x 1.14 x 100, and

Wb% = (Sf - Sd)/Sd x 1.23 x 100,

Where:

Wu% - Water potential, expressed as the percentage of water mass in relation to the total mass (soil + water), in soil from under the canopy

Wb% - Water potential, expressed as the percentage of water mass in relation to the total mass (soil + water), potential in soil from between-canopies

Sf - Net mass of soil at field capacity (subtracted pot weight)

Sd - Net mass of dry soil (subtracted pot weight)

Values of 1.14 and 1.23 are the soil bulk densities for soils from under and between the canopies respectively.

Curves of daily changes in mass equivalent to changes in water content were obtained for the two soils, in shade and full sunlight conditions, using the mean of three pots per treatment.

5.2.1.2 Seed germination

The germination of seeds of *D. leptophyllus* TQ 88 and *D. virgatus* CPI 78382 was tested from 23 September 1994 to 10 October 1994 in Petri dishes half filled with vermiculite and covered with double sheet soft filter paper. Fifty seeds per Petri dish, replicated three times for each genotype, were laid on top of filter paper and watered.

twice a day with distilled water. The numbers of germinated seeds were recorded daily; no new germination occurred after 7 October 1994.

5.2.2 Main trial

The effects of four shading levels (polythene sheet and shade cloth allowing transmission of 100%; 45 %; 30%; and 15% of the incoming solar radiation), three watering intervals (3; 6; and 12 days), and two soils (collected from under the ironbark canopies, and from between canopies sites), were tested on germination, seedling survival, and dry matter production of roots and shoots of forty days old plants, of two *Desmanthus* genotypes (*D. leptophyllus* TQ 88 and *D. virgatus* CPI 78382). Treatments were organised in a three block, split-split-split plot randomised design (Kempthorne 1979), having shade levels in plots, water regimes in subplots, soil in sub-subplots, and genotypes in sub-sub-subplots. Shading levels were achieved by using green shade cloth screens of different light transmission characteristics stretched horizontally in the field at a height of about 1.8 m above the pots, and vertically on the east and west sides to prevent direct sunlight at sunrise and sunset (Fig. 5.1).

Figure 5.1 Field distribution of shade cloth and pots

Seventy-two square-mouthed black plastic pots were each filled with 2.0 kg of sun-dried, 2 mm sieved and homogenized soil, from under canopy sites and seventy-two with soil from between canopy sites; each set of pots was split into two sets of 36 pots. On 13 October 1994 one set of 36 plots per soil type was sown with *D. leptophyllus* TQ 88 and the other with *D. virgatus* CPI 78382, at the rate of 30 seeds per pot; the seed had been hot water scarified (80 ^{0}C for 5 minutes). Sowing was done by laying six seeds per row into five parallel rows, scratched on the soil surface. Seeds were covered with a fine layer of soil from the same pot. The pots were distributed in blocks into shade, water, soil, and genotype treatments. All treatments were randomised by being drawn sequentially: first, shade levels into each block; second, water frequencies into each shade level; third, soil origins into each water regime; and fourth, genotypes into each soil type. On the same day, each pot was watered to field capacity, as previously determined in the pre-experimental test. From this date watering for each watering regime was done to field capacity. The amount of water necessary to reach this point was taken by weighing each pot on the watering day of that frequency and subtracting each value from the weight of a pot with soil at field capacity. The mean mass of water necessary was applied to the pots in the same shade level, soil, and genotype respectively in each block. These data were also used to assess the actual water balance in soils under the shade and watering frequency treatments. Regression curves were drawn to determine the effects of shade x watering frequency interaction on the soil water content. Seedling emergence and seedling survival after emergence were recorded at three day intervals. All pots were harvested on the fortieth day after sowing. There was no rainfall occurrence during the experimental period.

5.2.2.1 Statistical analysis

Analyses of variance of cumulative percentage of seedling emergence, survival, and dry matter production (mg/plant) of forty day old plants were carried out using STATIX Analytical Software Version 4.1. Means were compared by LSD tests at 5% confidence level ($p < 0.05$).

5.3 Results

5.3.1 Preliminary tests

5.3.1.1 Soil density and daily water balance in soil

Soil from the open areas between the tree canopies had a higher bulk density (1.23 g/cm^3) than that collected from under the canopies (1.14 g/cm^3). Such a difference is probably due to the higher content of organic matter in the under-canopies environment, and its effects on soil aggregation and habitats for the soil macro and microbiota.

Soil collected from under the canopies also retained a higher volume of water at saturation point (day 1); again possibly because of its higher organic matter content (Fig. 5.2). By day 14, soils from both under and between-canopies, kept under shade conditions, had lost 63% and 70%, respectively of the water held at saturation; those kept in the sun lost 86% and 90% of the saturated soil moisture content from under- and between-canopies sites, respectively.

The curves of soil water loss (Fig. 5.2) show that soils from all treatments lost water in a linear fashion from day 6 or 7 to day 14. Pots in the shade lost water somewhat slowly initially (days 1 to 6), while those in the sun lost their water more rapidly.

Watering intervals were based on the curves shown in Fig. 5.2. In the 3rd day low and medium water stresses were observed in soil, respectively for under shade and full sunlight treatments (Table 5.1). At day six water stresses were light for shaded and severe for full sunlight treatments. Severe and very severe water stress was registered at day twelve for shade and full sunlight treatment, respectively.

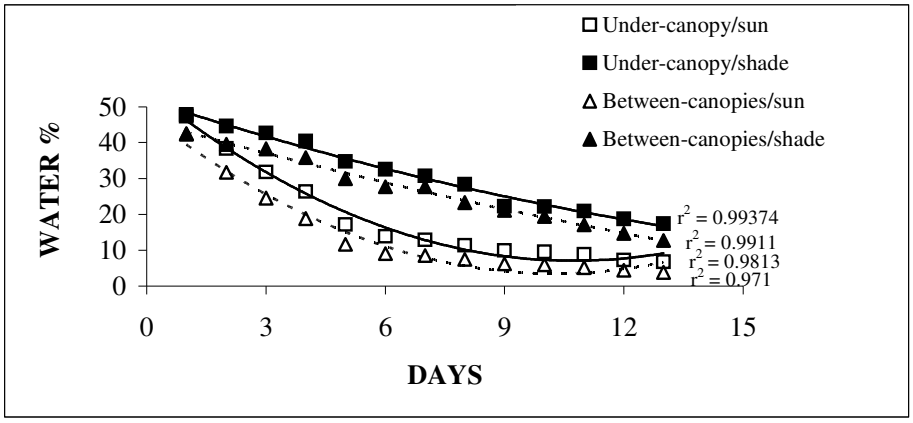


Figure 5.2 Curves of decreasing water content (weight %) in soils collected from under and between tree canopies, submitted to treatments of 50 % shade (■ and▲) and full sunlight (□ and Δ).

Table 5.1 Water stress in soil related to the interval between pot watering.

Watering interval (days)	Stress under shade	Stress in full sun
3	Low	Medium
6	Medium	Severe
12	Severe	Very severe

5.3.1.2 Seed germination

The *Desmanthus* genotypes showed different trends in their germination patterns. While 78 % of total germination of TQ 88 occurred on the second day of the test, germination of CPI 78382 occurred uniformly through the period (Table 5.2). No seed germinated after day 10. All the remain no germinated seeds were observed to be imbibed and rotted at the end of the germination test (10 October 2004).

Table 5.2 Mean percentage germination of 3 replicates of 50 seeds of *D. leptophyllus* TQ88 and *D. virgatus* CPI 78382, from 3 September – 19 October 1994. Seeds maintained in moist Petri dishes (filter paper on vermiculite) at 28 ^{0}C.

	Germination %										
Accession	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day10	Total
TQ 88	53	9	1	1	2	1	1	0	0	0	68
78382	0	5	1	6	9	4	3	4	5	5	42
Mean	26.5	7	1	3.5	5.5	2.5	2	2	2.5	2.5	55

5.3.2 Main trial

Actual water balance in soil

Water contents in the soil were drastically and linearly reduced by increasing the watering intervals (Fig. 5.3). The soil collected from under tree canopies did not have a significantly higher water holding capacity in any of the studied watering intervals than soil collected from between tree canopies (Fig. 5.3). Soil water content, averaged for the three watering intervals, on the other hand, increased significantly with increasing shade level (Fig. 5.4). Analysis of variance of linear regressions for the effect of watering regimes upon the soil water content, for four levels of sunlight, are shown in Table A.2.13 where genotypes and soils were both averaged in the analysis. Watering frequencies against soil water content significantly fitted the linear regressions for all levels of sunlight (Table A.2.13; $p < 0.01$).

Scatter plots of water content estimated by the regression (Table A.2.13), for the four levels of sunlight, are shown in Fig. 5.5. The slope of the regression line increased with increasing shade level, having predicted interceptions of the Y axis at 24%; 28%; 30%; and 35% of water content in soil, respectively for shading levels of 100%; 45%; 30%; and 15% of full sunlight.

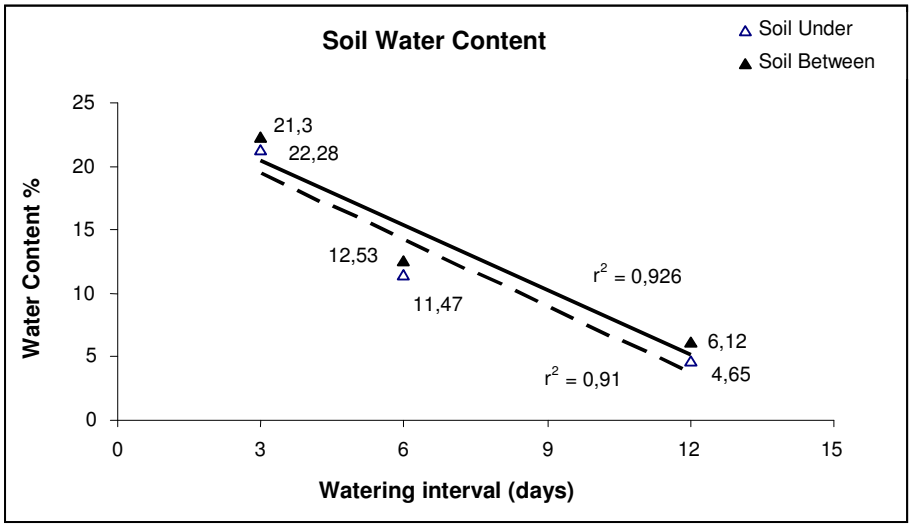


Figure 5.3 Linear regressions of the effects of 3, 6, and 12 day watering intervals on the water content in soils collected under (▲) and between (Δ) tree canopies, averaged for the studied levels of 15%, 30% , 45%, and 100% of the incoming solar radiation.

The decreasing slope of the regression line with decreasing shade levels (Table A.2.13) reflects the decreasing effect of shade with the postponement of irrigation. Shade was more effective in reducing water losses in soils under more frequent irrigation than under prolonged periods without watering. Three days after irrigation, the water content of the soils (averaged for soils and genotypes) was 38% and 58%, respectively of their content at soil field capacity, for pots exposed to 100% and 15% sunlight, respectively (Fig. 5.5 (a) and 5.5 (d)). Twelve days after irrigation, it was just 8 % and 15%, respectively for the same levels of sunlight (Fig. 5.5 (a) and 5.5 (d)). Water in this experiment consistently moved out of the soil faster than in the preliminary tests, this being more noticeable under shaded conditions. It may be due to differences in environmental conditions in the field and in the glasshouse where the preliminary work was done (Jackson *et al*. 1999), and/or differences in plant transpiration in the field.

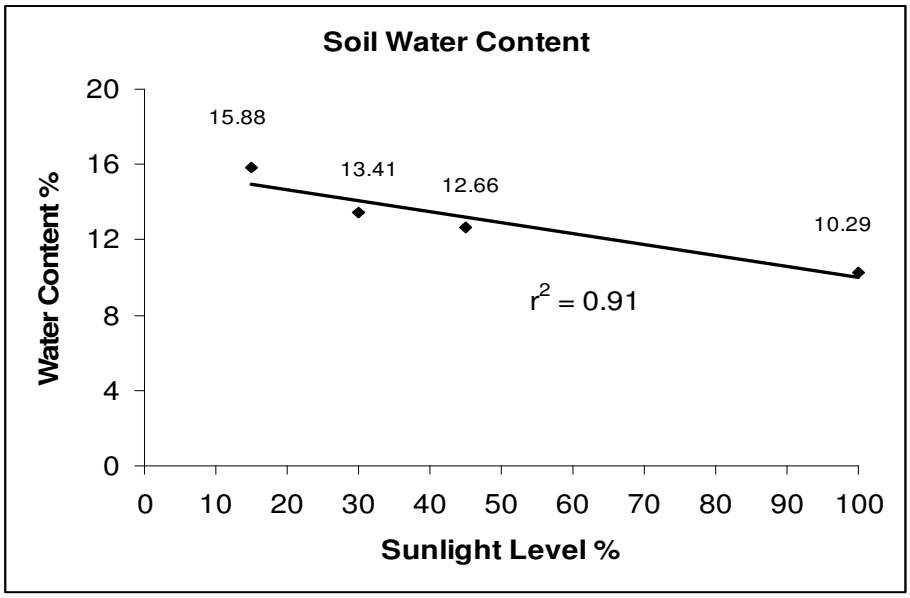


Figure 5.4 Linear regression for the studied levels of 15%, 45%, 70%, and 100% of the incoming solar radiation on the water content in soil, averaged for soils collected from under and between tree canopies.

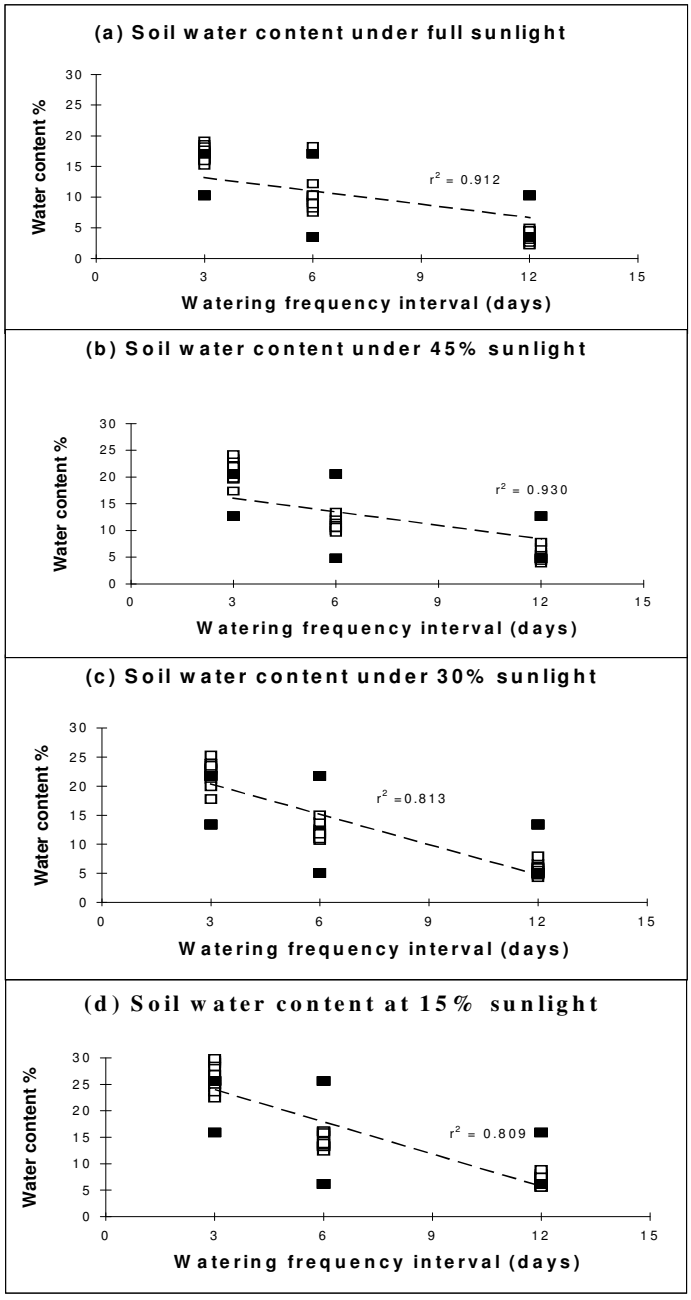


Figure 5.5 Linear regressions of water content in soil under 3, 6, and 12 day watering interval, and different levels of sunlight, averaged for *Desmanthus* genotypes and soil origins.

□ Original points
■ Predicted points from the regressions (Table A.13)

(a) Pots in full sunlight
(b) Pots in shade (45% of full sunlight)
(c) Pots in shade (30% of full sunlight)
(d) Pots in shade (15% of full sunlight)

5.3.2.2 Seedling emergence

Seedlings in general had poor emergence reaching, for both genotypes, only half of the potential values obtained from the percentage germination tests (68% and 42%, Table 5.2). The analysis of variance of seedling emergence after ten days, showed significant effects ($p < 0.01$) for levels of sunlight interception, watering interval, and genotypes, but the soil types, from under and between trees, did not significantly affect seedling emergence (Table A.2.14).

Few seedlings emerged after day 15 in the pot trial time by which well defined trends had been established (Figs. 5.6 to 5.9). Seedling emergence was highest for TQ 88 in shaded treatments; at 15 % light transmission twice as many seedlings emerged as under full sunlight (Table 5.3, Figs. 5.6 and 5.7). The reduced watering treatments resulted in reduced seedling emergence, with a statistical significant difference between 6 and 12 watering intervals (Table 5.3, Figs. 5.6 to 5.9).

Interactions were significant only for shade x genotype (Table A.2.14). Increasing the shade level influenced seedling emergence when its effects were analysed for each genotype (Table 5.4).

The shade effect on seedling emergence probably can be explained by higher water availability in the top layers of the soil for germinating seeds under shaded conditions than at full sunlight. This is supported by Fig. 5.5, in which the water content in soil watered every three days was clearly enhanced by shade treatments.

5.3.2.3 Desmanthus plant survival

5.3.2.3.1 TQ 88

The number of survived seedlings per pot, expressed as a percentage of the emerged seeds, was plotted against time for the various shade levels and for each combination of genotype, soil, and watering frequency (Figs.d 5.10 to 5.13).

Table 5.3 LSD pairwise comparisons of means of seedling emergence (% of emerged seedlings from the sown seeds) by shade, soil, watering frequency, and genotype.

% of full sunlight		Soil		Watering frequency		Genotype	
Level	Emergence	Origin	Emergence	Days	Emergence	Name	Emergence
100 %	17.22 b	Under	24.49 a	3	25.76 ab	TQ 88	32.41 a
45 %	23.98 ab	Between	25.23 a	6	27.57 a	CPI 78382	17.32 b
30 %	26.11 ab	--	--	12	21.25 b	--	
15 %	32.13 a	--	--	--	--	--	
LSD (p< 0.05)	9,00		4.56		5.02		8.34

Means in the same column followed by the same lower case letter are not significantly different by LSD (p < 0.05)

Table 5.4 LSD pairwise comparisons of means of seedling emergence (% of emerged seedlings from the sown seeds) for the interaction shade x genotype.

Genotype name	100% of full sunlight	45% of full sunlight	30% of full sunlight	15% of full sunlight	Mean
TQ 88	22.96 a C	33.15 a AB	30.74 a BC	42.78 a A	32.41
CPI 78382	11.48 b A	14.82 b A	21.48 b A	21.48 b A	17.32
Mean	17.22	23.98	26.11	32.13	24.86

Means followed by the same lower case letter in a column, and by the same CAPITAL case letter in a row, are not significantly different by LSD (p< 0.05)

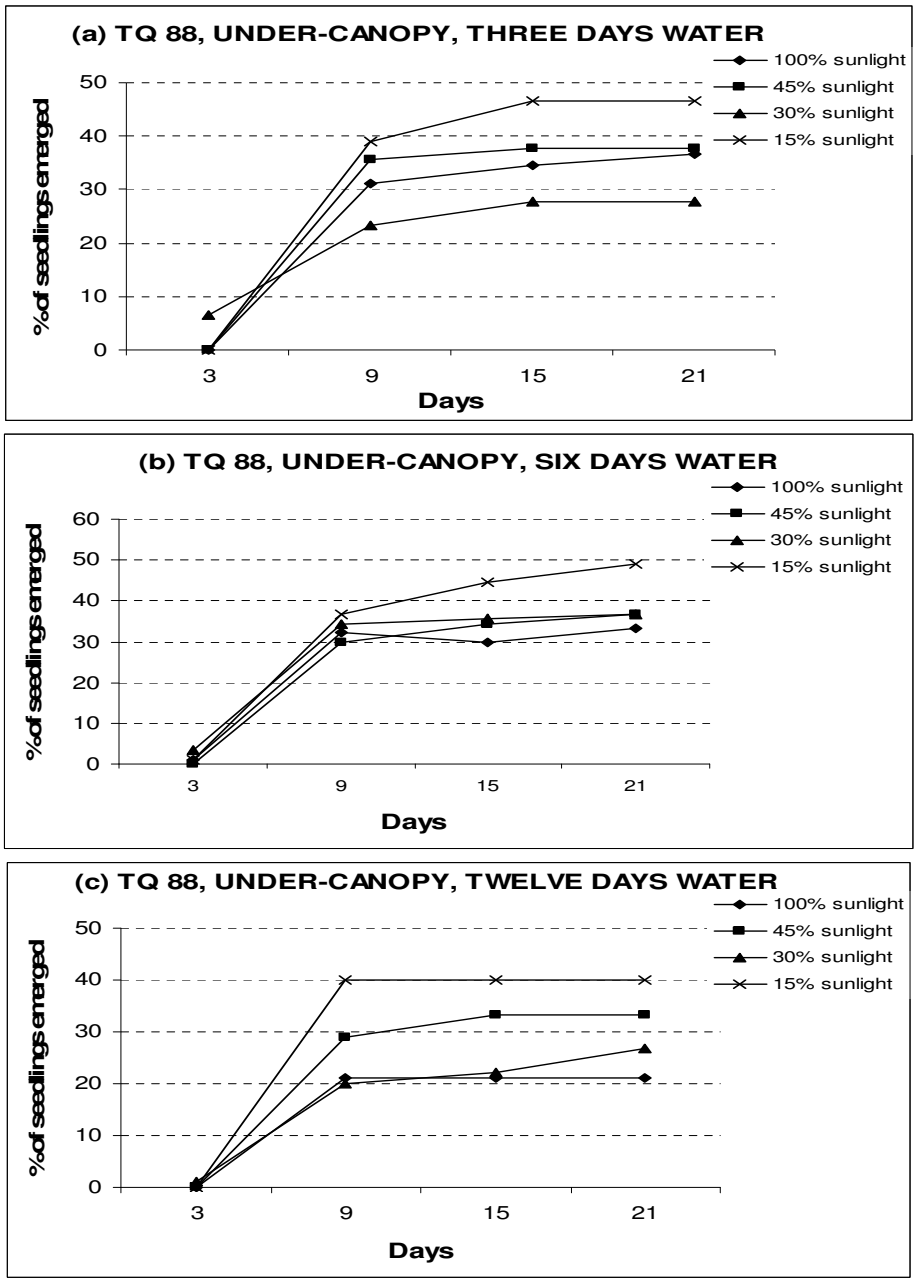
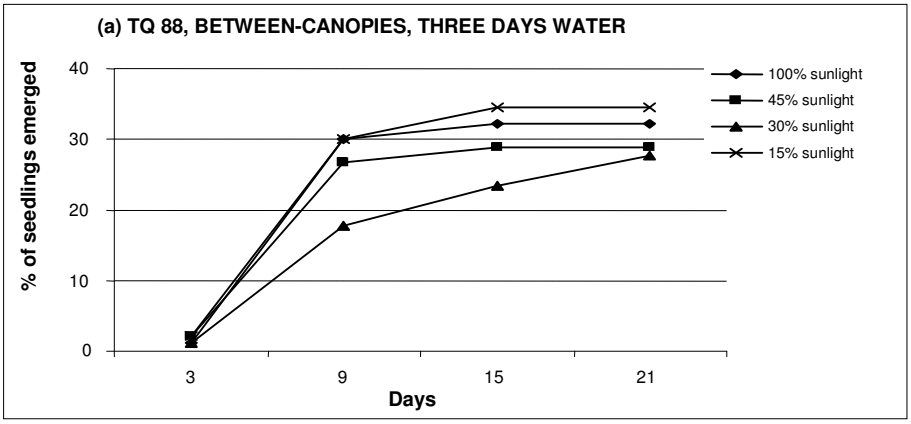
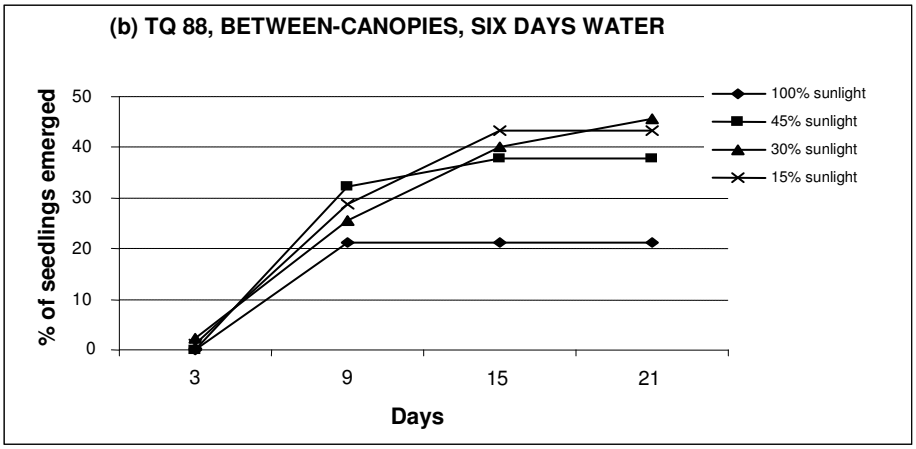


Figure 5.6 Percentage of seedling emergence curves of *D. lpetophyllus* TQ 88 in soil from under-canopy, at four levels of light incidence, and three different watering regime intervals.
100% = Emergence curve of plants growing at 100 % of full sunlight
45% = Emergence curve of plants growing at 45% of full sunlight
30% = Emergence curve of plants growing at 30% of full sunlight
15% = Emergence curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days





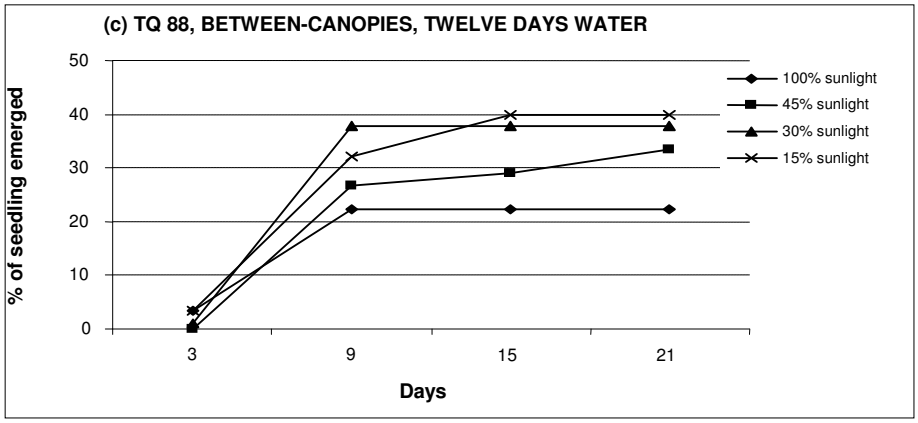


Figure 5.7 Percentage of seedling emergence curves of *D. leptophyllus* TQ 88 in soil from between-canopies, at four levels of light incidence, and three different watering regime intervals.
100% = Emergence curve of plants growing at 100% of full sunlight
45% = Emergence curve of plants growing at 45% of full sunlight
30% = Emergence curve of plants growing at 30% of full sunlight
15% = Emergence curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days

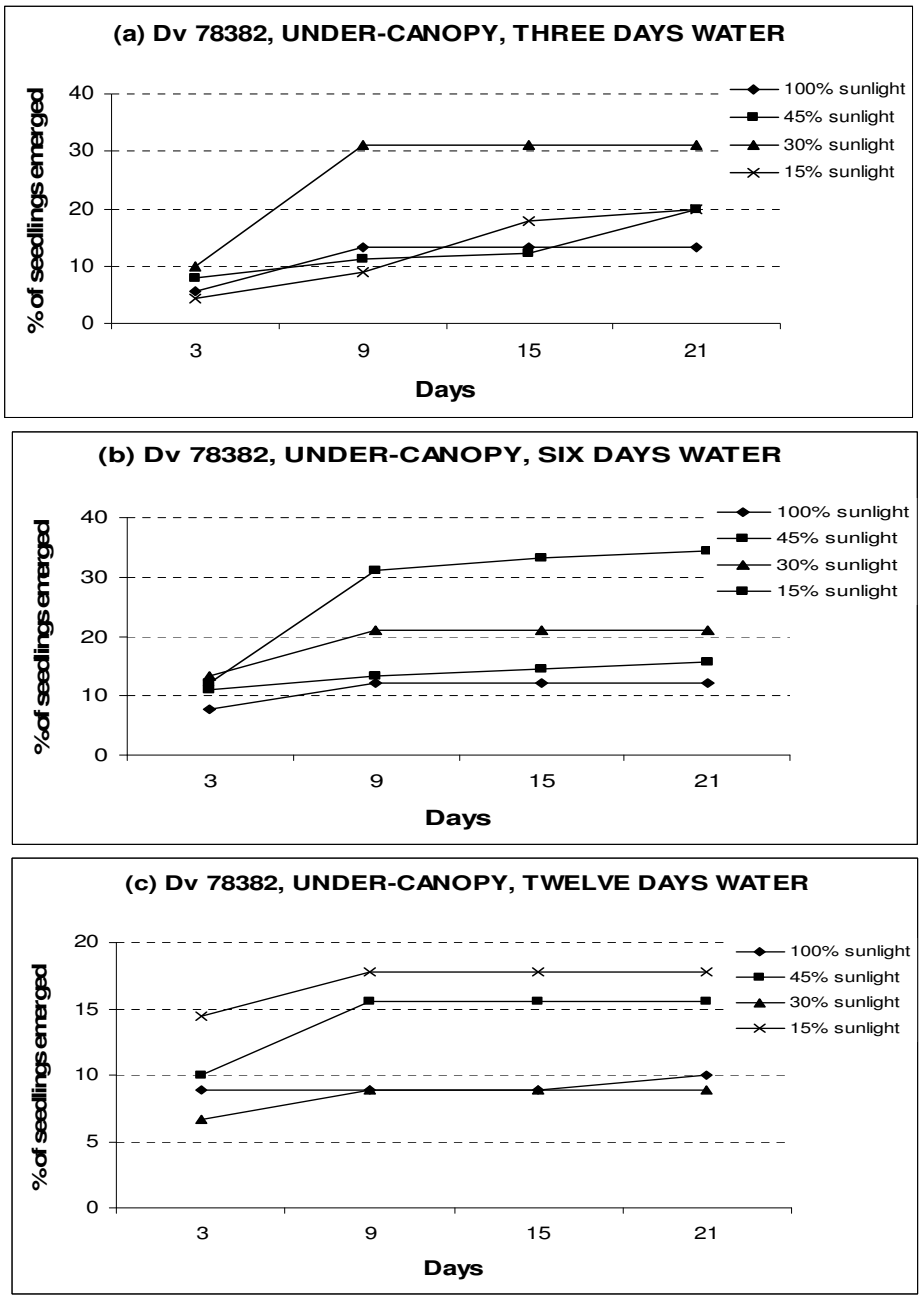


Figure 5.8 Percentage of seedling emergence curves of *D. virgatus* CPI 78382 in soil from under-canopies, at four levels of light incidence, and three different watering regime intervals.
100% = Emergence curve of plants growing at 100 % of full sunlight
45% = Emergence curve of plants growing at 45% of full sunlight
30% = Emergence curve of plants growing at 30% of full sunlight
15% = Emergence curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days

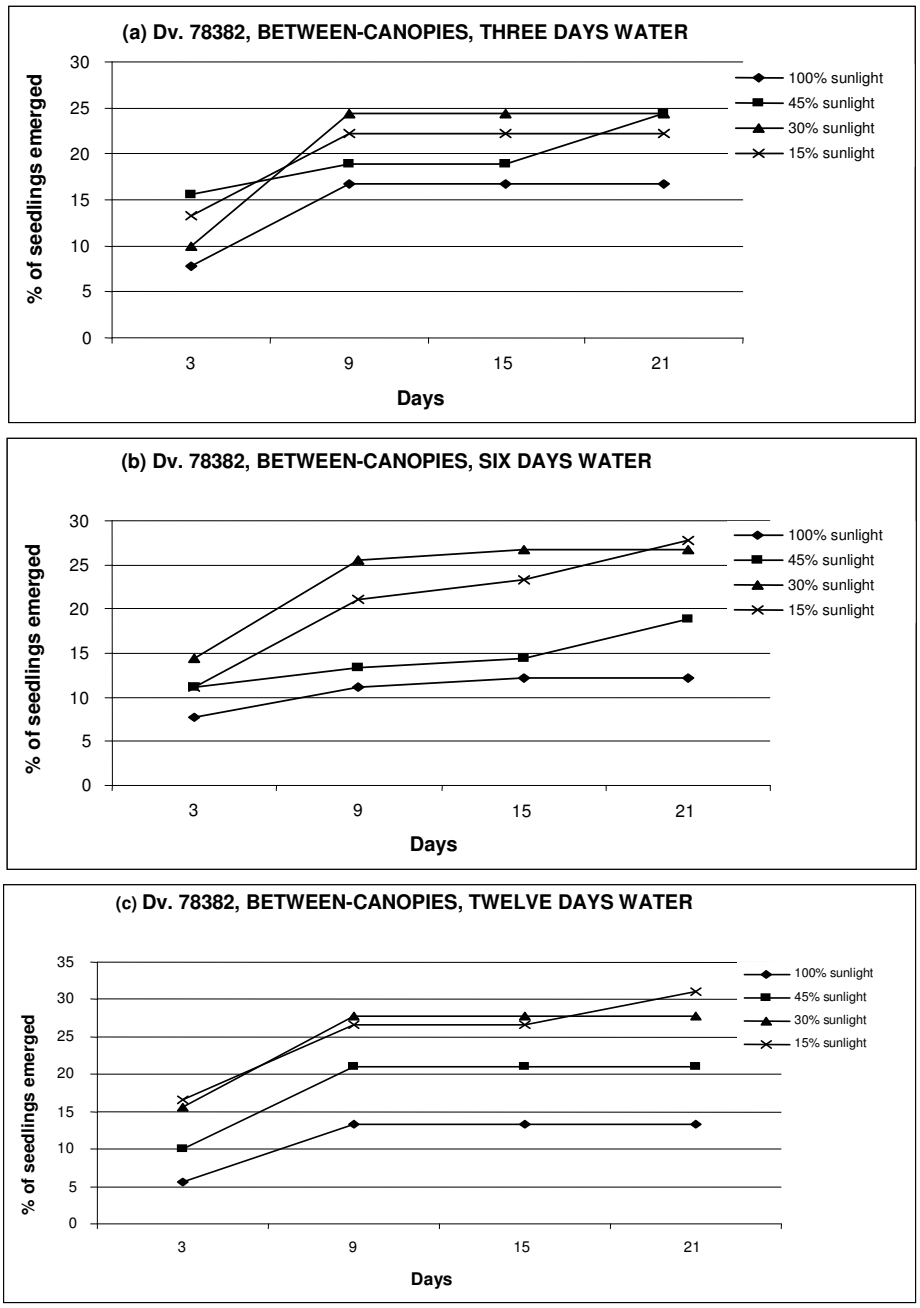


Figure 5.9 Percentage of seedling emergence curves of *D. virgatus* CPI 78382 in soil from between-canopies, at four levels of light incidence, and three different watering regime intervals.
100% = Emergence curve of plants growing at 100 % of full sunlight
45% = Emergence curve of plants growing at 45% of full sunlight
30% = Emergence curve of plants growing at 30% of full sunlight
15% = Emergence curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days

The greatest impact on seedling survival was that of TQ 88 growing in soil collected from between the trees, in pots placed in full sunlight, and watered every 12 days (Fig. 5.11). Only a few plants of TQ 88 were alive at the end of the experiment under niches of the full sunlight and 12 day water frequencies. Neither the 3 nor 6 day water frequency induced a large number of deaths in seedlings of TQ 88 in any combinations of soil and shade treatments (Figs. 5.10 and 5.11).

5.3.2.3.2 CPI 78382

Very few CPI 78382 seedlings died when growing in soil from under-canopies, regardless of shade or watering levels (Fig. 5.12). Many of these plants died however, when grown on soil from between trees, and subjected to 6 or 12 day watering regimes in full sunlight (Fig. 5.13 b, c). In soil from between-canopies all plants had died by day 39, under full sunlight, when watered every twelve days (Fig. 5.13 c).

Fewer seedlings died under the 6 or 12 day watering regime in soil from between trees when light was reduced to 45% or 30% of the full sunlight. Seedling death was nil at 45% of the full sunlight (Fig. 5.13 a, b).

5.3.2.4 Dry Matter Production

Plants were harvested on day 40th after planting and the entire root and shoot materials were dried at 65 ^{0}C for 72 hours before weighing. The dry matter yields per plant of shoot, root, shoot + root, and shoot/root ratio were obtained by dividing the total yield of shoot or root in each pot by the number of surviving seedlings in that pot on the day of the harvest.

5.3.2.4.1 *Shoot Dry Matter*

Shoot dry matter was significantly affected by the main factors shade, soil, watering regime, genotype, and the interactions of shade x soil, soil x watering regime, soil x genotype, watering regime x genotype, and soil x watering regime x genotype (Table A.2.15).

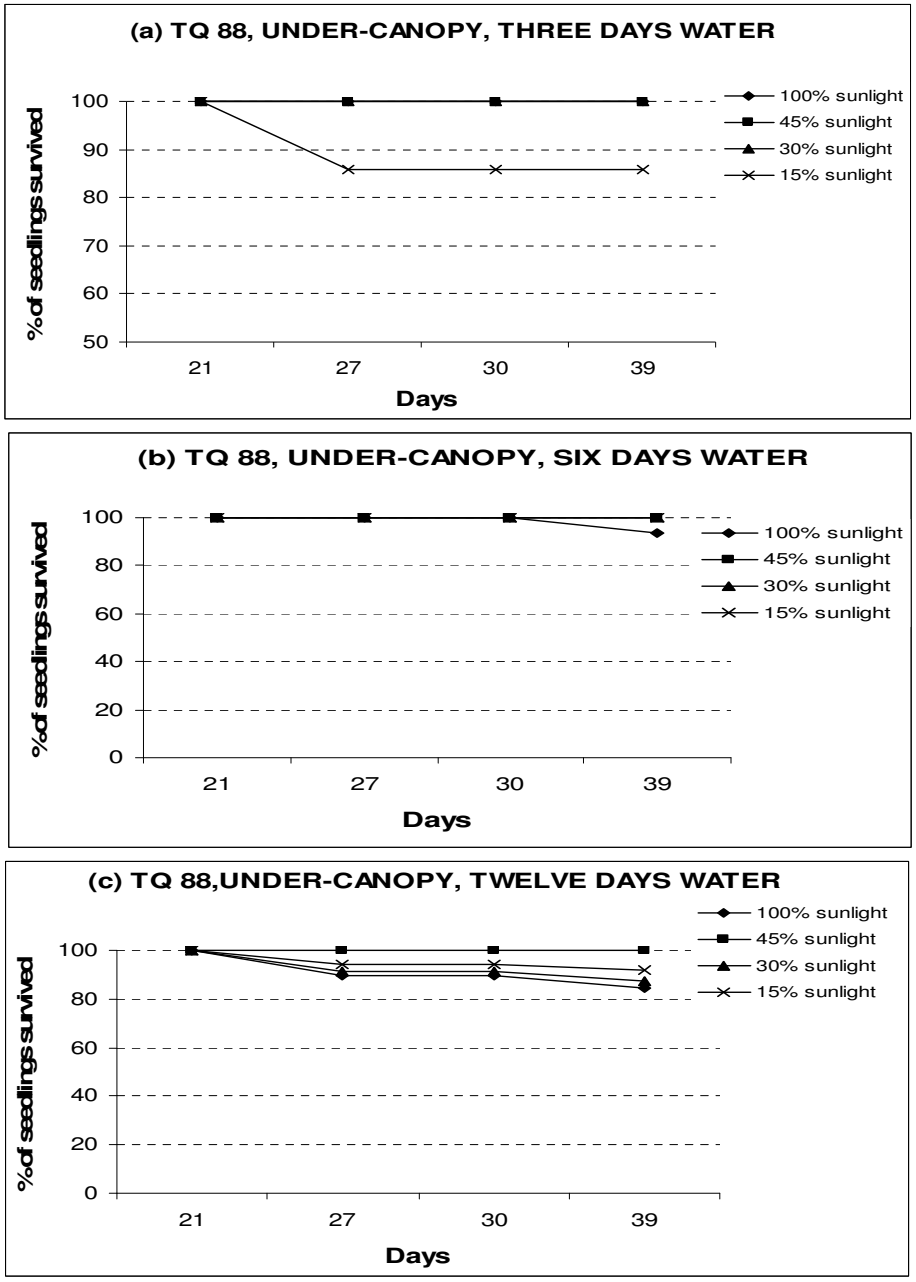


Figure 5.10 Percentage of seedling survival curves of *D. leptophyllus* TQ 88 in soil from under-canopy, at four levels of light incidence, and three different watering regime intervals.

100% = Emergence/survival curve of plants growing at 100% of full sunlight
45% = Emergence/survival curve of plants growing at 45% of full sunlight
30% = Emergence/survival curve of plants growing at 30% of full sunlight
15% = Emergence/survival curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days

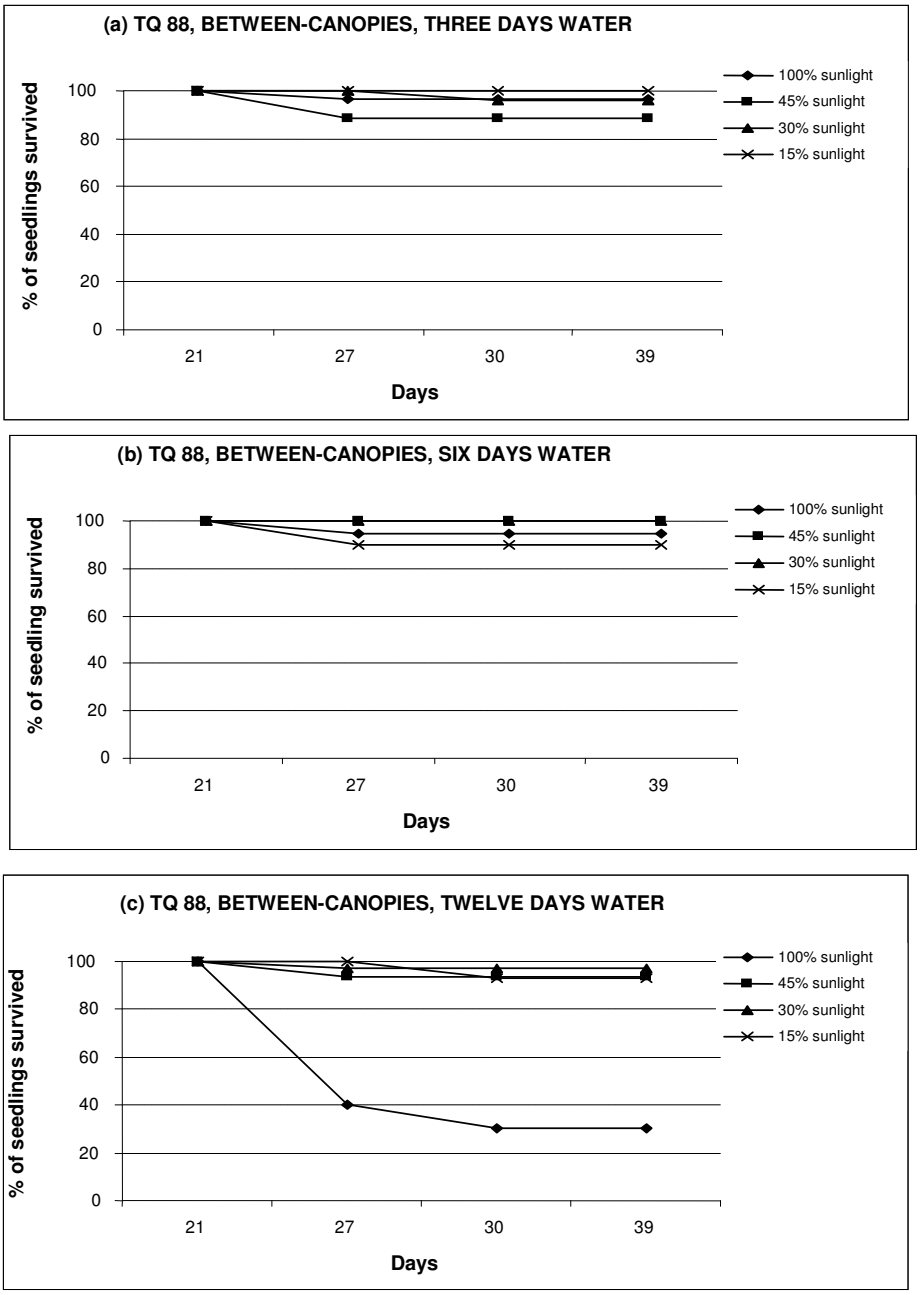


Figure 5.11 Percentage of seedling survival curves of *D. leptophyllus* TQ 88 in soil from between-canopies, at four levels of light incidence, and three different watering regime intervals.

100% = Emergence/survival curve of plants growing at 100% of full sunlight
45% = Emergence/survival curve of plants growing at 45% of full sunlight
30% = Emergence/survival curve of plants growing at 30% of full sunlight
15% = Emergence/survival curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days

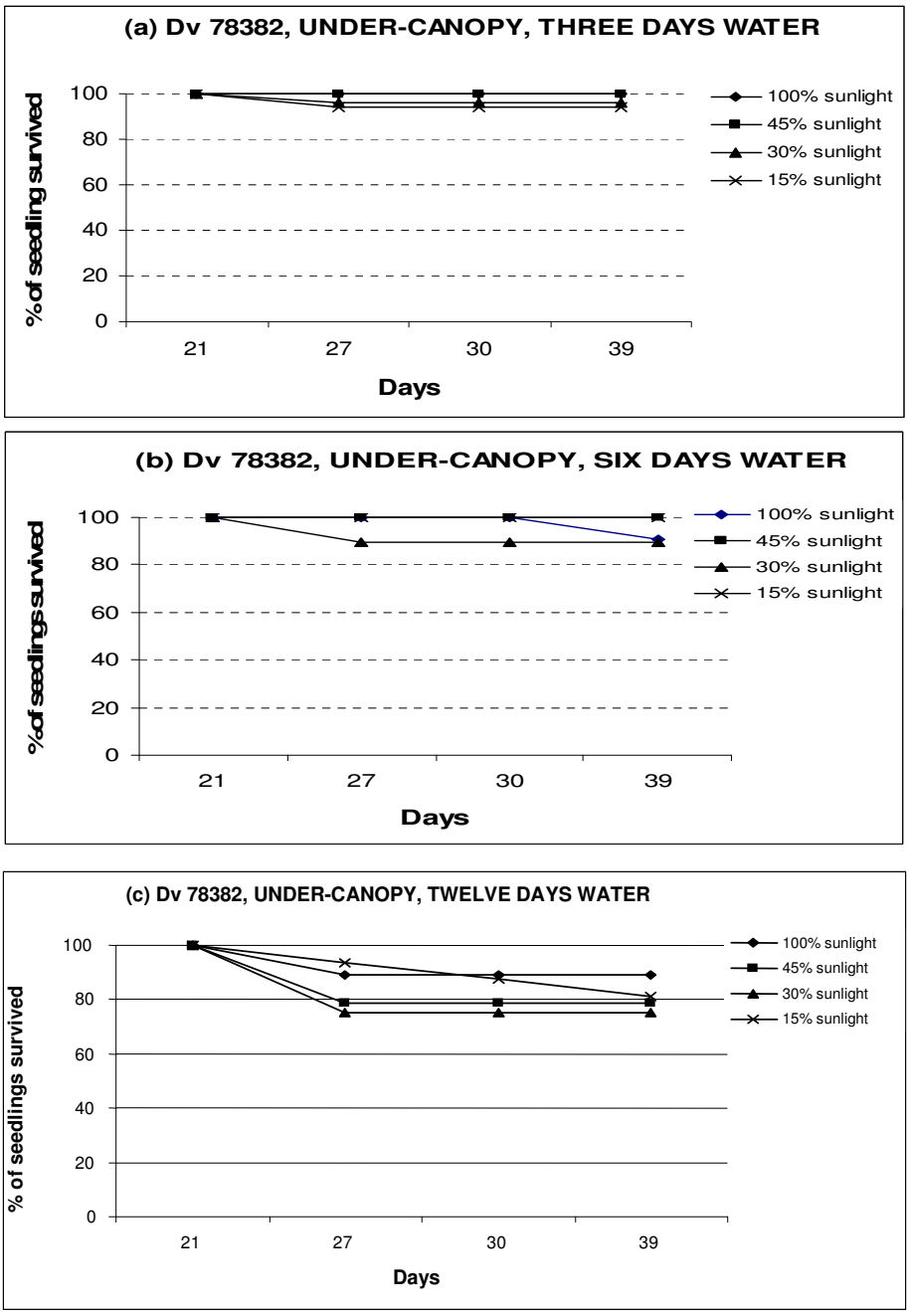


Figure 5.12 Percentage of seedling survival curves of *D. virgatus* CPI 78382 in soil from under-canopy, at four levels of light incidence, and three different watering regime intervals.

100% = Emergence/survival curve of plants growing at 100% of full sunlight
45% = Emergence/survival curve of plants growing at 45% of full sunlight
30% = Emergence/survival curve of plants growing at 30% of full sunlight
15% = Emergence/survival curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days

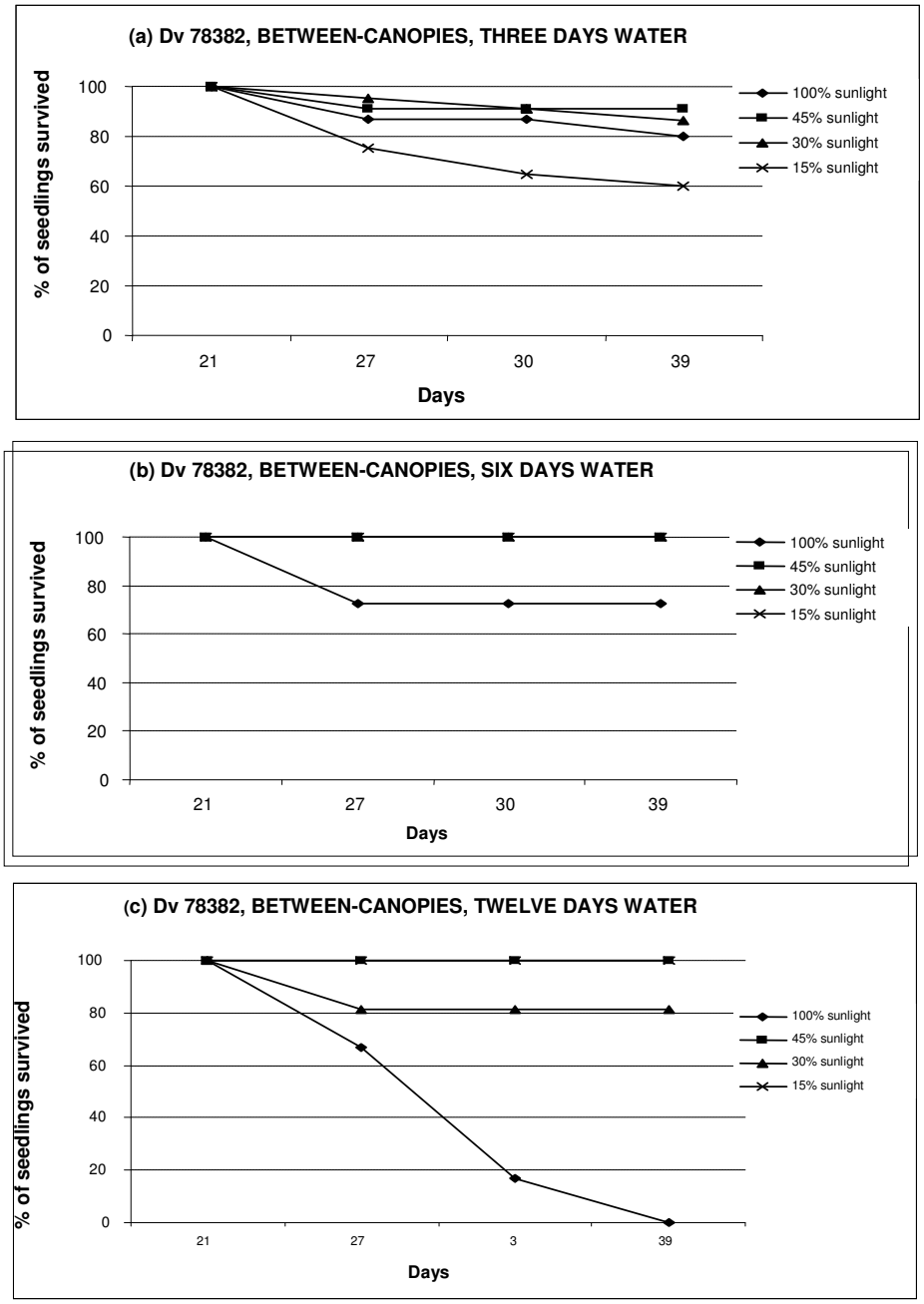


Figure 5.13 Percentage of seedling survival curves of *D. virgatus* CPI 78382 in soil from between-canopies, at four levels of light incidence, and three different watering regime intervals.

100% = Emergence/survival curve of plants growing at 100% of full sunlight
45% = Emergence/survival curve of plants growing at 45% of full sunlight
30% = Emergence/survival curve of plants growing at 30% of full sunlight
15% = Emergence/survival curve of plants growing at 15% of full sunlight

(a) Watering interval of 3 days
(b) Watering interval of 6 days
(c) Watering interval of 12 days

A summary of the effects of the main treatments compared by LSD ($p < 0.05$) is presented in Table 5.5. Shade enhanced dry matter production of plant shoots, where the seedlings grown in full sunlight yielded around 50% of the shoot dry matter obtained from the shaded treatments (Table 5.5). As the shade x watering frequency interaction was not statistically significant and the effect relatively small (Table A.2.15), it seems that shade increased shoot dry matter, regardless of its effect on water loss reduction. There is a dearth of information on this aspect of legume growth in the literature. Shade is frequently suggested as having a negative effect on dry matter production of tropical forage legumes (Ludlow 1980; Wong and Wilson 1980; Eriksen and Whitney 1981; Chen 1989; Shelton and Stur 1991). From thirty-two accessions of ten genera studied by Stur (1991) and Wong (1991), only *Calopogonium caeruleum* and *Desmodium ovalifolium* had higher dry matter at 50% sunlight than at 100% sunlight, and only *D. ovalifolium* did not have its yield depressed by shading to 20% of full sunlight.

The effect of the origin of the soil on shoot dry matter yield was even greater than the shade effect noted above. Indeed, seedlings grown in soil collected from under the tree canopies yielded as much as three times the dry matter of shoots than did seedlings grown in the soil from between the tree canopies (Table 5.5).

This last fact reflects the higher fertility of soils formed under eucalypt canopies (Polglase *et al.* 1992) than of soils from between canopies, as was shown above (section 4.3.1).

D. leptophyllus TQ 88 produced higher yields than *D. virgatus* CPI 78382 on both soils, with the former out-yielding the latter under all the conditions of shade and watering (Table 5.5). Shoot dry matter decreased significantly as time between watering increased (Table 5.5). The mean yield of shoot dry matter under twelve day watering intervals was less than 40% of that under three day watering interval (Fig. 5.14).

Table 5.5 Shoots: Pairwise comparisons of means of individual dry mass of shoot (mg/plant) by shade, soil, watering frequency, and genotype by LSD ($p < 0.05$)

Shade		Soil		Watering frequency		Genotype	
% of full sunlight	Dry mass	Origin	Dry mass	Days	Dry mass	Name	Dry mass
100 %	17.81 (51)* c	Under	44.39 (100) a	3	43.49 (100) a	TQ 88	34.42 (100) a
45 %	35.08 (100) a	Between	14.15 (32) b	6	28.31 (85) b	78382	24.12 (70) b
30 %	33.90 (97) ab	--	--	12	16.02 (37) c	--	--
15 %	30.30 (86) b	--	--	--	--	--	--
LSD($p < 0.05$)	4.74	--	3.63	--	3.84	--	3.85

Means in the same column followed by the same lower case letter are not significantly different by LSD ($p<0.05$)

** Numbers in parenthesis indicate the relative dry matter yield percentage whichwas a value of 100% for the highest value in the column

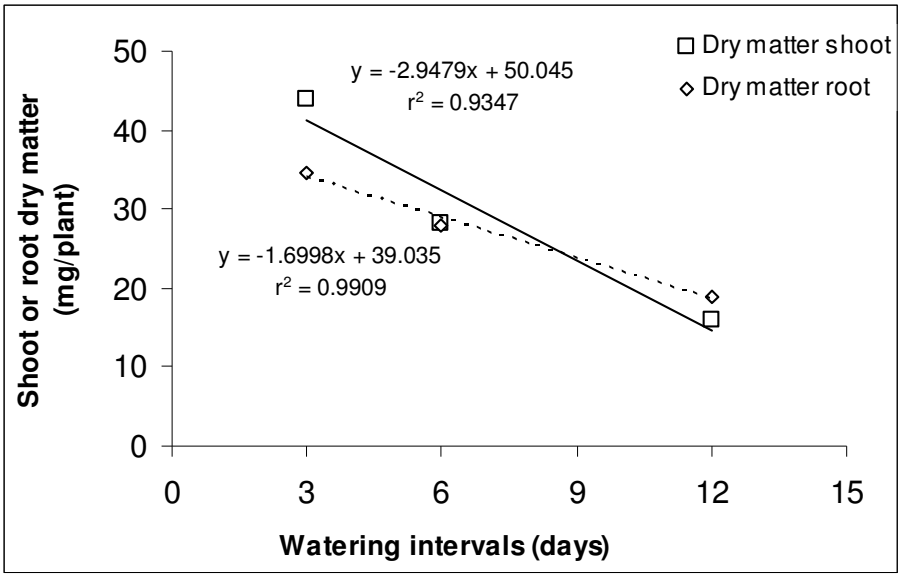


Figure 5.14 Linear regressions for the effect of watering interval on the shoot and root dry matter yields of *Desmanthus*, averaged for four levels of shade, two soils, and two genotypes (n= 48).

5.3.2.4.2 Root Dry Matter

Seedling root dry matter was significantly affected by the four main factors and by the combined effects of shade x watering regime, shade x genotype, and shade x soil x watering regime x genotype (Table A.2.17).

Seedlings produced well developed root systems under medium levels of light interception, but at 15 % light transmission, root dry matter did not differ significantly from the root mass obtained at full sunlight (Table 5.6). At these extreme levels of light and shade, root dry mass was about one half of that obtained under the intermediate levels of shade. High levels of shade (15% of light transmission) restricted the growth of root systems of both genotypes, regardless of the watering frequency (Table 5.6).

Despite the statistically significant differences in root dry mass obtained between soils and between genotypes, these factors exerted only a small effect on the variation of such data (Table 5.6). Root weights linearly decreased as the intervals between watering increased (Fig. 5.14; Tab. A.2.18).

Table 5.6 Roots: Pairwise comparisons of means of individual dry mass of root (mg/plant) by shade, soil, watering frequency, and genotype by LSD ($p < 0.05$).

Shade			Soil			Watering frequency			Genotype		
% of full sunlight	Dry mass		Origin	Dry mass		Days	Dry mass		CPI	Dry mass	
100 %	22.16 (63)*	b	Under	29.47 (100)	a	3	34.50 (100)	a	TQ 88	29.22 (100)	a
45 %	33.74 (96)	a	Between	24.81 (84)	b	6	27.99 (81)	b	78382	25.06 (86)	b
30 %	35.04 (100)	a	--	--		12	18.92 (55)	c	--		
15 %	17.61 (50)	b	--	--		--	--		--		
LSD ($p< 0.05$)	10.36		--	3.15		--	3.12		--	3.78	

Means in the same column followed by the same lower case letter are not significantly different by LSD ($p<0.05$)

** Numbers in parentheses indicate the relative dry matter yield percentage which was a value of 100% for the highest value in the collum

5.3.2.4.3 Biomass allocation between shoots and roots

Scatter plots of dry matter distribution of shoot and root, against total dry matter, as proposed by Browel (1962) and Burt (1968), were used to represent biomass allocation of *D. leptophyllus* TQ 88 and *D. virgatus* CPI 78382, growing under the different shade, soil, and watering treatments (Figs. 5.15 and 5.16). Seedlings of both genotypes, regardless of the level of shade and watering frequency, had different patterns of biomass allocation to shoots and roots in the different soils (Figs 5.15 and 5.16). Plants allocated most of their dry matter to the production of aerial, rather than to underground parts when grown in soil collected from the under-canopy environment. The opposite was true, however, with plants grown in soil from the between-canopies environment; here the larger proportion of the total dry matter was diverted to the underground parts. Reducing the data from Figs. 5.15 and 5.16 to the means of the shoot/root ratios (Table 5.7), a significantly higher value for the shoot/root ratio was obtained for plants growing in soil from under the canopy than between-canopies.

A model to explain how the shoot/root ratio may be controlled by the level of light has been proposed by Wilson (1988). According to this model, plants grown under increasing shade invest more carbon into maintaining leaf area through an increase in shoot/root ratio as if in an attempt to compensate for the decline in incident radiation. This model, however, may not explain the reduction in shoot/root ratio observed in the present study for plants growing in soils from between the canopies. Lower levels of nutrients in soil from between-canopies (section 4.3.1) compared with soils from under-canopies, may better explain such a result. Contrary to the implications of the Wilson (1988) model, plants of the present study exposed to low contents of nutrients in the soil invested more carbon into roots, possibly to explore a greater soil volume in their quest for scarce plant nutrients.

The increase in the shoot/root ratio with increasing shade as a separate factor was also observed in the present study (Table 5.7) and is in accord with the general trend found by Wong *et al*. (1985b) for 14 tropical legumes.

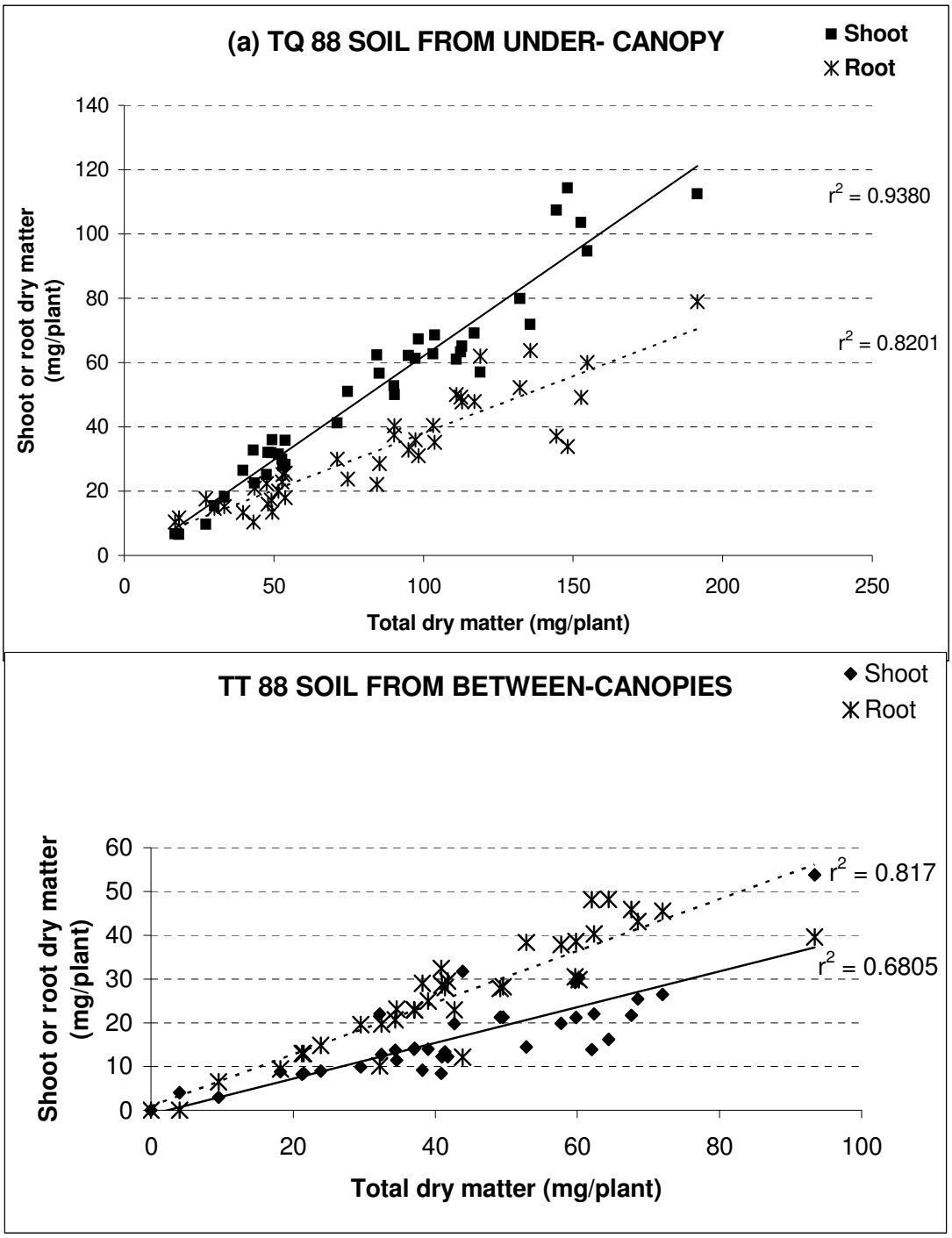


Figure 5.15 Scatter plot distribution of shoot and root dry matter of *D. leptophyllus* TQ 88, in relation to total shoot or root dry matter.

(a) Soil from under tree canopy
(b) Soil from between tree canopies

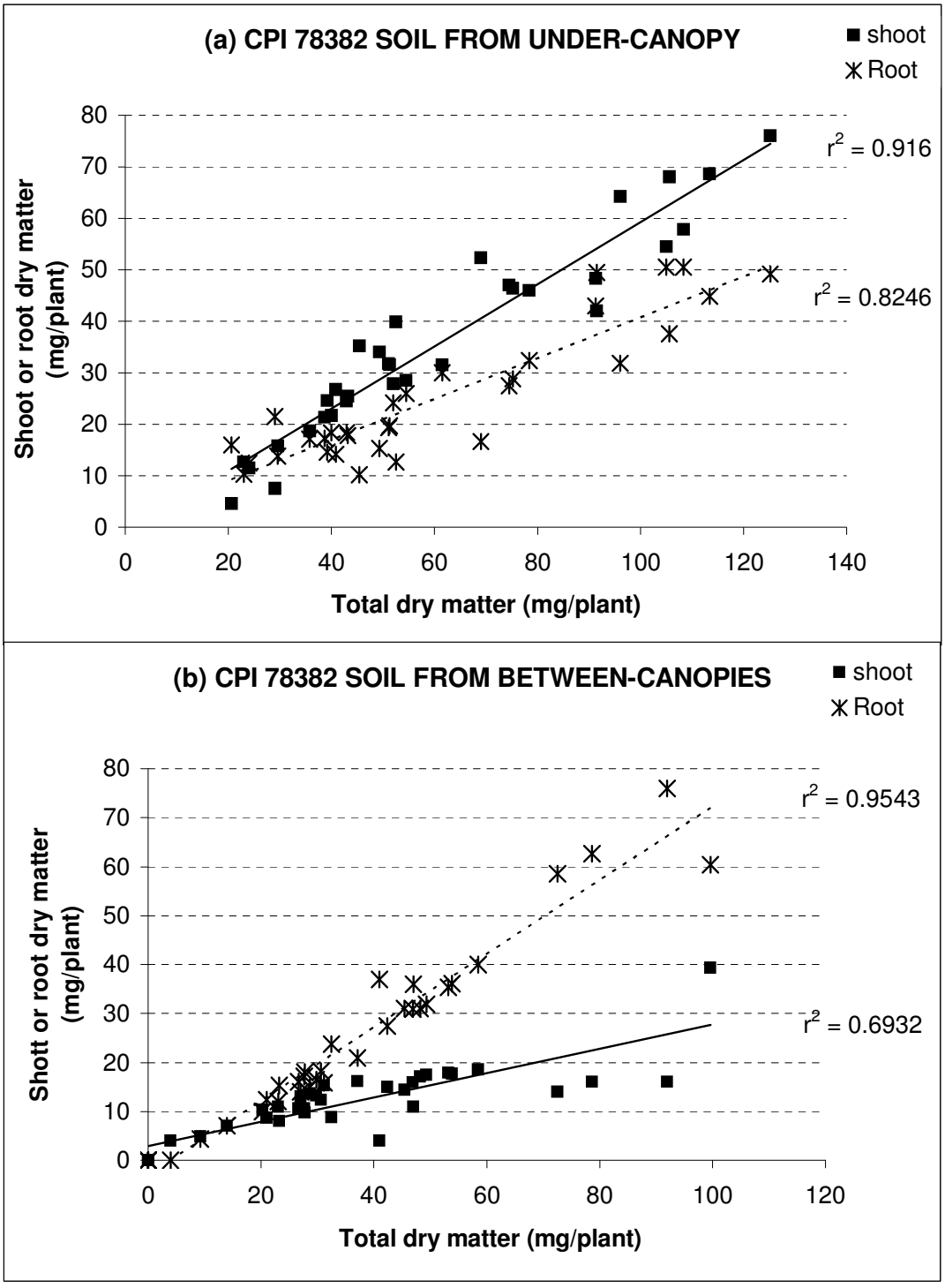


Figure 5.16 Scatter plot distribution of shoot and root dry matter of *D. virgatus* CPI 78382, in relation to total shoot or root dry matter.

(a) Soil from under tree canopy
(b) Soil from between tree canopies

Table 5.7 LSD pairwise comparisons of means of individual shoot/root dry matter ratio by shade, soil, watering and genotype.

Shade		Soil		Watering frequency		Genotype	
% of full sunlight	Dry matter	Origin	Dry matter	Days	Dry matter	CPI	Dry matter
100 %	0.80 b	Under	1.51 a	3	1.26 a	TQ 88	1.18 a
45 %	1.04 b	Between	0.57 a	6	1.01 b	78382	0.96 b
30 %	0.97 b	--		12	0.85 b	--	--
15 %	1.72 a	--		--	--	--	--
LSD ($p < 0.05$)	0.46	--	0.1	--	0.17	--	0.09

Means in the same column followed by the same lower case letter are not significantly different by LSD ($p < 0.05$)

There was also a genotypic effect in that *D. leptophyllus* TQ 88 allocated more dry matter to shoot growth than did *D. virgatus* CPI 78382 (Table 5.7). In two instances, with TQ 88 growing in the under-canopy soil, and with CPI 78382 in the between-canopies soil, the regression lines crossed each other indicating that root and shoot growth of the respective genotypes were favoured at low levels of dry matter production (Figs 5.15 and 5.16).

5.3.2.4.4 *The interactive effect of treatments on shoot and root dry matter*

The interactive effect of watering regimes and level of light interception, on shoot and root dry matter yield of *D. leptophyllus* TQ 88 and *D. virgatus* CPI 78382, growing on soils from under-and between-canopies is presented in Figs. 5.17 to 5.20.

When exposed to full sunlight, shoots and roots of TQ 88 were significantly reduced as the time between watering increased from 3 days to 12 days. Plants of TQ 88, when grown in soil from between the canopies produced less shoot than root (Tables 5.5 and 5.6). When the plants in full sunlight were watered at 12 day intervals, shoot and root yields from plants grown in the soil collected from between the canopies were reduced almost to zero (Figs 5.17 and 5.18). Watering frequency, however, was not such a limiting factor on shoot and root growth of shaded plants, despite occasional reductions observed at the 12 day watering intervals (Figs. 5.17 and 5.18).

Such a beneficial effect of shade on the maintenance of seedling growth under restricted soil water supplies has not previously been noted for tropical forage legumes.

Significantly higher shoot dry matter yields of plants of CPI 78382, under the 6 days watering interval, relative to 3 and 12 days intervals occurred in plants grown in soil collected from between-canopies, for all levels of effective shade, but not in full sunlight (Fig. 5.19). Such a response was also observed for root dry matter at 30% of light transmission (Fig. 5.20).

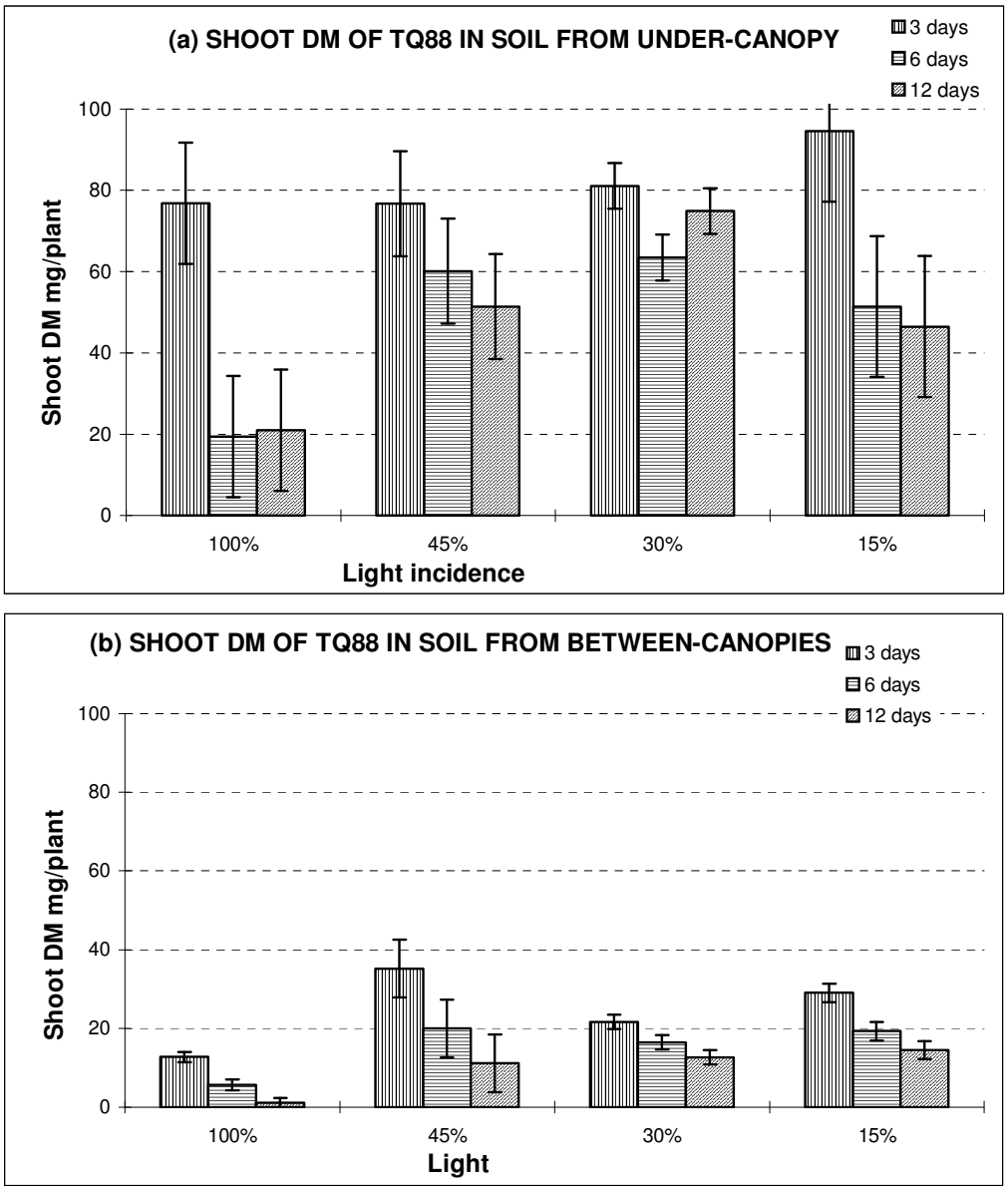


Figure 5.17 Effect of watering intervals (3, 6, and 12 days) on the dry matter yield of shoot (mg/plant) of *D. leptophyllus* TQ 88 grown on soil from under and between-canopies, submitted to four levels of shade.

(a) Plants grown in soil from under-canopy
(b) Plants grown in soil from between-canopies

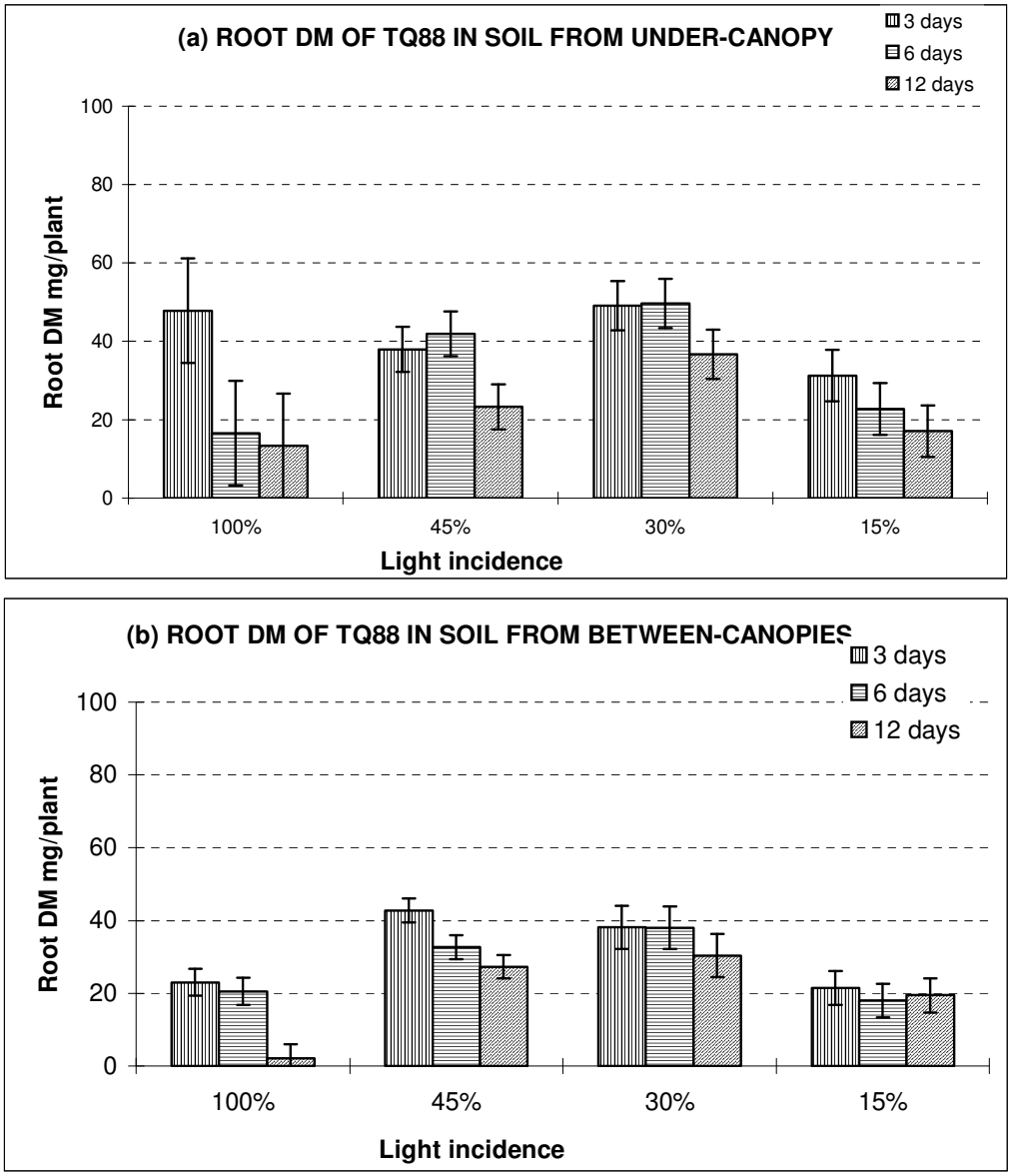


Figure 5.18 Effect of watering intervals (3, 6, and 12 days) on the dry matter yield of root (mg/plant) of *D. leptophyllus* TQ 88 grown on soil from under and between-canopies, submitted to four levels of shade.

(a) Plants grown in soil from under-canopy
(b) Plants grown in soil from between-canopies

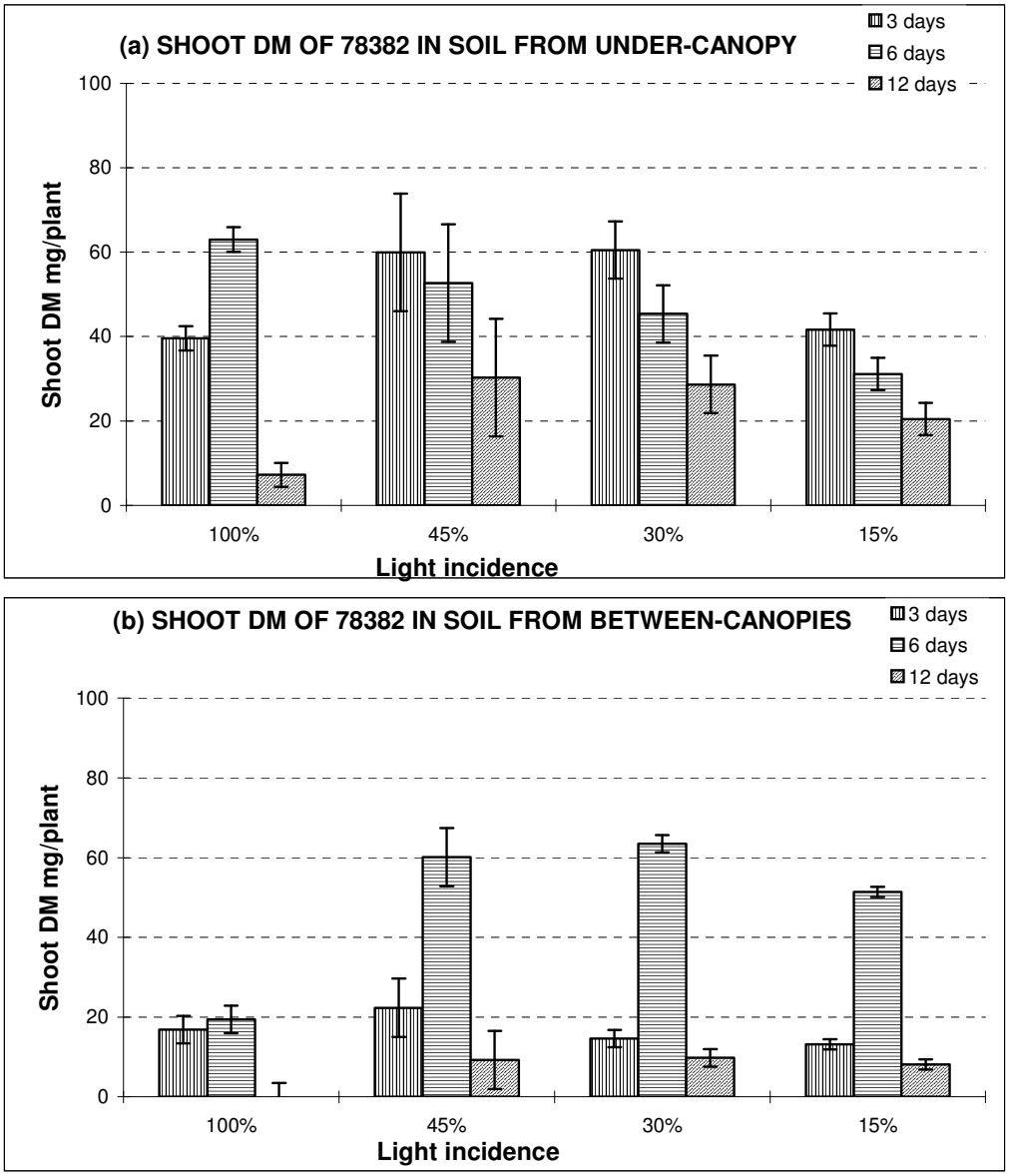
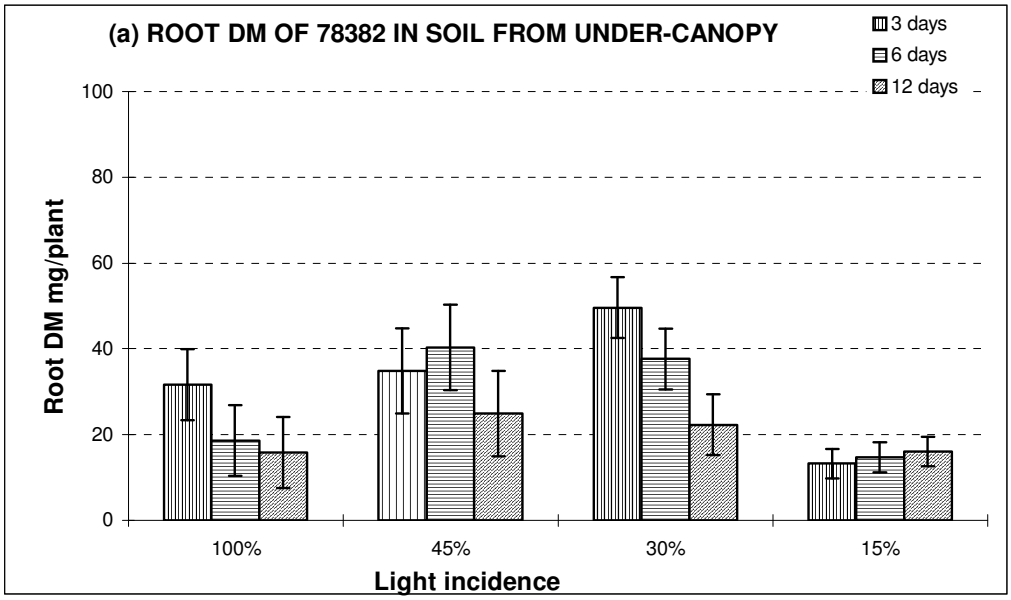


Figure 5.19 Effect of watering intervals (3, 6, and 12 days) on the dry matter yield of shoot (mg/plant) of *D. virgatus* CPI 78382 grown on soil from under and between-canopies, submitted to four levels of shade.

(a) Plants grown in soil from under-canopy
(b) Plants grown in soil from between-canopies



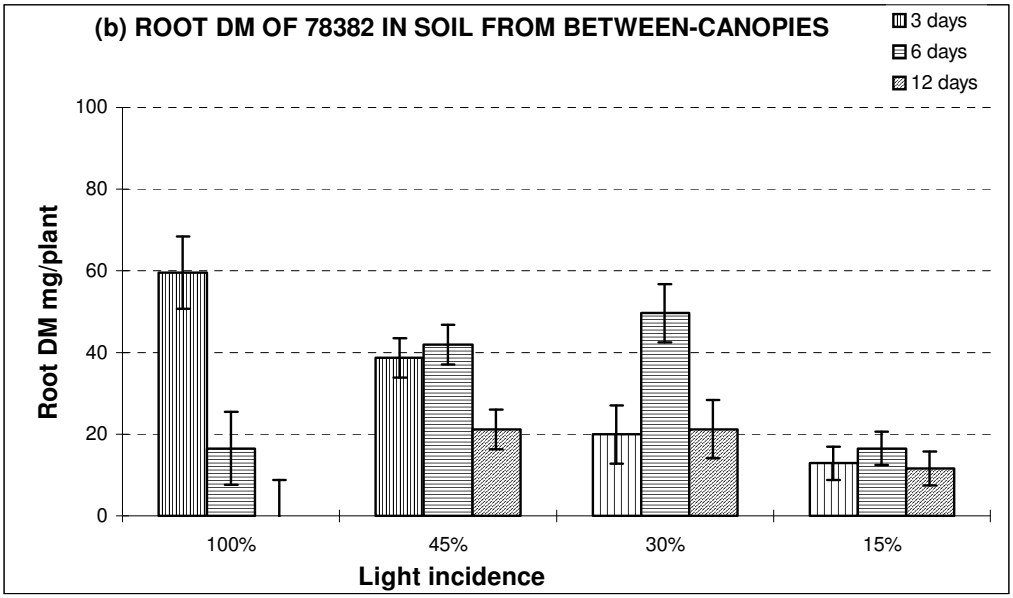


Figure 5.20 Effect of watering intervals (3, 6, and 12 days) on the dry matter yield of root (mg/plant) of *D. virgatus* CPI 78382 grown on soil from under and between-canopies, submitted to four levels of shade.

(a) Plants grown in soil from under-canopy
(b) Plants grown in soil from between-canopies

Poor growth was observed in *D. virgatus* CPI 78382, on soil from under-canopy, at full sunlight, and watered every 12 days, but the plants survived. On the other hand, shoot and root dry matter of CPI 78382 in full sunlight, on soil from between-canopies and watered every 12 days, was zero, as the plants died. This is important agriculturally in that droughts of 12 days or longer commonly occur at the start of the growing season in tropical environments.

5.3 Discussion

Before seed sowing, evaporation and deep drainage were the only ways in which water escaped from the soil in the experimental pots (section 5.3.1.1), and the level of shading was the major factor governing water loss from the soil at this stage; a reduction of incident sunlight reduced water loss 14 days after irrigation to one-thirteenth of that recorded under full sunlight (Fig. 5.2 and Table 5.1). Similar reductions in water losses were found in soils under the canopy of an *Acacia cavens* plantation by Ovalle and Avendano (1987), and *Acacia tortilis* and *Adansonia digitata* by Belsky *et al.* (1989, 1993a; b). The strong water retention by soils in pots in shaded treatments may explain the significantly superior seedling emergence observed later in these soils relative to those in a full sunlight.

Under field conditions, evaporation from the top 2 cm of the soil, where most of the seeds had been sown, can occur quickly during the intervals between rainfall or irrigation events, unless some soil cover such as mulch, a tree canopy, or a herbaceous stratum is present. A live herbaceous stratum, however, can intensify rather than reduce water losses from the soil layer by plant transpiration under some circumstances (Belsky *et al.* 1989, 1993a, b). Embryo desiccation and consequent germination failure would certainly result under such conditions.

Some of the physical characteristics of the soil such as its bulk density and natural permeability would have been changed during the process of soil collection and pot filling. In the present study, such changes appear to have had little effect on water evaporation; it was only 10 % higher in the between-canopies soil compared with that from under the canopy, in both heavily shaded and full sunlight treatments (Fig.

5.2). Such a reduction in evaporation from soil from under the canopies was clearly not sufficient to cause differences in seedling emergence, which was similar in both soils when the other treatments taken into consideration and were averaged (Section 5.3.2.2).

When plants started to grow and evapotranspiration conditions became established (section 5.3.2.1), shade was not as effective in reducing water loss from the soil (Figs. 5.3, 5.4 and 5.5) as it was when only evaporation was involved (Section 5.3.1.1, Fig. 5.2 and Table 5.1); this was especially so when water was applied at intervals longer than 6 days. However, the different soil and shading treatments still affected seedling survival when soil moisture levels were very low (section 5.3.2.3). Under a 12 day watering frequency, for instance, seedling mortality was very high for both genotypes when grown in the between-canopies soil and exposed to full sun (Figs 5.11 and 5.13); very few seedling deaths were observed, however, when the soil was shaded regardless of the watering frequency, the genotype, or the origin of the soil (Figs 5.10 to 5.13). It would appear that, under full sunlight and even high soil moisture, desiccation causing wilting that accompanied by high leaf temperatures led to seedling death. Leaf temperatures were not measured in the present study. From the works of Wong and Wilson (1980), Eriksen and Whitney (1982), Wong *et al.* (1985b), Barrios *et al.* (1986), and Wilson and Wild (1991a; b), it is presumed that plants shaded by shade-cloth would have lower leaf temperatures than those exposed to full sunlight. Similarly, Wilson and Ludlow (1991) observed that 5% shading lowered the temperatures of soil and leaf litter in a pasture by some 12-15%, although air temperatures were barely altered above the pasture. The reasons for improved survival of seedlings grown in the more fertile soil taken from under the tree canopies are not totally clear and the temperature effect warrants further study.

In the present study, seedlings of both genotypes produced more dry matter being grown in the under-canopy soil than in the between-canopies soil, regardless of shade or watering treatments (Tables 5.5 and 5.6). Bearing in mind that the water holding capacity was not different between the two soils (session 5.3.2.1), different nutrient levels in the two soils were clearly the only component able to promote the differences found in plant dry matter yields (Tables 5.5 and 5.6). Higher levels of

nitrogen and phosphorus in the soils collected under the tree canopy than in the soils collected from between the canopies were also shown by Eriksen and Whitney (1981),

Sophanadora (1989), and Durr and Rangel (2000), to be responsible for the higher dry matter yields obtained from herbaceous plants cultivated in under-canopy soils. Increases in the water use efficiency of plants in response to higher contents of nutrients, especially nitrogen in under-canopy soils, was suggested by Durr and Rangel (2000) as the mechanism by which plants growing in such soils could maximise photosynthesis and increase their dry matter yields.

Shoot and root growth were similarly affected by levels of shade (Table 5.7, Figs. 5.17 to 5.20). Yields of both plant parts were significantly higher at 45% and 30% of light incidence than at full sunlight and under heavy shade with only 15% of light transmission, regardless of the soil, watering, or genotype treatments. The shoot and root yields of the two studied genotypes of *Desmanthus,* under increasing levels of light interception, agree with the general trend found by Wong *et al.* (1985b), for the tropical forage legumes *Calopogonium mucunoides*, *Calopogonium caeruleum* and *Desmodium ovalifolium*, which had an optimal yield at 43% of light incidence in relation to full sunlight. Eleven other forage legumes included in the trial of Wong *et al*. (1985b) which did not include *Desmanthus* genotypes, produced lower yields and shoot/root ratios, under increasing shade levels.

Despite a general trend for decreasing shoot/root ratios with increasing shade, plant biomass allocation was higher for shoots than for roots for plants growing in soil from under the canopy, and higher for roots than shoots in soil from between-canopies for both genotypes (Figs 5.15 and 5.16).

Although further study is required to explore results presented in this chapter, it is clear firstly, that legumes of the genus *Desmanthus* can be grown under trees in dry tropical conditions, and even in drought. This is very important for the establishment of silvipastoral systems in shaded tropical environments. Secondly, genotypes vary in their ability to grow under dry conditions.

Chapter 6

PASTURE ESTABLISHMENT STRATEGIES FOR *DESMANTHUS* GENOTYPES

6.1 Introduction

In Chapters 4 and 5, the growth differences in tropical legumes of the genus *Desmanthus* have been reported from microhabitats under and between tree canopies. In the present chapter the focus is on the competitive interference between genotypes of *Desmanthus* and other tropical pasture plants, disturbance by external factors, and interactive responses to stress, competition, and disturbance, from the early stages of plant establishment.

Erratic wet seasons and heavy grazing in semi-arid tropical areas produce marked changes in pasture growth and composition. Knowledge of the individual characteristics of **cohabitant species**, their responses to environmental stresses, their abilities to compete for the capture of available resources, and their responses to external disturbance are important for the sustainable management of such pastures. Noble (1986) suggested that an effective way to develop more sensitive and precise management systems for tropical rangelands is through an accurate description of what controls seedling recruitment, plant survival, and plant death.

Plant populations in tropical pastures show large variations in plant architecture, phenology, life span, growth rate, and growth habit of the cohabitant species. Such variations have been shown to be related to the competitive ability of the individuals (Harper 1977d; Grime 1979; Warwick and Briggs 1980; Gardener and McIvor 1986; Reader *et al*. 1992). Seed size and the quantity of nutrient reserves stored in perennial organs (Grime 1979) may also affect the competitive ability of seedlings and established plants, respectively. Coexistence of species is the result of different life-forms or patterns of seasonal development, and complementarity of resource pattern use by the species (Veblen 1992).

Sarmiento (1984), Silva (1987), and Raventós and Silva (1995) showed that differences in the architecture and temporal asynchrony of vegetative and reproductive phenological patterns explained the coexistence of some neotropical savanna grass and forb species. Turkington (1975), Turkington *et al*. (1977), and Turkington and Harper (1979) suggested that individual legumes related to particular combinations of grass species in grass/legume pastures, are selected through the ability of the combinations of plants to utilise the soil environment more efficiently than simply random combinations of species. Raventós and Silva (1988) found that erect growth forms were competitively superior over prostrate growth forms, since their effects on other species were strongest, and their responses to other species were weak.

Regeneration from underground organs confers on *Desmanthus virgatus* CPI 78373 a great ability to compete with aggressive grasses on heavy clay soils of north-eastern Queensland (Clem and Hall 1994). Severe competition for water by the grass *Panicum maximum* var. *trichoglume* cv. Petrie, excluded the legume *Macroptilium atropurpureum* cv. Siratro and *Neonotonia wightii* cv. Cooper from a pasture on a **brigalow** clay soil after the second year of sowing (Silvey and Jones 1990).

The possession of some competitive attributes by a plant seems, however, not to be a helpful feature for all occasions. Preferential capture of environmental resources presented to a plant at sites with good reserves of plant nutrients and water might assure its dominance in such a habitat but, may be disadvantageous in poorer habitats (Berendse *et al.* 1992). An erect habit, which leads to a high competitive ability under nutrient-rich conditions, may adversely affect the competitive ability of plants in nutrient-poor grassland ecosystems (Berendse *et al.* 1992).

Desmanthus is a recently introduced pasture legume genus in Australia. The success of its genotypes to form legume-based pastures in the tropical rangelands of northern Australia will depend on their abilities to compete with both the native plants and the widespread and well adapted, introduced pasture plants.

The crucial phase of this competition is at seedling establishment, where young plants of *Desmanthus* have to face a strong competition for light, water, and nutrients with the local plant community. The following experiment was carried out to explore the ability of some genotypes of *Desmanthus* to establish from seeds or as transplanted seedlings into a typical tropical pasture plant community of north Australia, in response to different introduction strategies.

6.2 Materials and methods

6.2.4 Study site

The work discussed in this chapter was carried out in a wire netting fenced area of sown pasture on the campus of James Cook University, Townsville (site 4 of Fig. 2.3), and described in section 2.2 above.

6.2.2 Experimental design and procedures

The experiment was set out as a split-split-plot randomised block design (Kempthorne 1979). Areas where grasses and forbs had been killed by paraquat (herbicide-treated plots), and untouched vegetation (undisturbed plots), constituted the main plots (4.0 x 7.0 m). The introduction of *Desmanthus* by seed or as transplanted seedlings represented the sub-plots (2.0 x 7.0 m). Six genotypes, *Desmanthus virgatus* CPI 79653 and CPI 78382, *Desmanthus pubescens* CPI 92803, *Desmanthus leptophyllus* CPI 38351, and TQ88 and *Desmantus bicornutus* CPI 91162, were planted in rows and represented the sub-sub-plots (Fig. 6.1).

6.2.3 Field methods

Paraquat was sprayed over the vegetation on the herbicide-treated plots on 02 June 1992 at a rate of 60 mL per 20 L of water. *Desmanthus* seedlings were raised in a shade house at CSIRO Davies Laboratory, Townsville, from 13 March 1992 to 09 June 1992. On 10 June 1992 eight seedlings of each of the *Desmanthus* genotypes

BLOCK I

NOT DISTURBED		PARAQUAT	
SEEDLING	SEED	SEED	SEEDLING
TQ 88	CPI 92803	CPI 78382	TQ 88
CPI 79653	TQ 88	CPI 38351	CPI 78382
CPI 91162	CPI 79653	CPI 92803	CPI 91162
CPI 92803	CPI 38351	TQ 88	CPI 92803
CPI 78382	CPI 78382	CPI 79653	CPI 79653
CPI 38351	CPI 91162	CPI 91162	CPI 38351

BLOCK II

NOT DISTURBED		PARAQUAT	
SEED	SEEDLING	SEED	SEEDLING
CPI 38351	CPI 79653	CPI 91162	TQ 88
CPI 78382	TQ 88	CPI 78382	CPI 38351
CPI 92803	CPI 38351	TQ 88	CPI 91162
CPI 79653	CPI 91162	CPI 38351	CPI 79653
TQ 88	CPI 78382	CPI 92803	CPI 92803
CPI 91162	CPI 92803	CPI 79653	CPI 78382

BLOCK III

PARAQUAT		NOT DISTURBED	
SEEDLING	SEED	SEEDLING	SEED
TQ 88	CPI 38351	CPI 38351	CPI 78382
CPI 91162	CPI 78382	TQ 88	CPI 92803
CPI 38351	CPI 92803	CPI 91162	CPI 79653
CPI 79653	CPI 79653	CPI 92803	CPI 91162
CPI 92803	TQ 88	CPI 78382	TQ 88
CPI 78382	CPI 91162	CPI 79653	CPI 38551

BLOCK IV

PARAQUAT		NOT DISTURBED	
SEEDLING	SEED	SEEDLING	SEED
CPI 79653	CPI 92803	CPI 78382	CPI 79653
CPI 78382	CPI 91162	CPI 38351	CPI 92803
TQ 88	CPI 78382	CPI 79653	TQ 88
CPI 91162	CPI 79653	TQ 88	CPI 91162
CPI 92803	CPI 38351	CPI 91162	CPI 38351
CPI 38351	TQ 88	CPI 92803	CPI 78382

Figure 6.1 Field spatial distributions of the applied treatments of vegetation management and introduction method on six *Desmanthus* genotypes. “Paraquat” indicates that the pre-existing vegetation of the block had been killed by herbicide before planting the *Desmanthus* genotypes.

were transplanted, or 50 seeds that had been scarified with concentrated sulphuric acid to break dormancy were sown, into 2 m long rows that were 1 m apart. Seedlings and seeds were spaced 25 cm apart along each row. Because of the absence of rain during almost all the experimental period, sprinkler irrigation was used twice a week for three hours, representing the application of 20 mm of water per week.

The number of emerged seedlings was counted on 26 July 1992 (14 days after sowing). The total number of surviving seedlings was counted at approximately monthly intervals for the 8 months of the experiment. Immediately after the last plant survival count, the top parts of all of the plants were clipped at 10 cm above the soil. Leaves and stems of all of the plants in the same row were placed in a paper bag and dried at 65^0 C in a forced draft oven. After drying, each sample was weighed and the mass divided by the number of surviving plants in that specific row to obtain the individual, above ground, plant dry matter yield.

6.2.4 Statistical analysis

The Kaplan-Meier Survival Analysis of SPSS Advanced Statistics 6.1 (1994) statistical computer program was used to analyse the individual behaviour of genotypes within the treatments, and the treatment behaviour within genotypes.

Long Rank, Breslow, and Tarone-Ware tests in "SPSS 6.1" were used to test equality of survival distribution for genotypes and treatments. The effect of disturbance (herbicide), plant establishment method (seeds or seedlings), genotype, and their possible interactions on initial germination (data on seedling emergence as a percentage of sown seeds) and on individual dry matter yields of plant [the data were transformed into $\sqrt{(x+1)}$ form], were analysed by the General ANOVA procedure of "Statistics 4.1" computer program. The transformed data means were compared by the LSD Pairwise Comparison test ($p < 0.05$).

6.3 Results

6.3.1 Seedling emergence

An analysis of variance showed that the emergence of seedlings from sown seeds was significantly affected by genotype but was not altered by the disturbance treatments (Table A.2.19)**.** Considering seedling emergence in relation to seed potential to germinate, regardless of the disturbance treatment, the 6 genotypes were classified into three groups (Table 6.1): Group 1- comprising *D. bicornutus* CPI 91163 and *D. leptophyllus* CPI 38351, where emergence was about 60% of their potential germination, which means the percentage of emerged seedlings related to the percentage of germinable seeds, obtained in the germination test (Table 6.1); Group 2 - comprising *D. leptophyllus* TQ 88 and *D. pubescens* CPI 92803, where emergences were 40-45% of their potential germination; and Group 3 - comprising *D. virgatus* CPI 79653 and CPI 78382, whose emergences were about 20 – 30% of their potential germination. These results appear to reflect differences in plant type and seed size and their relation to seedling emergence. The Group 1 plants, *D. bicornutu*s CPI 91162 and *D. leptophyllus* CPI 38351, are fast growing, suffruticose shrubs with large seeds and had the highest emergence rate. Genotypes in Group 2 with intermediate emergence rates are erect shrubs but with slower growth than *D. bicornutus* CPI 91163 and *D. leptophyllus* CPI 38351, and all have large seeds. The components of Group 3, both showing very low emergence rates, are herbaceous or semi-herbaceous plants with small seeds. Such a general classification has the potential to be extremely useful in deciding sowing rates in the field.

Table 6.1 LSD pairwise comparisons of means of germination (%) by genotype.

Genotype	Mean
D. bicornutus CPI 91162	53.68 a
D. leptophyllus CPI 38351	44.43 ab
D. pubescens CPI 92803	34.31 bc
D. leptophyllus TQ 88	31.61 bc
D. virgatus CPI 79653	18.00 cd
D. virgatus CPI 78382	12.92 d

Means followed by the same letter are not significantly different by LSD ($p < 0.05$)

6.3.2 Seedling survival

The Kaplan-Meier Survival Analysis and the Long Rank, Breslow, and Tarone-Ware Statistical Tests (SPSS Advanced Statistics Version 6.1 1994) for equality of survival distribution of genotypes, for the two levels of vegetation disturbance and plant introduction treatments, are shown in Tables A.2.20 and A.2.21. Survival of genotypes did not differ statistically in any of the four possible interactions in the three tests applied (Table A.2.20). Such statistical tests compare the entire survival curve, rather than any isolated points on it (as does a normal test of means which normally uses only the last point for comparison). The lack of significant differences is to be expected because of the close percentage of censored cases for genotypes within each treatment (Table A.2.20).

The survival curves resulting from the possible combinations of disturbance and establishment treatments for each genotype are presented in Fig. 6.2. The survival curves of genotypes considered individually were significantly affected by the herbicide treatment or plant establishment method (Tables A.2.21 and A.2.22). Introduction by seed into the undisturbed areas had the strongest influence on plant mortality. Such an effect was not, however, observed uniformly across the genotypes. Survival was high for *D. pubescens* CPI 92803 which is adapted to alkaline soil (R.L. Burt, personal communication), and very low for the herbaceous types *D. virgatus* CPIs 79653 and 78382. Survival was reduced to an intermediate level in the shrubby legume types (i.e. *D. leptophyllus* CPIs 38351 and TQ88, and *D. bicornutus* CPI 91162; Fig. 6.2). Genotypes introduced as seedlings maintained a very high level of survival through time either with or without competition from the original vegetation. The results from the present study on tropical legumes accord with those of Cavers and Harper (1967) and Turkington *et al.* (1977) in temperate environments: large differences in survival curves were produced when *Trifolium repens* was introduced into undisturbed plots in natural vegetation and into denuded plots.

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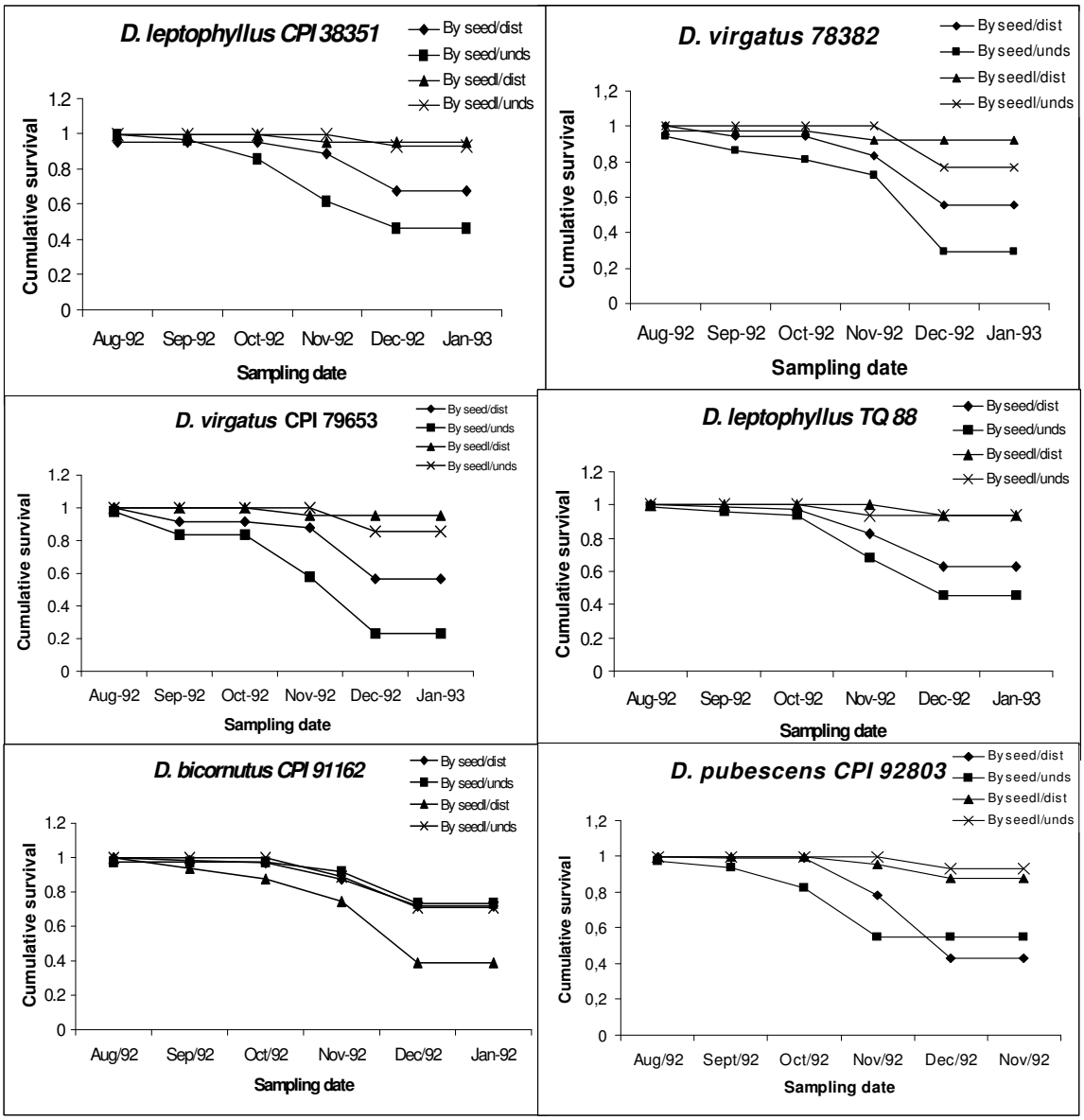

Figure 6.2 Kaplan-Meier survival curves of 6 genotypes of *Desmanthus*, introduced by seed or by seedling into herbicide treated pasture (no competition) or into the natural grassland complex.

Legend
By seed/dist – Introduced by seed into disturbed vegetation
By seed/unds – Introduced by seed into undisturbed vegetation
By seedl/dist – Introduced by seedling into disturbed vegetation
By seedl/unds – Introduced by seedling into undisturbed vegetation

6.3.3 Shoot dry matter

Analyses of variance for shoot dry matter production by individual plants are shown in Table A.2.23. Data were transformed into $\sqrt{(x+1)}$ because of the zero values in some plots. Back transformed means were significantly affected by the main effects of disturbance and genotype, and by their interactions, but were not affected by the method of establishment, or its interactions with other effects (Table A.2.23). Vegetation removal increased the transformed value of shoot dry matter in relation to the undisturbed vegetation, when averaged for genotypes and plant establishment method (Table 6.2). Such data represent a 19-fold increase in shoot dry matter for the original untransformed data. Competition pressure of natural vegetation reduced the final plant establishment and produced smaller plants than on the herbicide-treated plots. Plants initiated as seedlings did not produce heavier plants at harvest than those established from seed (Table 6.2).

These findings suggest the following hypotheses:

- competition in the early stages of life, by an introduced species, alien from the original vegetation, may drastically reduce establishment of such plants; or,
- establishment can be enhanced if seedlings are raised without competition and later transplanted into the natural undisturbed vegetation; or,
- surviving plants from transplanted seedlings, although more numerous, do not generate stronger individuals than those originating from sown seeds.

As is evident from their survival behaviour, shrubby and erect genotypes had higher final shoot dry matter masses than prostrate or decumbent types when other effects were averaged (Table 6.2). This was also true for the final dry matter yield of genotypes growing on herbicide-treated plots (Table 6.3). Under competition with the original vegetation, however, some shrubby types such as *D. leptophyllus* CPI 38351 produced less shoot dry matter than the herbaceous *D. virgatus* CPI 78382 (Table 6.3).

Table 6.2 LSD pairwise comparisons of means of shoot dry matter mass (g/plant) by the effects of vegetation management, establishment method, and genotype. Data subjected to √ (x+1) transformation before analysis and back transformed in this table.

Vegetation management		Establishment method		Genotype	
Disturbed	1.83 a	By seed	1.53 a	CPI 38351	1.92 a
Undisturbed	1.06 b	By seedling	1.36 b	CPI 79653	1.18 b
				CPI 92803	1.38 ab
				CPI 78382	1.10 b
				TQ 88	1.71 ab
				CPI 91162	1.39 ab

Means followed by the same letter in a column are not significantly different by LSD ($p < 0.05$)

Table 6.3 LSD pairwise comparisons of means of shoot dry matter by the interaction vegetation management x genotype. Data subjected to √ (x+1) transformation before analysis and back transformed in this table.

Genotype	Disturbed	Undisturbed
D. virgatus 38351	2.80 a A	1.04 b B
D. virgatus 78382	1.14 b A	1.07 ab A
D. virgatus 79653	1.32 b A	1.03 b B
D. bicornutus 91162	1.63 ab A	1.14 a A
D. pubescens 92803	1.74 ab A	1.02 b B
D. virgatus TQ 88	2.36 ab A	1.06 ab B

Means followed by the same low case letter in the column or upper case letter in the row are not significantly different by LSD ($p < 0.05$).

6.4 Discussion

Competition with the original vegetation for light, water, and nutrients are the main challenges faced by alien species in the early stages of their introduction to a new site. Establishment success of an alien species can be improved by removing or reducing the biomass of the existing herbaceous vegetation, or by raising seedlings outside the competition site for later planting into the site to be colonized. These introduction methods were used by Cavers and Harper (1967) and Turkington *et al.* (1977) for the temperate *Trifolium repens* introduced by seed or by transplants into

plots irrespective of whether the original herbaceous vegetation had been killed or not. Germination, establishment, and final dry matter yield of *T. repens* were all improved by removing the existing pasture vegetation. Establishment and dry matter production were also higher in transplanted plants than in plants grown from seeds. In the present study, establishment and final dry matter yields of *Desmanthus* genotypes from seeds sown into unaltered vegetation were significantly affected by competition (Fig. 6.2 and Table 6.3), similarly to the results obtained by Turkington *et al.* (1977) for *T. repens*. This factor, however, was not sufficient by itself to reduce germination (seedling emergence) across the range of *Desmanthus* genotypes studied.

Agronomists must consider in practical terms which method of introduction and what *Desmanthus* genotype should be used for a particular set of conditions. In regions where the rainfall regime permits the growth of a competitive biomass of the original pasture vegetation, shrubby types of legumes, introduced by sown seeds after an herbicide treatment of the live biomass, would probably lead to the best establishment. Reducing or removing the existing vegetation may be an expensive practice because of the cost of herbicides and loss of the forage biomass. Hence, an alternative method in such regions might be to sow pelleted and rhizobium-inoculated seeds of shrubby *Desmanthus* types into the intact vegetation followed by periodic and heavy grazing or slashing to reduce competition and improve the survival and establishment of the legume. In areas with lower rainfall, where natural vegetation is not highly competitive, herbaceous or shrubby *Desmanthus* types could be sown directly into the pasture in the wet season with the choice of genotype taking account of the adaptability of specific genotypes to local conditions of climate and soil.

The transplanting of seedlings into established grassland to form a concentrated nucleus for future dispersion is also a feasible but labour-intensive option for all conditions. Such methods would give a slower but more reliable alternative method for the introduction of *Desmanthus* genotypes into established pastures.

Chapter 7

STIMULATION OF WOOL GROWTH BY *Desmanthus* PASTURES

7.1 Introduction

Animal production in tropical areas is limited by shortages in animal feed of both energy and protein during the dry season. In inland tropical Australia, short and unreliable summer wet seasons, and cold conditions including frosts in the winter dry seasons are the critical factors in determining forage growth and animal production (Wheeler and Freer 1986). Wheeler and Freer (1986) observed differentiated water use efficiency between forage species and an exponential relationship between average rainfall and carrying capacity in Australian semi-arid lands. The rainfall regime becomes more variable and declines drastically with distance inland, producing a consequent diminution of pasture production (Williams 1974). The quality of grass-based pastures is highly susceptible to the rainfall decline and frost, and animal production, based on pasture production in tropical areas, becomes strictly seasonal.

Major improvements on productivity on the Australian rangelands have been achieved by the introduction of forage legumes. The most successful of the legumes are cultivars of the genus *Stylosanthes*: Verano (*Stylosanthes hamata* cv. Verano) and Seca (*Stylosanthes scabra* cv. Seca), which have been introduced from South America and have successfully adapted to a range of climates across the non-clay soils of the higher rainfall areas of tropical Australia. They provide ruminants with access to green forage with a high digestibility and high protein content for some weeks or months after the natural grasses have senesced at the end of the growing season (Gardener 1980; Burt 1993). The *Stylosanthes* cultivars are highly successful and have been sown into native pastures across approximately 1.5 million ha of the Australian tropics (Clements 1996). If the present trend in the area sown with *Stylosanthes* spp. is maintained, by the year 2010 the legume-based pastoral system area with this legume in northern Australia will be of the order of 2.5 million ha (Clements 1996). Improvements in meat and wool production by the introduction of

the stylo species over areas formerly occupied by natural grasses have been reported (Gillard *et al.* 1980; Gillard and Winter 1984). The *Stylosanthes* spp. however does not thrive at sites where the annual rainfall drops below 750 mm, nor on clay soils (Burt 1993). Thus there are currently no introduced legumes for the approximately 28 million ha of Mitchell grass (*Astrebla* spp.) plains in western North Queensland (Fig. 7.1), grazed predominately by wool- producing Merino sheep that represent 40 to 50 per cent of Queensland's sheep flock (Phelps, 1999).

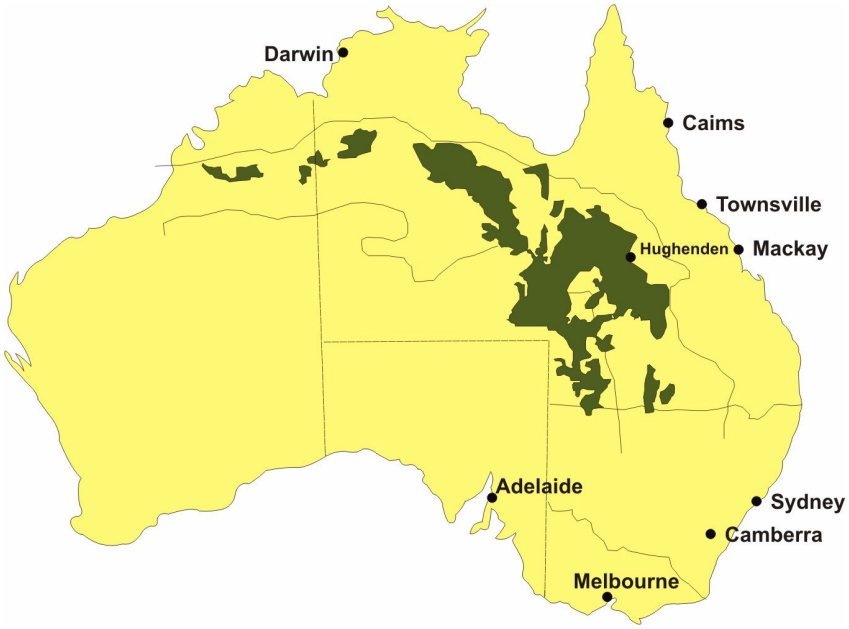

Figure 71. Location map of Australian central plain showing the Mitchell grass country (shaded) and the 600 mm and 300 mm annual rainfall isohyets across the northern rangelands. Source: Orr (1978)

Members of the *Desmanthus* genus offer the possibility for filling the role of a legume in the Mitchell grass plains that are too dry and whose soils are too clay-rich for *Stylosanthes* (Gardiner *et al*. 2004). The agronomic potential of *Desmanthus*, its soil and climatic requirements, biomass yield, and forage quality are presently under study in Australia and overseas (Carvalho and Mattos 1974; Call 1985; Benson and Vail 1989; Epp 1989; Kulakow *et al*. 1989; Muir and Pitman 1991; Gardiner 1992; 1999; Burt 1993; Clem and Hall 1994; Adjei 1995; Gardiner and Burt 1995; Gardiner and Rangel 1996; Trujillo *et al.* 1996; Bahnich *et al*. 1998; Gardiner *et al.* 2004). However, there is a lack of information relating to the nutritional value of

Desmanthus and its influence on animal performance. Battad (1993) in the Philippines conducted a pioneer study testing animal performance in response to *Desmanthus* supplementation of pastures. He found that goats supplemented at 1.0 % live weight with *D. virgatus* had a daily gain of 34.8 g/head against 16.1 g/head in the control treatment of stargrass without a legume supplement.

Some *Desmanthus* genotypes have been shown to have high sulphur contents in their seeds (Schlink and Burt 1993). Sulphur when present in amino acids, plays an important role in wool growth (Weston & Hogan 1986). But the quality of most tropical pasture species is not sufficient to maintain high levels of wool production for extended periods of time (Weston & Hogan 1986). The availability of forage plants with improved quantity and quality of fodder production is highly desirable for the sheep-producing areas of tropical and subtropical Australia, where no such plant currently occurs.

The experiment reported in this chapter focused on the potential of four *Desmanthus* accessions, in comparison with Verano stylo, as supplements to Mitchell grass (*Astrebla* spp.) hay, on the wool growth of Merino wethers at the CSIRO Lansdown Research Station, 50 km south of Townsville.

7.2 Materials and methods

Thirty-six Merino wethers (average liveweight 33.96 kg, s.d. 1.82 kg), were individually housed in metabolism cages and daily fed with 800 g (3% of averaged metabolic liveweight) of Verano (*Stylosanthes hamata* cv. Verano) chaff as an exclusive diet, for a 3 week pre-treatment period (24 July 1991 to 14 August 1991). On the next day the animals (6 per treatment following the protocol of Weston & Hogan 1986) were offered one of six chaff diets in which the fodder was passed through a chaff cutter (particles 10 mm long) and thoroughly mixed before offered to

the animals:

- 600 g/head/day of Mitchell grass chaff (*Astrebla* spp.) – the grass diet;
- The grass diet + 200 g/head/day of cv.Verano chaff;
- The grass diet + 200 g/head/day of *Desmanthus leptophyllus* CPI 38351 chaff;
- The grass diet + 200 g/head/day of *D. pubescens* CPI 92803 cv. Uman chaff;
- The grass diet + 200 g/head/day of *D. virgatus* CPI 78382 chaff;
- The grass diet + 200 g/head/day of *D. virgatus* CPI 79653 chaff;

Mitchell grass and Verano hasy were 5 years old and *Desmanthus* genotypes hays 2 years old. The experiment was carried out over a 6 week period between 15 August 1991 and 25 September 1991. All the sheep were provided with 26 g/day of a mineral supplement mixture to provide essential minerals: phosphorus, calcium, sodium, magnesium, iron, zinc, copper, manganese, cobalt, molybdenum, sulphur, and iodate (Weston & Hogan 1968).Water was available *ad libitum.* Individual food residues were daily collected and bulked per sheep for the whole period to be used in the calculation of daily dry matter intake. Faeces were individually and daily collected over a 10 days period between 16 and 25 September 1991. Faeces of each sheep were so bulked for the 10 days collection period and kept in a freezer at 5 ºC for digestion calculations.

Analyses of neutral detergent fibre, acid detergent fibre (Van Soest and Moore 1966) and Kjeldahl nitrogen (AOAC 1970), on a dry matter basis, were performed on diet components, bulked residues, and bulked faeces, collected during a 10 day period during the experiment, from 16 to 25 September 1991. Food residues and faeces were dried for 48 hours at 105^0 C to determine their dry matter content. Metabolisable energy intake was calculated as dry matter intake x (0.147 x *in vivo* dry matter digestibility % - 0.72) (Ministry of Agriculture, Fisheries and Food 1975). *In vivo* digestion of dry matter, organic matter, and nitrogen (Ministry of Agriculture, Fisheries and Food 1975) were determined in the last 10 days of the experimental period where:

Digestion % = (Feed - Residues - Faeces) x 100 / (Feed – Residues)

The sulphur content of hays was analyzed by a semi quantitative x-ray fluorescence spectrometer in the Geology Department of James Cook University.

On 15 August 1991, the sheep were completely shorn and rectangular patches of 15 cm x 10 cm (150 cm^2) were superficially marked in the right side of the body of each wether and the wool grown inside the patches, from this date, was clipped on 29 August 1991, and 19 September1991. Wool resulting from the shearing (15 August) was discarded because it was produced before the diet treatments were established. Clean wool yield (mg/100 cm^2/day), wool yield % (percentage of clean wool in relation to greasy wool), and fibre diameter (microns), were determined by CSIRO Division of Animal Production, Wembley, West Australia.

Analyses of variance of the data were processed using the General ANOVA System of the STATISTIX 1985, 94, Analytical Software, Version 4.1. Means were compared by the Tukey test at the 5% confidence level.

7.3 Results

7.3.1 Diet components

The analytical results of the diet components used in the wool growth experiment are presented in Table 7.1 and 7.2. Diet components had similar contents of dry matter, except for *D. virgatus* CPI 78382 which may have had a shorter exposure to the sun during the hay drying process leading to the small differences shown in Table 7.1.

S. hamata cv. Verano and *D. virgatus* CPI 79653 had the lowest values of neutral detergent fibre and acid detergent fibre, and the highest nitrogen contents, followed

in nitrogen content by *D. leptophyllus* CPI 38351. The hay of *D. virgatus* CPI 79653 had also the highest content of sulphur, while the highest value of N/S was found in Verano (Table 7.2).

Table 7.1 Dry matter content of the diet components used in the wool growth experiment.

Diet Component	Dry Matter Content %
Mitchell grass chaff	94.3
S. hamata cv. Verano chaff	92.9
D. leptophyllus CPI 38351 chaff	93.6
D. pubescens CPI 92803 cv. Uman chaff	92.4
D. virgatus CPI 78382 chaff	82.3
D. virgatus CPI 79653 chaff	92.51

Table 7.2 Chemical compositions of diet components. Organic Matter, Neutral Detergent Fibre, Acid Detergent Fibre, Nitrogen and Sulphur contents are all expressed as percentage of the Dry Matter used as animal feed.

Parameter	Diet component					
	Mitchell Grass	Verano	CPI 38351	CPI 92803	CPI 78382	CPI 79653
Organic Matter %	90.70	93.90	95.4	96.7	94.7	92.8
Neutral Detergent Fibre %	79.32	66.19	69.06	76.46	71.31	58.48
Acid Detergent Fibre %	47.64	44.52	50.24	55.88	53.94	43.77
Nitrogen %	0.50	1.60	1.58	0.92	1.42	2.64
Sulphur %	0.08	0.13	0.22	0.11	0.18	0.36
Nitrogen/Sulphur ratio	6.25	12.3	7.18	8.36	7.89	7.33

7.3.2 Animal responses to diets

Tables A.2.24 to A.2.32 show the analysis of variance of data for liveweight

variation (kg/head), dry matter intake (g/kg of body weight/day), dry matter digestibility (%), organic matter digestibility (%), neutral detergent fibre digestibility (%), acid detergent fibre digestibility (%), calculated metabolisable energy intake (kJ/kg of body weight/day), nitrogen intake (g/head/day) and nitrogen digestibility (%) over the 3 months of the experiment. Comparisons of means of these parameters are shown in Table 7.3 (Tukey, $p < 0.05$).

Although the diets had been calculated to liveweight maintenance the sheep lost weight in all the treatments. Long period of storage of the hays after preparation may lead to a diminishing in their nutritive qualities. Despite the time of storage of the Mitchell Grass and Verano chaffs were not available they had the appearance to have being stored for a reasonable period of time before be used in the trial. All the *Desmanthus* chaff were prepared in the previous year. Significantly higher weight losses ($p < 0.05$) occurred on the control diet (Mitchell grass alone) compared with the other diets (Table 7.3). Dry matter intake showed no significant variation ($p < 0.05$) among the diets, except for the diet supplemented by *D. virgatus* CPI 79653, which had significantly higher ($p < 0.05$) feed intake than the control (Table 7.3). The feeds supplemented with Verano, *D. virgatus* CPI 79653, and *D. leptophyllus* CPI 38351 had the highest dry matter digestibility, and that with *D. pubescens* CPI 92803 the lowest, which was even lower than the control diet (Table 7.3). The highest organic matter digestibility, neutral detergent fibre, and acid detergent fibre levels were observed in the control and Verano-supplemented diets, and the lowest on the *D. pubescens* CPI 92803-supplemented diet (Table 7.3). Metabolisable energy intakes in the supplemented diets were higher than in the control diet in all the *Desmanthus* supplemented diets, except for *D. pubescens* CPI 92803, which also had a low nitrogen intake. The highest nitrogen digestibility was recorded however, in the basal Mitchell grass diet (the control), followed by the diets supplemented with *D. leptophyllus* CPI 38351 and Verano (Table 7.3).

Table 7.3 The composition of different diets (Mitchell grass chaff + legume supplement chaff as shown), and their effects on the nutritional parameters of Merino wethers over the period of the feeding trial.

	Diets: Mitchell grass plus the indicated supplement					
Parameter	Nil	Verano	*D. leptophyllus* CPI 38351	*D. pubescens* CPI 92803	*D. virgatus* CPI 78382	*D. virgatus* CPI 79653
Dry matter digestibility %	42.47 bc	46.52 a	43.75 abc	39.80 c	42.22 bc	44.94 ab
Organic matter digestibility %	49.05 a	51.42 a	48.38 ab	45.32 b	48.58 ab	48.30 ab
Neutral detergent fibre %	55.34 a	54.99 a	51.24 ab	48.66 b	52.79 ab	51.64 ab
Acid detergent fibre %	52.72 a	50.28 a	45.01 b	43.44 b	48.16 ab	43.79 b
Liveweight variation (kg/head)	-5.83 b	-1.15 a	-1.53 a	-2.33 a	-1.33 a	-2.28 a
Total dry matter intake (g/kg^{-} BW/day)	19.91 b	22.82 ab	23.00 ab	22.82 ab	23.01 ab	24.66 a
Calculated metabolisable energy intake (KJ/kg of body weight/day)	109.81 c	139.47 ab	131.30 abc	117.21 bc	126.66 abc	144.93 a
Total nitrogen intake (g/day)	7.58 d	9.39 bc	10.17 b	8.73 c	10.19 b	12.43 a
Nitrogen digestibility %	68.07 a	61.87 ab	62.24 ab	55.21 bc	53.88 c	58.65 bc

Means in the same row followed by the same letter are not statistically different by Tukey ($p < 0.05$)

7.3.3 Diet efficiency and the stimulation of wool growth

Analysis of variance of the data of clean wool, wool yield, and wool fibre diameter in two periods of wool growth (14 and 21 days respectively) are shown in Tables A.2.33 to A.2.38. The efficiency of diets in producing quantitative and qualitative changes in such wool parameters are shown in Tables 7.4 and 7.5.

In the first period of 14 days of wool growth, the amount of wool produced (mg of clean wool/100 cm^2/day) by wethers on the diets supplemented with Verano, *D. pubescens* CPI 92803, and *D. leptophyllus* CPI 38351 was significantly higher ($p < 0.05$) than that from the rest of the diets (Table 7.4). In the second period of 21 days of wool growth, there were no significant differences ($p < 0.05$) in wool production among the supplemented diets, all of which produced significantly more wool than did the control (Table 7.5).

A significantly ($p < 0.05$) higher wool yield in comparison with the other diets, was obtained from *D. pubescens* CPI 92803-supplemented diet during the first period of wool growth (Table 7.4). In the second period however, the wool yield from the control diet was significantly higher than that from all the other diets (Table 7.5).

No significant differences in fibre diameter were found in the wool produced by the different diets in the first period of wool growth (Table 7.4). In the second period, however, fibre diameter was significantly higher ($p < 0.05$) from the diets supplemented with Verano, *D. virgatus* CPI 79653, and CPI 78381 (Table 7.5).

Wool fibre diameter and wool quality are associated parameters; wool quality is increased with the decreasing of its fibre diameter (Turner *et al.* 1986). However, low fibre diameter is also associated with a low wool productivity which is also associated with a poor nutritional status of the sheep. Inversely, high fibre diameter in wool is associated with a high wool productivity and rich nutritional status of the sheep. Graziers in western Queensland generally make their economic decisions to produce higher wool mass from their flocks to the detriment of wool quality.

Table 7.4 The effect of the different diets (Mitchell grass chaff + legume supplement chaff as shown) on the quantitative and qualitative parameters of wool growth. First growth period: wool collected on 29 August 1991, 14 days after the experiment was initiated.

Parameter	Diets: Mitchell grass hay plus indicated supplement						
	Nil	Verano chaff	*D. leptophyllus* CPI 38351 chaff	*D. pubescens* CPI 92803 chaff	*D. virgatus* CPI 78382 chaff	*D. virgatus* CPI 79653 chaff	LSD value for comparison
Clean wool (mg/100 cm^2/day)	42.02 d	56.51 ab	55.25 abc	62.56 a	51.20 bcd	45.91 cd	10.24
Wool yield (%)	59.17 c	67.93 b	67.89 b	77.37 a	67.46 b	64.55 bc	8,02
Fibre diameter (microns)	18.90 a	20.42 a	19.76 a	19.08 a	19.80 a	20.47 a	2.53

Means in the same row followed by the same letter are not statistically different by Tukey ($p < 0.05$)

Table 7.5 The effect of the different diets (Mitchell grass chaff + legume supplement chaff as shown) on the quantitative and qualitative parameters of wool growth. Second growth period: wool collected on 19 September 1991, 35 days after the experiment was initiated.

Parameter	Diets: Mitchell grass hay plus indicated supplement						
	Nil	Verano chaff	*D. leptophyllus* CPI 38351 chaff	*D. pubescens* CPI 92803 chaff	*D. virgatus* CPI 78382 chaff	*D. virgatus* CPI 79653 chaff	LSD value for comparison
Clean wool (mg/100 cm^2/day)	53.57 b	66.67 a	63.24 ab	66.76 a	71.37a	70.01 a	12.82
Wool yield (%)	79.11 a	69.77 b	72.22 b	72.09 b	72.56 b	70.01 b	6.51
Fibre diameter (microns)	18.11 c	20.06 ab	18.88 bc	18.19 c	19.19 abc	20.73 a	1.67

Means in the same row followed by the same letter are not statistically different by Tukey ($p < 0.05$)

7.4 Discussion

Variations in the dry matter and organic matter contents of the hays used in the wool growth experiment were minimal, except for *D. virgatus* CPI 78382 which may have had a shorter exposure to the sun during the hay drying process leading to the small differences shown in Table 7.1.

Lower levels of neutral detergent fibre and acid detergent fibre found in *D. virgatus* CPI 79653 and Verano hays in relation to that of the other legumes, probably reflects their fine stems and a higher leaf/stem ratio compared with the woody characteristics of the *D. leptophyllus* CPI 38351 and *D pubescens* CPI 92803 hays (Table 7.2). No explanation was found for the high levels of neutral detergent fibre and acid detergent fibre present in *D. virgatus* CPI 78382 that also had fine stems.

The nitrogen contents of the legumes ranged from 0.92% (*D. pubescens* CPI 92803) to 2.64% (*D. virgatus* CPI 79653). These levels of nitrogen in legume hay are similar to those reported by Little *et al.* (1984) for mature Verano herbage and by Gardiner and Rangel (1996) for the same *Desmanthus virgatus* genotypes. Gardiner & Rangel (1996) also found similar high nitrogen contents in *D. virgatus* CPI 79653 leaves and stems.

A legume supplement to the Mitchell grass diet significantly reduced the liveweight losses of sheep in the present study in comparison with the weights of those on the control diet (Mitchell grass alone). The mean liveweight losses of sheep fed the Verano-supplemented diet (1.15 kg/head) here, were much higher than that of sheep fed 20% Verano and 80% speargrass (*Heteropogon contortus*) chaff, over a similar period of time (Schlink 1998), despite comparable dry matter intakes of approximately 730 g/head/day and dry matter digestions of approximately 320 g/head/day, in both studies. Such different results can be explained by a higher nutritional quality of the speargrass and Verano hays used by Schlink (1998) in

comparison with the Mitchell grass hay used in the present study. Livewieght losses from the Mitchell grass diet (5.85 kg/head) were 43 times higher than losses from the speargrass diet (0.135 kg/head), despite a higher dry matter intake and dry matter digestion of Mitchell grass diet (657 and 279 g/head/day, respectively) than that from the speargrass diet (544 and 218 g/head/day, respectively). Increases in the total dry matter intake by supplementation of the basal Mitchell grass hay diet with hays of either Verano or the four *Desmanthus* genotypes averaged 16% and rose to 24% in the *D. virgatus* CPI 79653 diet. Lack of increase in dry matter intakes by the supplementation of low quality grass diets with legumes was also shown in sheep in Trinidad and Tobago by Lascano and Palacios (1993), Fassler and Lascano (1995), and was thought to have resulted from high tannin levels and low digestibility of the legumes, functioning as integrated factors or in a separately way.

In the present study, despite a relatively low digestibility of the supplemented diets, intakes were always above 90% of that on offer, rising to 96% for the *D. virgatus* CPI 79653 supplemented diet. The presence of poorly chopped, thick stems in the residues of all but the *D. virgatus* CPI 79653 and CPI 78382 supplemented diets is thought to be responsible for the small differences in intakes between the legume supplemented diets. Levels of condensed tannins were not analysed in the present study.

Mean dry matter intakes from the five supplemented diets (23 g/kg body weight/day) were similar to those of sheep fed a 34.7% *Trifolium tembense* and 63.3% maize straw diet (Mosi and Butterworth 1985), and were higher than the intakes reported by McMeniman *et al*. (1988) for sheep fed rice straw *ad libitum* plus 250 g of one of five legume hays: mung bean (*Vigna radiata*), cowpea (*Vigna unguiculata*), lablab bean (*Lablab purpureus*), pigeonpea (*Cajanus cajan*), and lucerne (*Medicago sativa*). The Mitchell grass hay basal diet in the present study, different from the rice straw control diet used by McMeniman *et al*. (1988), was responsible for a relatively high level of metabolizable energy intake; supplementation with legume hay improved the calculated metabolizable energy intake significantly ($p < 0.05$) only for the diets supplanted with *D. virgatus* CPI 79653 and Verano (Table. 7.3). The level

of metabolizable energy intake in the Mitchell grass control diet of the present study was much higher than that obtained by McMeniman *et al*. (1988) with a rice straw control diet.

The mean wool production in the present study was higher than that obtained by Schlink (1998) and smaller than that of Godfrey *et al.* (1993). Regardless of some significant ($p < 0.05$) increases obtained from diets supplemented with Verano, *D. leptophyllus* CPI 38351 and *D. pubescens* CPI 92803, the mass of clean wool from the first growth period showed weak correlations with the nutritional parameters studied (Table 7.6). A residual effect of the pre-experimental period where all the animals were fed 800 g/day of Verano hay may explain the weak correlations.

Table 7.6 Correlation coefficients between clean wool yield during the first wool growth period and nutritional parameters.

Nutritional Parameter	Correlation with clean wool yield (r^2)
Organic matter intake	0.35
Dry matter intake	0.32
Nitrogen intake	0.10
Digested dry matter	0.32
Metabolisable energy intake	0.06
Sulphur intake	0.25

In the second wool growth period, however, wool production was significantly increased by all of the supplemented diets in relation to the control, with no significant differences among them. In this period, when the residual effect of the Verano pre-experimental diet was no long evident, the clean wool production was significantly correlated with nutritional parameters (Table 7.7) such as: dry matter intake, digested dry matter, organic matter intake, metabolizable energy intake, nitrogen intake, and sulphur intake. Positive relationships between increasing intakes of dry matter and nitrogen in the diet and wool production have been frequently reported in the literature (Piper and Dolling 1969; Hynd 1989; Saul and

Channon 1990; Cronje and Weites 1990; Combe 1992; Lee and Williams 1993).

Table 7.7 Correlation coefficients between clean wool yield during the second wool growth period and nutritional parameters.

Nutritional Parameter	Correlation with clean wool yield (r^2)
Organic matter intake	0.95
Dry matter intake	0.88
Nitrogen intake	0.72
Digested dry matter	0.67
Metabolisable energy intake	0.66
Sulphur intake	0.61

The *Desmanthus* genotypes generally had higher sulphur contents in the hay than did Verano (Table 7.2). Schlink and Burt (1993) also found higher sulphur contents in the seeds of 6 *Desmanthus* genotypes compared to those of 5 other forage legumes. Adequate contents of sulphur-based amino acids in the diet have been identified by many authors as fundamental to high levels of wool production (Reis 1967; Rogers *et al*. 1980; Weston and Hogan 1986; Weston *et al*. 1988; Hume and Bird 1997). A nitrogen/sulphur ratio of 10/1 in the diet was suggested by Morrison *et al*. (1986) as being adequate to maintain the equilibrium of microbes in the animal rumen. Nitrogen/sulphur ratios observed in the present study for individual components of the diets revealed a sulphur deficiency in the Verano chaff, and adequate sulphur contents in the *Desmanthus* genotypes, to support an ideal microbial population in the rumen. The mixture of each legume with Mitchell grass hay in the supplemented diets produced N/S ratios that were all very close to the recommended N/S ratio of 10/1 (Table 7.3).

Sheep receiving a maintenance diet of wheat straw increased their wool growth volume by 86% through the addition of 10 g of a mixture of essential amino acids containing 3 g methionine, or 1g methionine and 2 g cysteine (Reis *et al*. 1990). Fenn and Leng (1989) also obtained an increase in wool growth of 16% by supplementation of a roughage-based diet with methionine via drinking water.

Sown pasture plants with high levels of protein and rich in sulphur-containing amino acids should provide an economical approach to improving wool production in certain areas of the tropics. The use of engineered plants to produce proteins high in sulphur and resistant to rumen degradation has been proposed by Higgins *et al*. (1989) as a solution to improve wool growth in sheep. The levels of nitrogen and sulphur present in some *Desmanthus* genotypes used in the present study (especially *D. virgatus* CPI 79653 with 2.64% and 0.36% of nitrogen and sulphur respectively on a dry matter basis), are associated with increases of wool growth, and show the potential of these plants for wool production. More detailed studies are still needed on the nature of the amino acids, the proportion of proteins, and protein degradability in such fodder.

Wool yield from the control diet in the second period of wool growth was higher than that from the supplemented diets (Table 7.5). No reference was found in the literature to such outcome. It is possible that higher grease production, stimulated by the legume, may explain the reduction in wool yield from the supplemented diets during the second period of wool growth.

The wool production results obtained here, associated with the agronomic adaptation of *Desmanthus* genotypes (Gardiner 1999; 2003; Gardiner *et al*. 2004) to the black clay soils of the Mitchell grass plains of northern Queensland, show the very strong potential of such legumes to improve wool growth in the area to which there are no adapted *Stylosanthes* cultivars.

Chapter 8

CONCLUSIONS AND FUTURE RESEARCH DIRECTIONS

8.1 Conclusions

Since the accidental introduction of *Stylosanthes humilis* (Townsville stylo) into northeastern Australia in the late 19th century, introduced forage legumes in the pastoral zones of tropical Australia have played an important role in pasture development. Other species of the genus *Stylosanthes (*notably *S. hamata*, *S. scabra,* and *S. guianensis)* and of other genera such as *Macroptilium* (*M. atropurpureum* cv. Siratro), *Centrosema* (*C. pascuorum cv.* Cavalcade*)*, *Clitoria* (*C. ternatea*) and *Leucaena* (*L. leucocephala*), have been successfully introduced into various niches of rangeland pastures. The legumes have promoted increases in the carrying capacity of native and introduced grass pastures, ensuring substantial increases in animal productivity in different ecosystems throughout tropical Australia. Propagation of indigenous forage legumes in improved or sown pastures in Australia was thought by Wheeler and Freer (1986) to be worthless, because throughout the country they have proved inferior to deliberately or accidentally introduced species. In 1986 there were around 130 introduced legume species and cultivars available for forage production in Australia (Wheeler and Freer 1986). Stylos are presently cultivated over approximately one million hectares across tropical Australia and they have sustained an estimated increase in beef production of some $ 40 millions over the last 25 years (Clements 1996). Other species, such as leucaena (*Leucaena leucocephala*) with 50,000 ha in production in Queensland and butterfly pea (*Clitoria ternatea*) with 12,000–20,000 ha sown in Queensland, have been successfully used in the past 10 years (Pengelly and Conway 2000).

The most recent challenge faced by Australian scientists has been the development of well-adapted perennial legumes for the large areas of clay soils throughout the semiarid rangelands of tropical Australia. Soil water extraction, water-logging, root disturbance with swelling and shrinking by soil wetting and drying, alkalinity, salinity, and seedling

establishment are common problems of clay soils (Keating and Mott 1987), and are barriers to be overcome by legumes that are adapted to these soils. Since 1990, legumes of *Desmanthus* genus (Desmanthus Jaribu – a commercial mixture of 3 cultivars: *Desmanthus virgatus* cv. Marc, *D. leptophyllus* cv. Bayamo, and *D. pubescens* cv. Uman), butterfly pea (*Clitoria ternatea*) and caatinga stylo (*Stylosanthes seabrana* cvv. Primar and Unica), were developed for permanent pastures on clay soils in northern Australia (Pengelly and Conway 2000). It is estimated that 12-20000 ha have been sown with on a wide range of soils of central Queensland (Pengelly and Conway 2000). Strategies of grazing management to ensure only occasional set of seeds in butterfly pea may enable it to persist as a permanent pasture species (Pengelly and Conway 2000). Specific rhizobial bacteria for effective legume nodulation have short lives when seed is sown into dry soils with high surface temperatures, and seedling establishment on soils that develop surface crusts, were pointed out by Pengelly and Conway (2000) as drawbacks for caatinga stylo in such soils.

Pengelly and Conway (2000) identified *Desmanthus* as one of the very few legume genera which have been consistently observed to persist under heavy grazing on clay rich rangeland soils. Pengelly and Conway (2000) have suggested that limitations in the commercially available Jaribu Desmanthus relate to differences in persistence, seed production, seedling recruitment and dry matter production, among the three component cultivars (Marc, Bayamo, and Uman). Pengelly and Conway (2000) also showed the necessity for more experimental works focused on the biological and environmental relationships of introduced species and genotypes, before any recommendations may be made regarding their use in pasture systems.

Some important conclusions set out below have been drawn from the present study and provide basic knowledge for future agroecological studies of the existing collection of *Desmanthus* for specific environments throughout the tropics of Australia and of the world.

8.1.1 **The studied accessions of *Desmanthus* spp. are able to form permanent and varied soil seed banks.** (Section 3.1.2).

8.1.2 **Variations in seed bank sizes below studied *Desmanthus* spp. are closely associated with genotypes.** (Section 3.1.2).

8.1.3 **Large seed banks are associated with *Desmanthus* accessions that have high seed production and low levels of seedling recruitment, and *vice-versa*.** (Section 3.1.2).

8.1.4 **Seed-coat dormancy is present in all the studied *Desmanthus* accessions and seedling recruitment is highly dependent on overcoming of this type of dormancy.** (Sections 3.2.2.1 and 3.4.1).

8.1.5 **The strophiole seems to be a natural structure in the control of water penetration into the seed of the studied *Desmanthus* accessions. An impermeable strophiole is a common feature in viable, non germinated seeds deposited in soil seed bank.** (Sections 3.2.2.1 and 3.4.1).

8.1.6 **High temperatures may be used to overcome seed-coat dormancy in *Desmanthus* genotypes. Seeds of different accessions, however, have different responses to the amount of heat necessary to promote seed germination, and in relation to maximum resistance to heat without reducing germination.** (Sections 3.2.2.2 and 3.4.1).

8.1.7 **Natural soil heat during summer in grassland areas of tropical savannas may not be sufficient to break down the seed-coat dormancy of some D*esmanthus* accessions deposited in the soil seed bank, but fire will do so.** (3.4.1).

Preservation of existing natural forests and woodlands, both in Australia and overseas, is a current and growing overall concern (Cameron *et al*. 1989). Hence, there is an urgent need to develop cultivars to support modern ecological approaches to silvipastoral systems. Despite the existence of some published studies exploring the survival and productivity of tropical forage legumes growing under natural or artificial shade conditions, none of them have been conducted under eucalypt woodlands typical of Australian savanna environments. Therefore, experiments were carried out in the present study to explore the ability of some accessions of legumes of the *Desmanthus* genus to grow in the micro-environments under and between iron bark (*Eucalyptus crebra*) trees, and in controlled shade environments. The results obtained from these experiments provide the following conclusions:

8.1.8 **Soils from under eucalypt trees were found to have better fertility, and lower bulk densities, than soils from between tree canopies.** (Sections 4.3.1 and 4.3)

8.1.9 **Combinations of shade-tolerant *Desmanthus* accessions and favourable soil conditions found under eucalypt tree canopies enhanced seedling establishment and plant survival in this micro-environment, compared to those in the open areas between tree canopies in the same woodland.** (Section 4.4).

8.1.10 **Some *Desmanthus* accessions were shown to be well adapted to shaded environments where the level of light was as low as 15% of full sunlight irradiance.** (Section 5.4).

8.1.11 **Higher levels of nutrients in the soils from under tree canopies is a key factor promoting higher dry matter yields in plants growing in such soils, compared to those grown in soils from between canopies.** (Section 5.4). Research is needed to identify whether a relationship exists between the higher nutrient levels in the under-canopy soils, and the increasing of water use efficiency of plants that may be reflected in higher dry matter yields.

8.1.12 **The availability of *Desmanthus* genotypes adapted to conditions of low levels of light irradiance will enhance pasture productivity, reduce costs of tree clearing, and help the preservation of the ecological integrity of savanna regions.** (Section 5.4).

The present study also explored the establishment and persistence of some accessions of *Desmanthus* introduced into established grass pasture and showed that:

8.1.13 **The percentage of plant establishment and survival of *Desmanthus* accessions can be significantly increased by reducing natural herbaceous vegetation by use of herbicides, transplanting of previously rooted seedlings, or by a combination of both techniques.** (Section 6.4).

Sheep and wool are part of the Australian pastoral tradition. In northern Australia, a large part of the flocks graze on native or introduced pastures established over clay soils. Feed supplementation, although not a common practice in such areas, is necessary for special groups such as ewes at mating and lambing, lambs after weaning, stud rams, and for all sheep during drought (Turner *et al*. 1986). Forage legumes provide a suitable feed supplement because of their association with nitrogen-fixing bacteria, high nutritional value of feed components, and the convenience of being grown in adjacent areas, or in native or introduced grass pastures on a property. Considering the scarcity of forage legumes adapted to clay soil conditions, *Desmanthus* provides an excellent alternative pasture plant for farmers to use in supplementing the quantity and quality of fodder for their flocks in northern Australia. In the present study, the hay of four accessions of *Desmanthus* and Verano stylo (*Stylosanthes hamata* cv. Verano) were tested as supplements for a Mitchell grass hay diet of sheep and their influence on wool growth. The study showed:

8.1.14 **Desmanthus, the subject of the thesis, has a better N/S ratio than Verano.** (Sections 7.3.1 and 7.4).

8.1.15 ***Desmanthus*-supplemented sheep feed increased clean wool growth. The enhanced wool production was significantly correlated with fodder nutritional parameters, especially organic matter and dry matter intake.** (Sections 7.3.2 and 7.4).

8.1.16 **The levels of nitrogen and sulphur present in some *Desmanthus* genotypes were associated with increased wool growth.** (Sections 7.3.2 and 7.4).

8.1.17 **The results obtained, associated with the agronomic adaptation of *Desmanthus* genotypes to the heavy clay soils of the Mitchell grass plains, show the strong potential of the genus to improve wool growth in that area.** (Section 7.4).

8.2 Future research direction

The present study has explored the responses of an agronomically important, but poorly known and underutilised species of a tropical legume; it has identified the response of several different forms of the legume to some key ecological factors. Several important applications of the work remain to be completed, including the following: In the great majority of the early agronomic studies conducted with accessions of the genus *Desmanthus,* very little attention had been paid on the identification of the accession used. The general term ”*Desmanthus virgatus”* was frequently used without any reference to the accession or cultivar under study (Pengelly and Liu 2001). Such a misclassification has created barriers for readers who attempt to compare the results that have been obtained by different authors. The more detailed studies of Lukow (1993), Burt (1993), and more recently Pengelly and Liu (2001) into the systematics of the genus *Desmanthus*, now provide agronomic studies of legumes of this genus with a sound basis for identifying the accession used in trials (Gardiner 1992, 1999, Gardiner and Rangel 1996, Gardiner *et al.* 2004).

With the release of the cultivars *D. virgatus* cv. Marc, *D. leptophyllus* cv. Bayamo, and *D. pubescens* cv. Uman as a mixture known as Jaribu Desmanthus in 1991 (Cook *et al*. 1993) as a forage legume suitable for heavy clay soils, the genus went beyond the phase of experimental studies and became a commercial plant for legume-based pastures. Since its release, Jaribu Desmanthus has been established in at least 3000 ha of permanent pasture systems in clay soils of northern New South Wales and the Central Highlands of Queensland (Pengelly and Conway 2000). However, given the low level of dry matter production in Marc, low seedling recruitment in Bayamo, and low seed production and seedling recruitment in Uman, associated with problems of requirements of specific rhizobia and a small gains in diet and soil nitrogen, Jaribu Desmanthus is often seen as small reward for the effort of establishing as *Desmanthus*-based pastures (Pengelly and Conway 2000). Based on these considerations Pengelly and Conway (2000) stressed that "if desmanthus is to be used in permanent pasture systems, then Marc or a similar cultivar with higher dry matter production might be the only alternative".

Focused on the considerations above and in the results obtained in the present study the following future research and development actions are proposed:

- **The commercial release of new, promising, competitive accessions, such as *D. leptophyllus* TQ88 and *D. bicornutus* CPI 91162.**

- **A search for accessions adapted to conditions of clay soils other than alkalinity to which the released cultivars (Uman, Bayamo, and Marc) are adapted.**

- **Explore the potential of some accessions of *Desmanthus* in promoting wool growth with more detailed studies on the value of their amino acids, their proportions of proteins, and their protein degradability.**

- **More detailed studies on the influence of environmental (soil, rainfall, temperature) and management (fire, grazing system, grazing pressure) factors on seedling recruitment, plant establishment and persistence of different accessions in *Desmanthus*-based pasture systems.**

- **Testing the adaptability of some shade-tolerant accessions, such as *D. leptophyllus* TQ 88 and *D. bicornutus* CPI 91162, as components of silvipastoral systems.**

8.3 Possible applications of this research

The experimental work carried out in the present study had two main objectives. The first was to establish the influence of environmental (fire, temperature, rainfall, competition) and management (introduction methods, competition) factors on the soil seed bank, seedling recruitment, plant establishment, and persistence of a range of accessions of the genus *Desmanthus.* The results obtained from the trials conducted with this objective are of general knowledge and, although they can not be immediately incorporated into the production systems, they form an important scientific support for new research. The second aim was to identify *Desmanthus* accessions that might be used in a legume-based pasture for the Mitchell grass plains of semiarid north-western Queensland to enrich the diet of sheep and improve wool production in this region. The results obtained here were promising and await confirmation by broad-scale agronomic trials to test the extent of adaptation of the genus *Desmanthus* to the conditions of the Mitchell grass plains. It is anticipated that the results will be incorporated into the wool production system of the region.

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APPENDICIS

Apendix 1 Meteorological data recorded at Townsville Airport and experimental sites.

Apendix 2 Statistical analyses of data collected in trials carried out in field and laboratory.

Appendix 3 Scientific papers and conference presentations arising from the present study

Apendix 1 – Meteorological data recorded at Townsville Airport and experimental sites

Table A.1.1 - Monthly Data for Townsville Airport. Source: Bureau of Meteorology, Department of Environment and Territories. 1991

1991	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Annual Mean
Mean daily Maximum Temperature (C°)	30.0	30.4	31.0	29.7	27.9	26.7	25.5	26.7	28.5	29.6	30.7	32.4	-	29.1
Mean Daily Minimum Temperature (C°)	24.4	24.5	22,2	20.5	17.8	14.2	12.4	14.5	16.3	20.7	23.2	24.3	-	19.6
Mean 9am Relative Humidity (%)	85.0	83.0	63.0	61.0	65.0	72.0	61.0	66.0	54.0	53;0	67.0	74.0	-	67.0
Mean 3pm Relative Humidity (%)	73.0	74.0	57.0	53.0	54.0	51.0	43.0	51.0	43.0	49.0	55.0	55.0	-	54.8
Total Monthly Precipitation (mm)	458.2	865.4	14,8	1.2	17.2	9.6	0.6	0.0	0.0	3.2	21.2	138.8	1530.2	-
Rain Days (number)	24	21	8	2	8	3	1	0	0	2	5	9	83	-

Table a.1. 2 - Monthly Data for Townsville Airport. Source: Bureau of Meteorology, Department of Environment and Territories. 1992

1992	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Annual Mean
Mean daily Maximum Temperature (C°)	32.2	32.2	31.7	30.1	28.5	25.8	25.4	26.5	29.0	28.9	31.1	30.4	-	29.4
Mean Daily Minimum Temperature (C°)	25.7	25.1	23.3	21.0	20.2	13.4	13.2	16.1	20.6	20.6	22.6	23.9	- -	20.5
Mean 9am Relative Humidity (%)	72.0	81.0	64.0	63.0	73.0	64.0	58.0	61.0	62.0	63.0	57.0	70.0	-	64.8
Mean 3pm Relative Humidity (%)	57.0	63.0	53.0	55.0	61.0	44.0	46.0	53.0	53.0	49.0	53.0	65.0	-	54.3
Total Monthly Precipitation (mm)	27.0	227.8	7.2	5.4	54.6	6.6	1.8	1.8	33.2	3.8	49.4	179.2	597.8	-
Rain Days (number)	24	21	8	2	8	9	1	0	0	2	5	9	83	-

Table A.1. 3 - Monthly Data for Townsville Airport. Source: Bureau of Meteorology, Department of Environment and Territories. 1993

1993	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Annual Mean
Mean daily Maximum Temperature (C°)	30.4	31.0	32.0	29.9	28.1	26.3	26.1	26.2	27.5	28.8	30.2	31.1	-	29.0
Mean Daily Minimum Temperature (C°)	24.1	24.1	22.9	20.0	18.0	15.4	16.5	14.8	19.8	21.0	23.5	24.0	- -	20.3
Mean 9am Relative Humidity (%)	69.0	69.0	63.0	58.0	66.0	63.0	75.0	61.0	66.0	64.0	64.0	64.0	-	65.2
Mean 3pm Relative Humidity (%)	64.0	61.0	53.0	54.0	57.0	50.0	58.0	50.0	55.0	55.0	58.0	58.0	-	56.1
Total Monthly Precipitation (mm)	66.2	109.2	36.6	8.2	13.8	3.2	17.6	14.2	30.8	49.4	102.8	44.0	496.0	-
Rain Days (number)	15	10	8	6	5	4	7	5	8	6	8	11	93	-

Table A.1.4 - Monthly Data for Townsville Airport. Source: Bureau of Meteorology, Department of Environment and Territories. 1994

1994	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Annual Mean
Mean daily Maximum Temperature (C°)	34.1	31.3	31.3	29.9	27.9	26.3	25.4	25.6	27.4	29.5	31.1	30.8	-	29.2
Mean Daily Minimum Temperature (C°)	25.4	25.0	22.9	20.0	17.9	14.0	14.0	13.3	16.2	20.8	23.0	23.6		19.7
Mean 9am Relative Humidity (%)	60.0	73.0	63.0	57.0	64.0	59.0	59.0	55.0	57.0	59.0	58.0	64.0	-	60.7
Mean 3pm Relative Humidity (%)	57.0	69.0	55.0	50.0	55.0	48.0	49.0	45.0	51.0	49.0	53.0	60.0	-	53.4
Total Monthly Precipitation (mm)	148.2	142.4	44.8	0.6	1.0	5.4	1.2	0.0	5.6	1.2	16.4	102.8	469.6	-
Rain Days (number)	8	12	8	1	1	3	1	0	3	3	5	10	55	-

Table A.1.5 – Monthly Data for Townsville Airport. Source: Bureau of Meteorology, Department of Environment and Territories. 1995

1995	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Annual Mean
Mean daily Maximum Temperature (C°)	32.0	31.4	31.7	31.4	28.3	26.6	26.1	25.1	27.3	29.2	31.2	32.6	-	29.4
Mean Daily Minimum Temperature (C°)	24.8	24.9	24.1	20.9	19.2	15.4	12.6	16.6	18.0	20.7	23.7	24.7	-	20.5
Mean 9am Relative Humidity (%)	66.0	76.0	70.0	59.0	68.0	60.0	62.0	65.0	61.0	65.0	63.0	66.0	-	65.1
Mean 3pm Relative Humidity (%)	59.0	71.0	63.0	52.0	60.0	48.0	49.0	57.0	57.0	66.0	61.0	58.0	-	57.9
Total Monthly Precipitation (mm)	49.3	223.4	20.2	3.0	19.6	4.0	0.2	104.0	0.6	91.2	73.4	120.6	709.8	-
Rain Days (number)	12	14	5	1	9	5	1	7	2	8	7	13	84	-

Table A.1.6 - Monthly Data for Townsville Airport. Source: Bureau of Meteorology, Department of Environment and Territories. 1940 -1998

1940-1998	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Mean
Mean daily Maximum Temperature (C°)	31.3	31.0	30.8	295	275	25.5	25.0	25.9	27.6	29.3	30.7	31.4	-	28.7
Mean Daily Minimum Temperature (C°)	24.2	23.9	22.8	20.5	17.6	14.4	13.6	14.7	17.2	20.6	22.8	24.0	-	19.7
Mean 9am Relative Humidity (%)	71.6	74.9	72.3	68.1	67.6	66.2	66.4	63.0	59.7	60.9	63.0	66.6	-	66.7
Mean 3pm Relative Humidity (%)	65.3	66.9	63.7	59.5	56.9	51.4	51.3	51.1	51.8	52.2	57.2	60.2	-	57.5
Total Monthly Precipitation (mm)	276.4	282.6	197.1	61.8	37.0	20.7	13.8	17.6	10.9	24.6	53.0	129.7	1116.8	-
Rain Days (number)	14.5	15.7	12.9	7.8	6.3	4.0	3.0	2.8	2.4	4.9	6.6	9.9	90.5	-

Table A.1.7 - Experimental area - Total Monthly Precipitation (mm)

YEAR	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total	Mean
1992		163.0	5.0	9.5	37.5	0.0	0.0	0.0	44.5	4.5	54.0	138.0	456.0	45.5
1993	61.5	141.5	34.5	10.0	10.0	2.7	13.7	8.0	20.0	36.5	71.5	49.0	458.9	38.2
1994	118.0	173.0	96.5	4.5	0.0	5.7	0.0	0.0	2.0	0.0	16.5	113.5	530.5	44.2

APENDIX 2 Statistical analyses of data collected in trials carried out in field and laboratory.

Table A.2.1- Analysis of variance of number of seed recovered from soil seed bank of nine genotypes of *Desmanthus* spp.

SOURCE	DF	SS	MS	F	P
Genotype (A)	8	4098000	512200	4.47	0.0053
Replication (B)	3	1504000	501300	4.37	0.0198
A*B	16	1833000	114600		
TOTAL	27	7435000			

Table A.2.2 - Analysis of variance of percentage of raised seed strophioles as affected by heat treatments in nine genotypes of *Desmanthus* spp.

SOURCE	DF	SS	MS	F	P
Temperature (A)	5	210200	42038.5	171.62	0.0000
Block (B)	2	1806.27	903.136	3.69	0.0277
Genotype (C)	8	16248.2	2031.02	8.29	0.0000
A*C	40	21036.7	525.917	2.15	0.0010
A*B*C	106	25965.1	244.953		
TOTAL	161	27520			

Table A.2.3 - Analysis of variance of percentage of germinated seed as affected by heat treatments in nine genotypes of *Desmanthus* spp.

SOURCE	DF	SS	MS	F	P
Temperature (A)	5	65890.0	13178.0	37.39	0.0000
Block (B)	2	789.040	394.520	1.12	0.3310
Genotype (C)	8	11098.5	1387.32	3.94	0.0004
A*C	40	21751.7	543.791	1.54	0.0413
A*B*C	106	37361.5	352.467		
TOTAL	161	136900			

Table A.2.4 - Analysis of variance of number of seeds remained hard as affected by heat treatments in nine genotypes of *Desmanthus* spp.

SOURCE	DF	SS	MS	F	P
Temperature (A)	5	186300	37250.1	134.41	0.0000
Block (B)	2	2641.32	1320.66	4.77	0.0104
Genotype (C)	8	20472.4	2559.05	9.23	0.0000
A*C	40	20662.3	516.557	1.86	0.0062
A*B*C	106	29377.3	277.145		
TOTAL	161	259400			

Table A.2.5 - Analysis of variance of number of leaves at under-canopy in December 1992 as affected by *Desmanthus* genotypes and quadrant.

SOURCE	DF	SS	MS	F	P
Genotype (A)	6	456.429	76.0715	3.51	0.0177
Quadrant (B)	3	157.287	52.4292	2.42	0.0994
A*B	18	389.631	21.6462		
TOTAL	27	1003.35			

Table A.2.6 - Analysis of variance of number of leaves at between-canopies in December 1992 as affected by *Desmanthus* genotypes and quadrant.

SOURCE	DF	SS	MS	F	P
Genotype (A)	5	47.5671	9.51342	5.14	0.0060
Quadrant (B)	3	14.7446	4.91486	2.66	0.0862
A*B	15	27.7579	1.85053		
TOTAL	23	90.0696			

Table A.2.7 - Analysis of variance of height of plant at under-canopy in December 1992 as affected by *Desmanthus* genotypes and quadrant.

SOURCE	DF	SS	MS	F	P
Genotype (A)	6	47757.6	7959.61	5.76	0.0017
Quadrant (B)	3	21310.4	7103.48	5.14	0.0097
A*B	18	24884.3	1382.46		
TOTAL	27	93952.4			

Table A.2.8 - Analysis of variance of height of plant between-canopy at December 1992 affected by *Desmanthus* genotypes and quadrant.

SOURCE	DF	SS	MS	F	P
Genotype (A)	5	2788.71	557.742	1.44	0.2682
Quadrant (B)	3	2047.12	682.375	1.76	0.1986
A*B	15	5828.12	388.542		
TOTAL	23	10664.0			

Table A.2.9 - Analysis of variance of relative plant survival under-canopy affected by *Desmanthus* genotypes and quadrant.

SOURCE	DF	SS	MS	F	P
Genotype (A)	6	0.29219	0.04870	30.54	0.0000
Quadrant (B)	3	0.02293	0.00764	4.79	0.0126
A*B	18	0.02870	0.00159		
TOTAL	27	0.34381			

Table A.2.10 - Tests of significance of equality of survival distributions of *Desmanthus* genotypes for quadrant under-canopy.

Test	Quadrant South-East	Quadrant south-west	Quadrant North-West	Quadrant North-East
Log-Rank	82.97**	99.39**	65.47**	115.74**
Breslow	56.93**	84.92**	57.90**	63.86**
Tarone-Ware	69.96**	94.05**	62.87**	88.35**

**Significant at 1%

Table A.2.11 - Analysis of variance of relative plant survival of *Desmanthus* genotypes between-canopy at December 1992.

SOURCE	DF	SS	MS	F	P
Genotype (A)	5	0.01064	0.00213	1.59	0.2229
Quadrant (B)	3	0.01005	0.00335	2.50	0.0989
A*B	15	0.02008	0.00134		
TOTAL	23	0.04077			

Table A.2.12 - Tests of significance of equality of survival distributions of *Desmanthus* genotypes for quadrant between-canopy.

Test	Quadrant South-East	Quadrant south-west	Quadrant North-West	Quadrant North-East
Log-Rank	20.64**	16.31**	59.06**	7.16ns
Breslow	17.49**	19.54**	32.12**	14.59*
Tarone-Ware	19.93**	20.82**	43.37**	10.61ns

** - significant at 1%; * - significant at 5%, ns - not significant

Table A.2.13 - ANOVA regressions analysis of soil water content under 100, 45, 30, and 15% sunlight, and three watering frequencies. Averaged for soil and genotype.

100% sunlight

	DF	*SS*	*MS*	*F*	*Significance F*	
Regression	1	1109.66	1109.66	350.4031	1.76E-19	
Residual	34	107.6715	3.166809			
Total	35	1217.331				
	Coefficients		*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	23.89988		0.784709	30.45699	2.91E-26	22.30516	25.4946
X Variable 1	-6.79969		0.36325	-18.7191	1.76E-19	-7.5379	- 6.06148

45% sunlight

	DF	*SS*	*MS*	*F*	*Significance F*	
Regression	1	1503.613	1503.613	452.8478	3.14E-21	
Residual	34	112.8919	3.320349			
Total	35	1616.505				
	Coefficients		*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	28.48761		0.803507	35.45409	1.94E-28	26.85469	30.12053
X Variable 1	-7.91521		0.371951	-21.2802	3.14E-21	-8.6711	-7.1593

30% sunlight

	DF	*SS*	*MS*	*F*	*Significance F*	
Regression	1	1672.236	1672.236	569.364	8.13E-23	
Residual	34	99.85884	2.937025			
Total	35	1772.095				
	Coefficients		*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	30.10202		0.755704	39.8331	4.05E-30	28.56625	31.6378
X Variable 1	-8.34725		0.349823	-23.8614	8.13E-23	-9.05817	-7.63632

15% sunlight

	DF	*SS*	*MS*	*F*	*Significance F*	
Regression	1	2270.749	2270.749	568.026	8.44E-23	
Residual	34	135.9188	3.997613			
Total	35	2406.667				
	Coefficients		*Standard Error*	*t Stat*	*P-value*	*Lower 95%*	*Upper 95%*
Intercept	35.33297		0.881654	40.07578	3.31E-30	33.54124	37.12471
X Variable 1	-9.727		0.408126	-23.8333	8.44E-23	-10.5564	- 8.89759

Table A.2.14 - Analysis of variance of seedling emergence % .

SOURCE	DF	SS	MS	F	P
SHADE (A)	3	4086.73	1362.24	13.93	0.0041
BLOCK (B)	2	381.019	190.509	1.95	0.2229
A*B	6	586.883	97.8138		
SOIL (C)	1	19.753	19.7531	0.49	0.5566
B*C	2	80.709	40.3549		
A*C	3	225.309	75.1029	0.49	0.7022
A*B*C	6	920.525	153.421		
WATER (D)	2	1017.13	508.565	5.36	0.0099
A*D	6	302.623	50.437	0.53	0.7804
C*D	2	155.710	77.8549	0.82	0.4495
A*C*D	6	860.340	143.390	1.51	0.2065
A*B*C*D	32	3038.27	94.946		
GENOTYPE (E)	1	8200.31	8200.31	88.44	0.0000
A*E	3	864.506	288.169	3.11	0.0339
C*E	1	79.0123	79.0123	0.85	0.3601
D*E	2	42.7469	21.3735	0.23	0.7949
A*C*E	3	72.2222	24.0741	0.26	0.8541
A*D*E	6	927.623	154.604	1.67	0.1461
C*D*E	2	62.1914	31.0957	0.34	0.7166
A*B*C*D*E	54	5006.94	92.7212		
TOTAL	143	26930.			

Table A.2.15 - Analysis of variance of individual dry matter of shoot.

SOURCE	DF	SS	MS	F	P
SHADE (A)	3	6752.97	2250.99	33.21	0.0004
BLOCK (B)	2	285.883	142.942	2.11	0.2025
A*B	6	406.687	67.7812		
SOIL (C)	1	32923.7	32923.7	368.08	0.0000
A*C	3	1260.83	420.278	4.70	0.0356
A*B*C	8	715.572	89.4465		
WATER (D)	2	18171.5	9085.77	106.27	0.0000
A*D	6	834.581	139.097	1.63	0.1719
C*D	2	5451.96	2725.98	31.88	0.0000
A*C*D	6	630.700	105.117	1.23	0.3174
A*B*C*D	32	2735.82	85.4945		
GENOTYPE (E)	1	3821.06	3821.06	28.94	0.0000
A*E	3	960.310	320.103	2.42	0.0771
C*E	1	1046.35	1046.35	7.93	0.0071
D*E	2	1766.22	883.108	6.69	0.0027
A*C*E	3	327.614	109.205	0.83	0.4854
A*D*E	6	411.818	68.6363	0.52	0.7904
C*D*E	2	847.299	423.649	3.21	0.0492
A*C*D*E	6	627.548	104.591	0.79	0.5806
A*B*C*D*E	48	6337.25	132.026		
TOTAL	143	86315.7			

Table A.2.16 - Analysis of regression of the effect of watering frequency on the individual dry matter of shoot.

	Df	*SS*	*MS*	*F*	*Significance F*
Regression	1	18104.73	18104.73	37.68999	7.85E-09
Residual	142	68210.99	480.3591		
Total	143	86315.72			

Table A.2.17 - Analysis of variance of dry matter of root.

SOURCE	DF	SS	MS	F	P
SHOOT (A)	3	7977.10	2659.03	8.24	0.0151
BLOCK (B)	2	1325.39	662.69	2.05	0.2092
A*B	6	1936.28	322.71		
SOIL (C)	1	780.77	780.77	11.56	0.0094
A*C	3	637.88	212.63	3.15	0.0865
A*B*C	8	540.34	67.54		
WATER (D)	2	5879.18	2939.5	52.35	0.0000
A*D	6	3535.85	589.31	10.49	0.0000
C*D	2	0.98	0.49	0.01	0.9912
A*C*D	6	1021.16	170.19	3.03	0.0184
A*B*C*D	32	1796.84	56.15		
GENOT (E)	1	622.27	622.27	5.26	0.0262
A*E	3	1081.26	360.42	3.05	0.0374
C*E	1	79.41	79.41	0.67	0.4165
D*E	2	2.98	1.49	0.01	0.9875
A*C*E	3	417.32	139.11	1.18	0.3284
A*D*E	6	416.55	69.43	0.59	0.7387
C*D*E	2	304.25	152.13	1.29	0.2854
A*C*D*E	6	1813.81	302.30	2.56	0.0314
A*B*C*D*E	48	5673.70	118.20		
TOTAL	143	35843.4			

Table A.2.18 - Analysis of regression of the effect of watering frequency on the individual dry matter of root.

	df	*SS*	*MS*	*F*	*Significance F*
Regression	1	5826.725	5826.725	27.56454	5.43E-07
Residual	142	30016.64	211.3848		
Total	143	35843.36			

Table A.2.19 - Analysis of variance of germination in function of *Desmanthus* genotypes and vegetation removal treatments.

SOURCE	DF	SS	MS	F	P
Disturbance (A)	1	10.8776	10.8776	0.16	0.7183
Block (B)	3	738.289	246.096	3.55	0.1626
A*B (E1)	3	207.686	69.2288		
Genotype (C)	5	9510.46	1902.09	19.34	0.0000
B*C (E2)	15	1475.55	98.3698		
A*C	5	273.436	54.6873	0.18	0.9657
A*B*C (E3)	15	4547.01	303.134		
TOTAL	47	16763.3			

Table A.2.20 - Kaplan-Meier survival analysis of *Desmanthus* Genotypes, where: Total = Cumulative number of plants counted at different dates; Number events = cumulative number of deaths; Number censored = Cumulative number of plants survived; Percentage censored = Percentage of survival

Introduced by seeds in herbicide treated plots.

	Total	Number Events	Number Censored	Percent censored
D. leptophyllus 38351	136	18	118	86.76
D. virgatus 79653	62	10	52	83.87
D. virgatus 92803	128	21	107	83.59
D. virgatus 78382	45	7	38	84.44
D. leptophyllus TQ88	102	13	89	87.25
D. *bicornutus* 91162	145	15	130	89.66
Overall	618	84	534	86.41

Introduce by seeds in untreated vegetation plots.

	Total	Number Events	Number Censored	PercentCensored
D.leptophyllus 38351	123	24	99	80.49
D. virgatus 79653	48	13	35	72.92
D. virgatus 92803	72	20	52	72.22
D. virgatus 78382	34	9	25	73.53
D.leptophyllus TQ88	85	16	69	81.18
D. *bicornutus* 91162	148	30	118	79.73
Overall	510	112	398	78.04

Introduced by seedlings in herbicide treated plots.

	Total	Number Events	Number Censored	Percent Censored
D.leptophyllus 38351	46	1	45	97,83
D. virgatus 79653	46	1	45	97,83
D. virgatus 92803	45	2	43	95,56
D. virgatus 78382	41	2	39	95,12
D. leptophyllus TQ88	47	1	46	97,87
D. *bicornutus* 91162	39	4	35	89,74
Overall	264	11	253	95,83

Introduced by seedling in untreated vegetation plots.

	Total	Number Events	Number Censored	Percent censored
D.leptophyllus 38351	47	1	46	97,87
D. virgatus 79653	46	2	44	95,65
D.virgatus 92803	47	1	46	97,87
D. virgatus 78382	45	3	42	93,33
D.leptophyllus TQ88	46	1	45	97,83
D. *bicornutus* 91162	42	4	38	90,48
Overall	273	12	261	95,60

Table A.2.21- Test statistics for equality of survival distributions for *Desmanthus* genotypes.

Introduced by seed in herbicide treated plots.

	Statistic	df	Significance
Log Rank	5.09	5	0.4048 [ns]
Breslow	3.27	5	0.590 [ns]
Tarone-Ware	3.38	5	0.6412 [ns]

([ns]=not significant at 0.005)

Introduced by seed in untreated vegetation plots.

	Statistic	df	Significance
Log Rank	9.20	5	0.1014 [ns]
Breslow	8.71	5	0.1213 [ns]
Tarone-Ware	8.47	5	0.1320 [ns]

([ns]=not significant at 0.05)

Introduced by seedling in herbicide treated plots.

	Statistic	df	Significance
Log Rank	5.92	5	0.3142 [ns]
Breslow	5.94	5	0.3120 [ns]
Tarone-Ware	5.97	5	0.3089 [ns]

([ns]=not significant at 0.05)

Introduced by seedling in untreated vegetation plots.

	Statistic	df	Significance
Log Rank	6.99	5	0.2214 [ns]
Breslow	7.83	5	0.1661 [ns]
Tarone-Ware	7.41	5	0.1916 [ns]

([ns]=not significant at 0.05)

Table A.2.22 - Survival analysis of treatment interactions where: Total=Cumulative number of plants counted at different dates; Number events= cumulative number of deaths; Number censored= Cumulative number of plants survived; Percentage Censored= Percentage of survival .

Inside *D.leptophyllus* 38351

	Total	Number Events	Number Censored	Percent Censored
By seed in herbicide treated plots	133	18	115	86.47
By seed in untreated plots	123	24	99	80.49
By seedling in herbicide treated plots	47	1	46	97.87
By seedling in untreated plots	47	1	46	97.87
Overall	350	44	306	87.43

Inside *D. virgatus* 79653

	Total	Number Events	Number Censored	Percent Censored
By seed in herbicide treated plots	61	10	51	83.61
By seed in untreated plots	48	13	35	72.92
By seedling in herbicide treated plots	46	1	45	97.83
By seedling in untreated plots	47	1	46	97.87
Overall	202	25	177	87.62

Inside *D. virgatus* 92803

	Total	Number Events	Number Censored	Percent Censored
By seed in herbicide treated plots	128	21	107	83.59
By seed in untreated plots	72	20	52	72.22
By seedling in herbicide treated plots	45	2	43	95.56
By seedling in untreated plots	47	1	46	97.87
Overall	292	44	248	84.93

Inside *D. virgatus* 78382

	Total	Number Events	Number Censored	Percent Censored
By seed in herbicide treated plots	45	7	38	84.44
By seed in untreated plots	34	9	25	73.53
By seedling in herbicide treated plots	41	2	39	95.12
By seedling in untreated plots	45	3	42	93.33
Overall	165	21	144	87.27

Inside *D. leptophyllus* TQ88

	Total	Number Events	Number Censored	Percent Censored
By seed in herbicide treated plots	102	13	89	87.25
By seed in untreated plots	84	16	68	80.95
By seedling in herbicide treated plots	47	1	46	97.87
By seedling in untreated plots	46	1	45	97.83
Overall	279	31	248	90.98

Inside *D. bicornutus* 91162

	Total	Number Events	Number Censored	Percent Censored
By seed in herbicide treated plots	145	15	130	89.66
By seed in untreated plots	148	30	118	79.73
By seedling in herbicide treated plots	41	4	37	90.24
By seedling in untreated plots	44	3	41	93.18
Overall	378	52	326	86.24

Table A.2.23 - Analysis of variance of shoot dry matter of *Desmanthus*, affected by vegetation removal, sowing methods, and genotypes treatments. Data transformed into (√x+1).

SOURCE	DF	SS	MS	F	P
Vegetation removal (A)	1	14.4168	14.4168	38.42	0.0085
Block (B)	3	0.85227	0.28409	0.76	0.5878
A*B (E1)	3	1.12581	0.37527		
Sowing method (C)	1	0.69046	0.69046	2.53	0.2098
B*C (E2)	3	0.81822	0.27274		
Genotype (D)	5	7.86647	1.57329	4.47	0.0016
A*D	5	8.30908	1.66182	4.72	0.0011
C*D	5	0.66310	0.13262	0.38	0.8636
A*C	1	0.14829	0.14829	0.42	0.5187
A*C*D	5	0.85423	0.17085	0.49	0.7878
A*B*C*D (E3)	63	22.1801	0.35207		
TOTAL	95	57.9248			

Table A.2.24 - Analysis of variance of liveweight variation of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	90.8439	18.1688	7.12	0.0002
RESIDUAL	29	73.9630	2.55045		
TOTAL	34	164.807			

Table A.2.25 - Analysis of variance of total dry matter intake of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	67.1977	13.4395	3.05	0.0249
RESIDUAL	29	127.972	4.41283		
TOTAL	34	195.170			

Table A.2.26 - Analysis of variance of dry matter digestibility of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	164.102	32.8203	0.51	0.0006
RESIDUAL	29	120.915	5.03935		
TOTAL	34	285.017			

Table A.2.27 - Analysis of variance of organic matter digestibility of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	113.957	22.7915	5.61	0.0010
RESIDUAL	29	117.830	4.06310		
TOTAL	34	231.787			

Table A.2.28 - Analysis of variance of neutral detergent fiber digestibility of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	187.891	37.5782	5.45	0.0012
RESIDUAL	29	199.950	6.89482		
TOTAL	34	387.841			

Table A.2.29 - Analysis of variance for acid detergent fiber digestibility of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	415.982	83.1964	9.82	0.0000
RESIDUAL	29	245.720	8.47311		
TOTAL	34	661.703			

Table A.2.30 - Analysis of variance of metabolisable energy intake of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	4981.87	996.374	5.14	0.0017
RESIDUAL	29	5623.98	193.930		
TOTAL	34	10605.9			

Table A.2.31 - Analysis of variance of total nitrogen intake of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	80.3733	16.0746	31.9657	0.0000
RESIDUAL	29	14.5832	0.50287		
TOTAL	34	95			

Table A.2.32 - Analysis of variance of total nitrogen digestibility of sheep fed with six different diets

SOURCE	DF	SS	MS	F	P
DIET	5	23.8811	4.77622	10.49	0.0000
RESIDUAL	29	13.20094	0.45521		
TOTAL	34	37.08204			

Table A.2.33 - First period analysis of variance for clean wool of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	0.73938	0.14788	4.45	0.0039
RESIDUAL	30	0.99739	0.03325		
TOTAL	35	1.76677			

Table A.2.34 - Second period analysis of variance of clean wool of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	1.19557	0.23911	2.05	0.1016
RESIDUAL	30	3.39042	0.11691		
TOTAL	35				

Table A.2.35 - First period analysis of variance of wool yield of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	1054.51	210.902	4.56	0.0033
RESIDUAL	30	1386.52	46.2172		
TOTAL	35	2441.03			

Table A.2.36 - Second period analysis of variance of wool yield of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	433.840	86.7680	2.86	0.0323
RESIDUAL	30	880.381	30.3580		
TOTAL	35				

Table A.2.37 - First period analysis of variance of wool fiber diameter of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	13.0193	2.60327	1.25	0.3104
RESIDUAL	30	62.4239	2.08080		
TOTAL	35				

Table A.2.38 - Second period analysis of variance of wool fiber diameter of sheep fed with six different diets.

SOURCE	DF	SS	MS	F	P
DIET	5	32.1039	6.42078	2.99	0.0271
RESIDUAL	30	62.3569	2.15024		
TOTAL	35				

Appendix 3 Scientific papers and conference presentations arising from the present study

A.3.1 Rangel, J.H de A., Schlink, A.C. and Gardiner, C.P. (1996). Simulation of wool growth by *Desmanthus virgatus*: a legume for tropical savannas. In, J.Brown (ed), *The Future of Tropical Savannas: An Australian Perspective* (Poster Papers), CSIRO Publishing, 41-42.

A.3.2 Rangel, J.H de A. and Gardiner, C.P. (1996). Effect of fire on seed germination of *Desmanthus virgatus* accessions. In, J.Brown (ed), *The Future of Tropical Savannas: An Australian Perspective* (Poster Papers), CSIRO Publishing, 43-44.

A.3.3 Rangel, J.H. de A. (2001). Competitividade de quatro gramíneas e seis leguminosas forrageiras tropicais em resposta a freqüência de desfolhamento. *Revista Científica Rural*, 6 (1), 13-21.