Environmental and plant factors causing low legume seedling establishment following oversowing into drought-prone hill swards

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ABSTRACT

Legumes are a valuable component of pastures since they tend to have higher feed quality than grasses and can also fix atmospheric nitrogen in the soil. The technology for oversowing legumes has had many improvements but the success rate is poor and the legume contribution to hill country pasture production remains low. It was concluded that unpredictable weather and plant factors were the major factors causing poor pasture legume establishment from oversowing.

To determine the environmental and plant factors responsible for poor pasture legume establishment from oversowing, a series of seven trials were carried out at AgResearch, Poukawa near Hastings. The five annual and seven perennial legume species oversown in order of establishment success were; subterranean clover (*Trifolium subterraneum*), barrel medic (*Medicago trunculata*), birdsfoot trefoil (*Lotus corniculatus*), white clover (*T. repens*), strawberry clover (*T. fragiferum*), murex medic (*M. murex*), arrow leaf clover (*T. vesiculosum*), lucerne (*M. sativa*), alsike clover (*T. hybridium*), persian clover (*T. resupinatum*), Maku lotus (*L. pedunculatus*) and caucasian clover (*T. ambiguum*). Seeds of each species were oversown in autumn, winter and spring, following defoliation with glyphosate and trodden with sheep. The greatest loss of potential seedlings after oversowing was non-appearance of seedlings, which accounted for about 80% of viable seed. Overall, the contribution of sown legume species to total herbage mass was less than 12% and seedling establishment success was typically between 5 and 30%.

The relationships between eight environmental factors and seedling establishment were explored and the main influences on establishment were found to be gravimetric soil water content, soil temperature, minimum air temperature and daily wind run. A simple model based on these four factors was developed from the field trial data and extrapolated to 10 years of Lawn Road, Hastings and 5 years of Poukawa climate data and the best time, on average, for oversowing was predicted.

To test the effect of high, medium and low soil surface moisture and also to find out the fate of oversown seed two experiments were carried out in a glasshouse using caucasian, strawberry and subterranean clovers. A simple and cheap technique

based on $CoCl_2$ saturated paper strips was developed to measure the changes in soil surface moisture. The soil moisture at depth was a poor indicator of seed germination compared with the surface soil moisture. The low soil surface moisture gave lowest seedling survival. The main cause of low soil surface was wind run. The percentage of ungerminated seed was significantly higher for oversowning than to the standard seed germination test.

Two trials were carried out at AgResearch, Ballantrae, to test the effect of seed rate and seed size. It was observed that sowing rates greater than those usually recommended would increase the seedling density and legume contribution to the total herbage mass and might produce more seed for re-establishment of annual legumes in the subsequent years. Seed size did not significantly affect establishment.

The effect of seed coating and seed dressing was also monitored in a trial at Poukawa. The seed of subterranean and white clovers dressed with fungicide, insecticide and two commercial seed coatings were compared with bare seed. The commercial seed coating increased the early seed germination by 30% but not the final seedling density compared with bare seed. Apron fungicide seed dressing had a deleterious effect on seed germination. The effect of glyphosate residue and litter phytotoxicity was tested in a glasshouse experiment with birdsfoot trefoil and subterranean and white clovers. The species were oversown onto sods sprayed with glyphosate 20 days earlier and onto ordinary sand. The glyphosate residue and dead material did not have any major effect on seed germination and seedling survival.

Overall, environmental factors were found to be the key determinants of successful establishment for pasture legumes by oversowing. Both, the likely environmental conditions at the time of oversowing, and during the first few months of seedling growth need to be considered. The establishment of legume species suited to oversowing can be improved by using high sowing rates and seed coating but ultimately it is the moisture level and temperature at the soil surface that determines germination, and wind run and minimum air temperature that determines seedling survival in drought-prone hill swards.

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Chapter 1

Chapter 1

GENERAL INTRODUCTION AND OBJECTIVES

Pasture and rangelands are often confined to the unploughable hilly areas of the world. Most of these areas have the potential for improvement (Riveros, 1993). The easiest and most economical method to improve the nitrogen level of hill country is to establish suitable legume species (Suckling, 1951; Ball *et al.*, 1982; During, 1984). Suckling (1965) pointed out that lack of legumes was a fundamental weakness in unproductive pasture on moist hill country in New Zealand. Legumes improve the productivity and palatability of the herbage and also fix atmospheric nitrogen (Suckling, 1949; Taylor, 1985; Langer, 1990). Also, they have a higher mineral content than grasses (Miller, 1984).

In hill country, the introduction of legumes by oversowing is accepted practice whereas oversowing of grasses is less common (Lambert *et al.*, 1985). Oversowing is commonly used to establish introduced pasture species on large areas of hill pastures throughout the world (White, 1977). Due to erosion, and cultivation on hill slopes oversowing is of increasing importance. Although technology for oversowing has been improved, the percentage of legume contributing to hill country pastures is still very low and there are many disappointing results from oversowing in drought-prone hill country (Campbell *et al.*, 1987; Campbell, 1992). The vagaries of the weather have an overriding effect on the establishment of seedlings by oversowing, so field experiments oversown in only one or two seasons are unlikely to cover the range of possible weather conditions. There is a need for long term experimentation, probably at least more than ten years, if the key environmental factors that determine the success of oversowing are to be elucidated.

There is a range of pasture legume species that appear potentially useful in dry hill country of New Zealand (Woodman *et al.*, 1992, Dodd and Orr, 1995a, 1995b) but these require further evaluation particularly with respect to their capacity to establish from oversowing.

This thesis examines the environmental and plant factors influencing legume establishment of a wide range of legume species in drought-prone hill country using a standard oversowing technique (Campbell *et al.*, 1987).

The objectives were to:

i) determine the different environmental and plant factors that affect the establishment from oversowing of a range of legume species, including comparisons of the fate of oversown seed and seedling survival,

ii) examine the effect of seed treatments, seed size and seed rate on establishment by oversowing, and to provide practical recommendations on the optimum field conditions for oversowing and the most effective time to oversow.

Chapter 2

Chapter 2

REVIEW OF LITERATURE

This chapter provides an overview of legume pasture establishment in hill country. More detailed reviews precede each chapter.

2.1 Introduction

Pasture establishment is a broad term and its definition varies according to situations and conditions. A frequently used definition in crop agronomy is that establishment has been achieved once the seedling becomes dependent on photosynthesis for its energy source (Naylor *et al.*, 1983) rather than seed reserves for further growth (Harper, 1977). That is, the seedling has achieved a positive carbon balance. Dowling *et al.* (1971) described pasture establishment only to the stages of germination, emergence and early seedling growth whereas Bellotti (1984) differed from this definition, and suggested that pasture establishment included all stages of seedling development from seed imbibition to a stage in seedling development when the surviving seedling persisted and the sown seedling population stabilized. Snaydon (1978) defined pasture establishment from an agronomic perspective as the period until a closed stand was formed. A better definition of establishment might be based on morphological criteria such as the attainment of stolons on white clover seedlings which could remove the confounding influence of season and fertility on the rate of development of seedlings and more closely approximate the true physiological state of the plant (Chapman *et al.*, 1985).

Leach *et al.* (1976) has taken establishment to include events of sowing and the early growth phases of pasture development, which include germination, seedling emergence and post emergence growth, to determine which stage most influenced establishment success. Many studies quote establishment levels from a count of seedlings after 2 - 4 months (e.g. Chapman *et al.*, 1985). This can clearly be misleading as seedlings are able to survive under stressful circumstances for some time making little or no net growth (Chippindale, 1948).

The weeks following oversowing are the most critical for seedling establishment whereas survival following establishment largely depends upon grazing management and summer moisture during the first growing season (Suckling 1959, 1965). The main aim of pasture establishment is to introduce a more persistent grass-legume pasture that will ensure long term botanical stability, control weeds, prevent erosion, fix atmospheric nitrogen and reduce the rate of soil acidification (Campbell, 1992).

In this thesis, pasture establishment is defined in terms of individual plant survival from imbibition and germination of the seed through to the leaf and root development of the seedling so that it can grow independent of seed reserves and can face a short term drought (Harper, 1977).

2.2 Hill country pastures

New Zealand is a mountainous area and much of its grassland occurs in hill and high country (White, 1990). The National Research Advisory Council (New Zealand) defined hill and high country as 'predominantly non-ploughable land' (Taylor, 1982). Throughout the world there are large areas of unploughable hill country that have potential for improvement by legume introduction (Riveros, 1993). The world's pasture lands are capable of providing their part of the increase in animal products predicted to be required to feed double the world's present population in the year 2020 (Brougham and Clark, 1985). Part of this increased production could be achieved by establishing high quality pastures in hill country.

In eastern Australia about 25% of high rainfall hill areas are suitable for pasture establishment by oversowing (Crofts, 1985) and in New South Wales the potential area for hill country pasture improvement is 5.7 million ha (Gruen and Pearse, 1959). In New Zealand out of 14 million ha of pasture area there are nearly 5 million ha of surface sown pasture on unploughable hill country, a major part of which is in the North Island (Anon, 1985; White, 1990). In addition, there are over 5 million ha of hill tussock grasslands in the eastem and central districts of the South Island (White, 1990). Scott (1981) reported about 54% of the total area of North Island is hill country and is estimated to carry around 25% of New Zealand stock.

Hill country in New Zealand can be divided into two main areas: 'summer moist' where rainfall during summer is reliable and 'summer dry' where rainfall is variable and drought often experienced (Thomson, 1983). New Zealand's dry hill country can be characterised as a combination of low annual rainfall (<1200 mm), high evapotranspiration (ET) (>900 mm annual potential ET), high runoff, and low water holding capacity soils (Stevens *et al.*, 1993).

In New Zealand about 2 million ha of permanent pastures were treated by aerial top dressing and oversowing between 1950 and 1960 (Robinson and Cross, 1960). Surface sowing of hill country pasture with clover has been practised in New Zealand for many years and it is undisputed that the introduction of new and improved strains of legumes assisted in pasture improvement and increased production (Suckling, 1951). Grasslands Tahora white clover has been the most successful hill country cultivar (Chapman *et al.*, 1993) and almost exclusively used in hill country pastures since its release in 1982 (Barker and Dymock, 1993a).

Hill pasture usually has a low legume content (less than 10%) and poor production and quality (Stevens *et al.*, 1993), with the result the yearly additions to soil organic nitrogen are very low (Campbell, 1974a; Grant *et al.*, 1978). The amount of legume nitrogen fixed in an unimproved hill pasture in one study was only 34 kg N ha^{-1} $year^{-1}$ (Grant and Lambert, 1979), which was considerably less than that measured in lowland pastures (Brock,-1973; Hoglund *et al.*, 1979). The importance of good legume production to hill country pasture cannot be over emphasised (Ball *et al.*, 1982). Introduced legumes play a vital role in soil fertility development as well as providing quality forage (Suckling, 1959; Levy, 1970; Grant *et al.*, 1978) and increased meat yield (Brougham, 1981).

Large increases in farm production from steep hill country are potentially possible. Although estimates suggest that stock numbers could more than double through land improvement especially by introducing legumes, the change is limited by economic and physical constraints (Chapman and Macfarlane, 1985). Hill country pastures are found on a wide range of soil types and climates, resulting in large variation in pasture composition and production. The introduction of improved species and cultivars into hill country is usually attempted by oversowing seed into established pasture. Every year about 100,000 ha of unploughable hill and high country in New Zealand are oversown from the air, with approximately 2000 tonnes of herbage seed (Ministry of Transport, 1975), but oversowing is very inefficient with only a low percentage of seedlings developing to maturity (Suckling, 1949; Madden, 1952; Charlton, 1977; Campbell, 1992).

2.3 Legumes

Legumes have been in agricultural use for at least 6,000 years and are the group of plants of the utmost value for good quality forage and developing fertility throughout the world (Duke, 1981). Legumes includes many species in the genus Trifolium, Medicago and Lotus and species adapted to sub-tropical and tropical climates like siratro (*Macroptilium atropurpureum*) and Townsville stylo (*Stylosanthes gracilis*) (Smetham, 1977). Out of these, the clovers generally inhabit temperate regions of the world (Taylor, 1985). In New Zealand 121 taxa of the Fabaceae were reported as naturalised (Webb, 1980). Half of the pasture legumes will probably be grazed by ruminants at some time but less than 10%

of total taxa of legume species are now deliberately and regularly sown in pasture mixtures (Lancashire, 1984).

Legumes are important not only in providing high quality animal feed but also because of other characteristics. Rhizobia fix atmospheric nitrogen which becomes immediately available for host plant growth and ultimately for associated grass growth (Taylor, 1985; Langer, 1990). In other parts of the world nitrogenous fertilizers are used extensively on pasture but in New Zealand reliance is largely placed on the nitrogen fixed by bacteria (During, 1984). Legumes such as white clover produced through nitrogen fixation approximately 1.3 million tonnes of nitrogen per year in New Zealand pastures, worth over $1.2 billion in saved fertilizer (Widdup, 1994).

Legumes tend to be higher in protein and minerals than grasses (Miller, 1984) and contain from 60 to 80% (w/w) digestible dry matter (Taylor, 1985). Legume herbage mass has less fibre than grass and a higher ratio of soluble to insoluble carbohydrates, and as a result high feed quality stock growth rates are high (Johns, 1966). Pastures containing red or white clovers exacerbate bloat in cattle and sheep (Waghom *et al.*, 1990). In contrast, bloat has not been recorded in cattle grazing lotus and sainfoin and other condensed tannin containing species (Jones and Lyttleton, 1971).

Despite all the benefits of legumes, survival following oversowing is a problem. The epigeal germination of legumes tends to handicap the chances of successful establishment from oversowing, as the forces of extending radicle and hypocotyl combine to push the germinating seed from the point of entry of the radicle, leaving the seedling exposed to desiccation and attack by pests and pathogens (Charlton, 1977).

Throughout New Zealand a wide range of rainfall occurs and the low producing pastures such as in dry hill country of Central Otago are dominated by annual species, while perennial species dominate where rainfall is high (Scott, 1979). About 18 legume species have been found to be environmentally suitable for South Island hill and high country (Scott *et al.*, 1985) and a large number of legume species have been tested at the AgResearch high country research farm, Tara Hills, by Woodman *et al.* (1992). Among all these species some species like white clover, red clover, birdsfoot trefoil and luceme are adapted over wide areas of South Island. Overall, the North Island is wetter than the South Island and has had more than 23 legume species / cultivars released (Rumball, 1983) and many more evaluated (Dodd and Orr, 1995a and 1995b). In the North Island, white and subterranean clovers are extensively used in hill country pastures whereas Maku lotus is largely used in forest areas. These species and other legumes species under evaluation for use in dry hill country will need to be able to establish from oversowing if they are to be widely used.

2.4 Oversowing

2.4.1 Methods

Sowing methods vary in the degree of soil preparation and on whether or not herbicide is used to suppress or kill the existing vegetation. Direct drilling, sod seeding, surface seeding, under-drilling and over-drilling, are all terms used to describe the sowing of seed without prior cultivation. White (1977) referred to sod seeding as a subset of over-drilling dealing only with pasture sowing and considered direct drilling as dealing only with over-drilling in association with herbicide application. In North America conservation tillage has been reviewed by Unger and McCalla (1980) and in Europe developments in zero tillage by Baeumer and Bakermans (1973). Campbell *et al.* (1987) thoroughly reviewed direct drilling and surface sowing in Australia. During the past two decades much research has been done on direct drilling (over-drilling, conservation tillage) machinery for use in renovation of deteriorated pastures in New Zealand (Baker, 1980).

Oversowing, surface sowing, broadcast seeding or aerial sowing refers to the surface broadcasting of seed into existing pasture with or without prior application of a herbicide (White, 1977). Oversowing of pasture species into undisturbed pasture has the potential to reduce establishment costs markedly and allow pasture improvement in areas such as hill country, where conventional cultivation is impracticable in New Zealand (Lambert *et al.*, 1985).

For the scope of this thesis, oversowing is defined as broadcasting of seed into existing vegetation with prior application of herbicide and removal of dead material with mowing or hard grazing.

2.4.2 Techniques

Successful establishment of an introduced species will depend on the seed reaching a favourable site, its ability to germinate and subsequent growth compared to neighbouring established plants (Harper, 1977). Oversowing of clover seed is not a new technique. About 70 years ago Williams (1922) found poor germination of uncovered clover seed when sown on the soil surface and Stapledon and Hanley (1927) found improved germination when using sheep to tread the seed.

Oversowing with treading still appears to be the cheapest and most practicable establishment method for hill country (Charlton and Thom, 1984; Macfarlane and Bonish, 1986). White (1977) recommended 'hoof and tooth' cultivation as a means of ensuring that seed to soil contact occurs. The treading usually controls the growth of the resident pasture and 'cultivates' a seed bed which also ensures seed soil contact. Pre- and post-sowing treading are a usual practice for oversown pasture. Although pre-sowing treading thins the pasture, it is a poor substitute for herbicide (Hume and Chapman, 1993). The post-sowing treading is important as it enhances seed and soil contact. Post-sowing treading is more effective than pre-sowing treading (White, 1977).

Competition from existing vegetation is a major factor limiting the survival of oversown seedlings. After germination of seed and commencement of seedling growth, competition with the resident pasture starts. Control of this competing vegetation appears to be essential to achieve establishment. The seedlings are required to establish and compete against existing plants with developed roots (Macfarlane, 1987).

Herbicide, fallowing, hard grazing and burning have all been shown to improve establishment from oversowing. Where herbicide is used before sowing, contact of seed with soil is more likely and interaction between treading and herbicide application was observed by Sithamparanathan *et al.* (1986). Establishment and survival of legume seedlings sown by broadcasting onto non-arable land can be significantly increased by a prior treatment of the site with herbicide to reduce competition from the existing vegetation (Blackmore, 1965; Douglas, 1970; Davies, 1969; Davies and Davies, 1981; Blowes *et al.*, 1985; Chapman *et al.*, 1985; Lambert *et al.*, 1985; Barker and Zhang, 1988; Awan and Kemp, 1994).

For improved legume establishment by oversowing on dense pasture hard grazing before and after sowing was useful (Suckling, 1954; During *et al.*, 1963; Cullen, 1966; Campbell, 1966; Charlton, 1977; Lambert *et al.*, 1985). Burning has also been used to reduce competition from existing vegetation and to promote legume establishment (Ehrenreich, 1959; Campbell, 1961; Davies and Davies, 1981; Awan and Kemp, 1994). Pastoral fallowing increases individual plant size, vigour and rooting depth, increases the presence and vigour of white clover by creating new niches for growth and improves nitrogen fixation by the legumes in the year after the fallow only (Mackay *et al.*, 1991).

Scott (1989) reviewed seed coating in relation to its effect on plant establishment. He emphasized the objectives of seed coating as protection of rhizobia, supply of micro- and macro-nutrients, protection from birds and rodents, supply of growth regulators, attraction of moisture, supply of oxygen, germination stimulation, germination delay, increase in seed weight or size and the supply of selective herbicide or herbicide antidotes. Coated seed increased establishment of surface sown pasture to some extent (Vartha and Clifford, 1973; Scott *et al.*, 1992) but Dowling (1978) did not find any benefit of seed coating, whereas coating was shown to assist establishment in some sowings (Douglas, 1970; Musgrave and Lowther, 1976). The availability of nutrients to seed germination as early as four days after sowing is important (McWilliam *et al.*, 1970). Given the varied nature of seed coats, and conflicting reports of their effectiveness further consideration to this subject is given in Chapter 7.

The application of rhizobia appropriate to a legume species will markedly increase that legumes establishment if never previously been present (Musgrave and Lowther, 1976; Lowther and Patrick, 1992). Some strains of rhizobia have been shown to result in better establishment than others for birdsfoot trefoil (Lowther and Patrick, 1992) and caucasian clover (Patrick *et al.*, 1994).

Other factors summarised by Charlton and Giddens (1983) that influence pasture establishment are consumption of surface sown seed by birds (Suckling, 1949), seed harvesting by ants (Campbell, 1989), pest damage particularly by porina (*Wiseana* spp.) and slugs (Blackmore, 1964; Charlton, 1978; Charlton, 1982; Chapman *et al.*, 1985), fungal diseases (Stovold *et al.*, 1980) and cyst nematode (*Heterodera trifoli*) and root knot nematode (*Meloidogyne hapla*).

Although technology for oversowing into hill country has had many improvements, its establishment remains poor. The reasons for the low success are not just technological but include other factors like unpredictable rainfall and harshness of the seed environment.

2.4.3 Time for oversowing

Seasonal variation in rainfall and temperature is likely to influence the success of oversowing (Chapman *et al.*, 1985). In southern North Island, Suckling (1949) obtained high and low establishment of ryegrass and legumes from sowings in autumn and spring, respectively. Musgrave (1977) and Scott and Charlton (1983) found that late winter and early spring were the optimum times for sowing clover in the South Island whereas in the North Island, autumn sowing gave better results than spring sowing (Madden *et al.*, 1949; Suckling, 1976; Charlton, 1977; and Lambert *et al.*, 1985). Legume species vary in their sensitivity to high and low temperature and moisture during establishment. Therefore, there is a need to obtain information on the response of legume species following sowing in different seasons. Given the significance of time of sowing, and uncertainty over the factors involved and their relative thresholds, various legume species new to hill country will be examined further in Chapter 3.

2.5 Environmental factors affecting establishment

Environmental and plant factors have a critical influence on the germination and early seedling survival and establishment of legume species. In the review of Chapman and Macfarlane (1985), soil moisture, soil fertility, soil temperature and grazing management were regarded as key factors although in no particular order of importance. They also suggested that the effects of these limitations could be reduced by appropriate species choice and pasture establishment techniques. The first few months in the life of a seedling are generally the most critical to its survival because seedlings have meagre carbohydrate reserves and are susceptible to a large variation in the environmental conditions (Troughton, 1954).

Competition from resident vegetation was found to be a major cause of seedling mortality for aerial sown temperate pasture species (Dowling *et al.*, 1971; Bellotti and Blair, 1989). Aerial, winter sown seedlings died during the following late spring, early summer period due to competition induced stress (Campbell and Swain, 1973a). The competition during seedling establishment is mainly between neighbouring plants. Competition commonly occurs for two or more factors of the environment like light and nutrients but in arid regions water is also another factor.

Under New Zealand pastoral conditions competition for light during pasture establishment may result in suppression of the sown species (Evans, 1973). This applies particularly to the effect of ryegrass cultivars on white clover as well as other grasses (Sears, 1953; Brougham, 1959; Sears and Brougham, 1963; Cullen, 1964). This effect can be controlled by frequent intensive defoliation which reduces shading of the less vigorous species. Adjusting the onset and severity of defoliation may therefore help in obtaining optimum root and shoot growth. This is important because just after defoliation or in the early stages of establishment competition for nutrients is likely to be of greater significance than competition for light (Brouwer, 1963; Seager *et al.*, 1992). Cook and Ratcliff (1984) also suggested that the main form of competition between native grasses and establishing seedlings was root competition for nutrients and that competition for light was secondary because of the open canopies of the native grasslands. Grazing, burning and cutting of the sward did not give sufficient reduction in root competition to allow establishment of surface sown perennial grasses (Cook, 1985).

Halsall *et al.* (1995) found allelopathic effects of a range of residues from pasture on subterranean clover, resulting in reduced germination, stunted growth of the radicle and reduced root growth and nodulation. Roots exposed to the extracts of pasture residue became discoloured and shortened with distorted and scant root hair formation which may result in reduced nodulation. Seedlings exposed to these allelopathic compounds were smaller, less robust and slower growing than the control seedlings and hence more susceptible to attack by diseases and pests (Halsall *et al.*, 1995).

The effect of environmental factors on seedling survival will be discussed further in Chapter 3.

2.6 Summary and conclusion

In this chapter aspects of legume pasture establishment from oversowing have been outlined. Pasture establishment is a complex system involving all stages of seedling development from seed soil contact to plant maturity. Considerable oversowing work has been done all over the world and particularly in Australia and New Zealand. Legume pasture establishment in hill country is still a problem and even with increased knowledge and improved oversowing technology, establishment is still less than 10% of the viable seed sown. Substantial improvements in oversowing technology have been made and may be reaching their possible limits. We can improve plant factors for oversowing and also benefit from improved understandings of the appropriate environmental factors. No pasture species / cultivars have been bred for establishment characteristics suited specially to oversowing. Opportunities for increased seed size and seedling growth rates, and radicle characteristics have not been fully identified and developed. Large areas in the hill and high country have the potential for development through oversowing of legume species.

In hill country the risk of seedling mortality during the germination phase is always high. Soil moisture and soil temperature have been given the most serious attention in previous studies and little consideration has been given to other factors such as excess soil moisture, maximum and minimum air temperature, wind run, degree days and evapotranspiration. There is a need to study the effect of these environmental factors over a wide range of annual and perennial legume species. Despite considerable work in the past there is still a need to understand the high failure of legume establishment from oversowing especially in drought-prone hill country.

It is concluded from the review of literature that:

* establishment from oversowing is very low,

* legumes have greater advantages than grasses for feed quality, and are more commonly oversown,

* environmental factors such as soil moisture and soil temperature are more critical for establishment from oversowing than from full cultivation,

* the environment on the soil surface affects both germination and early establishment processes and is a critical area to study,

* plant factors such as seed size, seed vigour, and seedling relative growth rate affect relative growth and germination,

* because of the harsh environment at the soil surface there is relationship between rate of germination and successful establishment.

The above statements identify the focus for the research in this thesis which will examine a range of pasture legume species under a range of environmental factors.

Chapter 3

Chapter 3

FACTORS AFFECTING LEGUME SEEDLING SURVIVAL AND ESTABLISHMENT[1]

3.1 INTRODUCTION

The hill country farmer needs high producing, vigorous and phosphorus-efficient pastures to support high quality animal production with a low maintenance cost for fertilizer (Daly and Manson, 1987). The key to hill country pasture improvement is the introduction of legumes to fix nitrogen and to provide high quality feed following the correction of the soil nutrient deficiencies (Levy, 1970). The predominant method for establishment of more productive legume species into unploughable hill country pasture has been hand and aerial oversowing due to the steepness of slope and poor accessibility preventing cultivation (Suckling, 1965; Cullen, 1971; Charlton, 1977; Charlton and Giddens, 1983; Lambert *et al.*, 1985).

Aerial oversowing has gained wide acceptance as a pasture establishment method but frequently the results are disappointing with only a small percentage (less than 10%) of sown seed establishing (Suckling, 1950; Cullen, 1970; Dowling *et al.*, 1971; Campbell, 1974a; Charlton, 1977; Campbell *et al.*, 1987). Hill country pastures are extremely diverse as a result of interactions between climatic, edaphic and biotic factors (Chapman and Macfarlane, 1985; White, 1990). In addition, plant and environmental factors affect the success of oversown legume species (Awan and Kemp, 1994).

Legume establishment on drought-prone hill country pasture requires a method that gives effective establishment and is also economically and ecologically

[1]Part of this work were published in the Proceedings of the New Zealand Grassland Association; 55:101-104 (Awan *et al.*, 1993)

effective (Awan and Kemp, 1994). Oversowing (also called surface or broadcast sowing or seeding) usually consists of various pre- and post- sowing techniques for the total, or temporary, suppression of the existing vegetation (Campbell, 1962; Cullen, 1966; Smith, 1966) and the improvement of seed soil contact (Campbell, 1968; Baars *et al.*, 1982). Methods for removal of existing vegetation include defoliant herbicide, burning and hard grazing. Treading of the sown seed with stock is used to improve seed soil contact and to burry at least some of the seed.

The herbicides most frequently used in connection with oversowing are glyphosate, paraquat and dalapon (Blackmore, 1957; Dowling *et al.*, 1971; Vogel *et al.*, 1983; Chapman *et al.*, 1985; Lambert *et al.*, 1985; Leroux and Harvey, 1985; Trimmer and Linscott, 1985; Barker and Zhang, 1988). Glyphosate being a systemic herbicide, suppresses the resident growing vegetation more effectively and efficiently than other herbicides (Cullen, 1969; Campbell, 1968; Naylor *et al.*, 1983; Malik and Waddington, 1990) and by killing the grasses and many flat weeds for up to two to three months after its spraying (Campbell, 1992). Seedlings usually develop and spread more rapidly when glyphosate rather than paraquat at equal rate of active ingredients is used (Chapman and Fletcher, 1985) but Barker *et al.* (1988) found little difference between effects of paraquat and glyphosate.

Cultural treatments such as burning and hard grazing have been used to reduce competition from existing vegetation (Campbell, 1961; Campbell and Annand, 1962; Davies and Davies, 1981; Awan and Kemp, 1994). However, burning reduces the insulating effect of the litter and duff and exposes the soil surface which can result in a high soil temperature on the burnt areas (Ehrenreich, 1959). Hard grazing has been successfully used to prepare pasture for oversowing (Suckling, 1954; During *et al.*, 1963; Hogg, 1965; Campbell, 1968; Charlton, 1977). Cullen (1966) stressed that swards oversown with pasture species should be closely grazed even if treated with herbicide. Hume and Chapman (1993) found that hard grazing was successful in preparing pasture for oversowing, but establishment was poor.

Good seed soil contact can be obtained by post-sowing treading with stock (Lambert *et al.*, 1985; Campbell, 1992). White (1990) recommended 'hoof and tooth' cultivation as a means of ensuring that seed to soil contact occurred. While studying the interaction between herbicide and treading Sithamparanathan *et al.* (1986) observed that the highest treading intensity, equivalent to 400 sheep ha^{-1}, applied after oversowing nearly doubled the survival of oversown legumes like white clover cv. G. Huia, red clover cv. G. Pawera, subterranean clover cv. Woogenellup, luceme cv. G. Wairau and lotus cv. G4703, and seedling establishment showed a linear, or curvilinear, response to treading. Where a dense turf mat exists, driving a mob of sheep or cattle back and forth across a hillside which has been previously hard grazed can result in the formation of an excellent seed bed, especially when soil is moist (Lambert *et al.*, 1985). Nevertheless, in steep hill country, a heavily disturbed surface soil is highly susceptible to severe soil erosion.

Despite the research undertaken on establishment by oversowing, legume establishment in hill country is still difficult and risky (Barker and Zhang, 1988). The establishment from oversowing is usually poor and has been reported to range from 1 - 27% of viable seed sown with a mean of 8% (Campbell, 1974b; Campbell, 1992). In New Zealand white clover showed poor establishment in a range of experiments, for example, percentage of sown seed establishment was recorded as 3% by Charlton and Giddens (1983), 6% by Charlton and Brock (1980) and 5% by Awan and Kemp (1994). Similarly, subterranean clover establishment was found to be 6% by Wedderburn *et al.* (1993) and 28% by Awan and Kemp (1994). These legume establishment experiments were carried out in moist areas, and establishment is likely to be worse in dry hill country with fluctuating soil moisture and unpredictable rainfall. Therefore, there is still a need to determine the most important factors affecting legume pasture establishment particularly in dry hill country pasture.

A number of environmental and plant factors influence germination and early seedling development of oversown temperate legume species and the need for adequate water, nutrient, light and temperature during the early stages of plant development is important (McWilliam *et al.*, 1970). However, some potentially important factors like wind run, minimum air temperature, degree days and evapotranspiration have not been thoroughly investigated. Research on soil moisture and temperature has often been done in controlled environments (McWilliam *et al.*, 1970; Dowling *et al.*, 1971, Campbell and Swain 1973b) rather than in the field.

McKell (1972) emphasized that the success of seedling establishment was due, in the initial stages, to a favourable environment, reduced competition and vigorous (adapted) seedlings. Water relations between the soil, seed and atmosphere have been identified as the dominant factor controlling germination (Harper and Benton, 1966; Campbell and Swain 1973a; Dowling and Robinson 1976). Adequate moisture is necessary to ensure imbibition and early seedling growth. Moisture is further necessary during seedling emergence to reduce soil strength and to permit unimpeded root growth (Barley, 1976). The radicle-entry of a surface sown seed is a vital stage in pasture establishment and was strongly related to soil surface moisture in a glasshouse experiment while using mist propagation equipment to maintain moisture (Campbell and Swain, 1973a). Losses of surface moisture during the radicle entry stage can seriously limit the success of oversowing (Campbell, 1968). Dowling and Smith (1976) found a significant relationship ($R^2 = 78\%$) between mean soil moisture and establishment (% of seed sown) using a water balance model similar to that of Smith and Jones (1975) but modified to incorporate a reduction in evapotranspiration due to dead resident cover.

In a moist environment, temperature is the most limiting factor for germination

(Gillingham, 1974; Suckling, 1975; Lambert, 1977; Bircham, 1977; Radcliffe and Lefever, 1981; Charlton *et al.*, 1986; Hampton *et al.*, 1987). Seed of most pasture species can germinate over a wide range of temperatures with the major effect of temperature being on the rate of germination (McWilliam *et al.*, 1970). White (1970) concluded that temperature is extremely important in controlling pasture growth in New Zealand because temperature varies with latitude, altitude, aspect and season.

A germinating legume seed is extremely vulnerable to desiccating wind and water stress when its radicle is on the soil surface (White, 1990). Rapid germination is another plant characteristic that may contribute to a vigorous and successful seedling establishment (McKell, 1972). Even after the entry of the radicle into the soil, death can occur if the surface dries out more rapidly than root growth (Leslie, 1965). For example, it was observed by Wilson *et al.* (1970) that cycles of wetting and drying resulted in a loss of seed viability possibly caused by the breakdown of biochemical products in the seed. The major reasons for the disparity between germination on the soil and in the laboratory are the environmental factors (Harper and Benton, 1966; Winkworth, 1969; Young *et al.*, 1970). Mark and McKee (1968) suggested that germination from standard laboratory tests x 0.33 provided an adequate estimate of field germination of phalaris (*Phalaris arundinacea*) but this formula was not accurate for lucerne (Campbell *et al.*, 1987). The differences between laboratory standard seed germination and seedling survival on the soil surface of a range of pasture legume species is given below:-

Species	Standard germination (%)	Net seedling survival (%)	Reference
White clover	90	3	Charlton and Giddens (1983)
Maku lotus	84	5	Awan and Kemp (1994)
White clover	65	5	Awan and Kemp (1994)
Subterranean clover	99	28	Awan and Kemp (1994)

In New Zealand, recommendations for legume sowing are for autumn sowing in North Island summer dry hill country, and east coast country, early spring sowing on South Island hill and high country and either autumn or spring for summer wet finishing country and North Island dairying country (Scott and Charlton, 1983; Burgess and Brock, 1985). Spring sowings of lucerne are more successful than summer and autumn sowings (Musgrave *et al.*, 1975) while the annual, subterranean clover should be sown in early autumn (White, 1977). Sithamparanathan *et al.* (1986) found spring sowing was much better for white, red and subterranean clovers, lucerne and lotus when oversown on Central North Island summer wet hill country, whereas Charlton and Giddens (1983) noted the high losses of seedlings that occurred during dry summers and concluded autumn sowing was superior to spring in a summer-dry environment. Chapman and Fletcher (1985) concluded that the optimum time for oversowing white and subterranean clovers was autumn for the summer moist hill country on the lower North Island. There is still a paucity of information on the oversowing requirements for a wide range of annual and perennial legume species so the oversowing seasons of autumn, winter and spring were used in the following experiments to find out the most appropriate time for oversowing in drought-prone hill country.

There has been relatively less research on oversowing in dry hill country than in moist hill country. There is also interest in a greater number of legume species in dry than wet hill country due to the relatively poorer performance of white clover in dry summers than in wet summers.

The main objective of this trial was to determine the environmental and plant factors that affect the successful establishment of legume species oversown into drought-prone hill country pastures. A secondary objective was to compare the survival of annual and perennial legume species under different sowing conditions.

3.2 Materials and methods

3.2.1 Physical description

The trial was situated at the AgResearch Research Farm, Poukawa, 12km south of Hastings, latitude 39° 45' S, longitude 176° 43' E, at an elevation of 55 m. This property was representative of the topography and climate of the Hawkes Bay region. The trial site was located on a south-eastem 21° slope. The soil was a Crownthrope silt loam, a moderately fertile yellow-grey earth of sedimentary origin (pH 5.7) (Table 3.1). There were two adjacent trials within the same paddock, site I was used in 1992 and site II in 1993. The pasture botanical composition was: barley grass (*Hordeum murinum* L.) 40% , vulpia hair grass (*Vulpia bromoides* (L.) S.F. Gray) 30%, white clover (*Trifolium repens* L.) 7%, subterranean clover (*T. subterraneum* L.) 3%, chickweed (*Stellaria media* L.) 8%, winged thistle (*Carduus tenuiflorus* Court.) 5% and others 7% (percentage by weight).

3.2.2 Climate

Hawkes Bay is on the east coast of the North Island, New Zealand and therefore subject to frequent drought. Dry periods during the summer months are common, and frost occurs during winter seasons (June, July and August). The major limitations to pasture growth are moisture deficit during summer and low temperature during winter. During the experimental period meterological data were collected about 500 m from the trial site. The mean rainfall, maximum and minimum temperature and wind speed were averaged over each 15 days after sowing (DAS) for each experiment.

3.2.3 Materials

A range of legume species (7 clovers, 2 lotus and 3 medics) was oversown (Table 3.2). These species varied in their growth characteristics, seed size, usage

Table 3.1 Soil analysis of the trial sites[1]

Site / year	pH	Olsen P µg P/g	$SO_4^{=}$ µg S/g	Exch K^+ meq/100g	Exch Ca^{++} meq/100g	Exch Mg^{++} meq/100g	Exch Na^+ meq/100g	CEC meq/ 100g
I / 1992	5.7	16	3.5	0.60	10.3	1.88	0.2	24
II / 1993	5.8	19	4.5	0.36	12.1	2.23	0.4	27

[1]Laboratory report, Fertilizer and Lime Research Centre, Massey University, Palmerston North, New Zealand.

Table 3.2 Legumes species sown at Poukawa trial area during 1992 and 1993 (The botanical names and cultivar of these species are given in Table 3.7 page 41)

Species	Autumn A 1992	Autumn B 1992	Winter A 1992	Winter B 1992	Autumn 1993	Winter 1993	Spring 1993
Annual legumes							
Subterranean clover	✓	✓	✓	✓	✓	✓	✓
Arrow leaf clover	x	✓	✓	✓	✓	✓	✓
Persian clover	✓	✓	✓	✓	✓	✓	✓
Barrel medic	✓	✓	✓	✓	x	x	x
Murex medic	✓	✓	✓	✓	x	x	x
Perennial legumes							
Alsike clover	✓	✓	✓	✓	✓	✓	✓
Birdsfoot trefoil	✓	✓	✓	✓		✓	✓
Caucasian clover	✓	✓	✓	✓	✓	✓	✓
Lucerne	✓	✓	✓	✓	x	x	x
Maku lotus	✓	✓	✓	✓	x	x	x
Strawberry clover	✓	✓	✓	✓	✓	✓	✓
White clover	x	x	x	x	✓	✓	✓

in different environments and ease of establishment. The botanical names with their cultivars are given in Table 3.7. A standard seed germination test was performed for all legume species (ISTA, 1985) and the moisture content and accelerated aging were tested for some of the species (Baskin, 1987). Seed of arrow leaf and persian clovers was scarified before testing.

The accelerated aging test apparatus consisted of an outer chamber with immersion-type heating element and inner chamber made up of plastic containers with lids. Seed was placed on a wire mesh basket inside the inner chamber. The temperature was thermostat controlled at 40°C and the aging time was 72 hrs (Baskin, 1987). Germination of aged seeds was evaluated according to International Seed Testing Association (ISTA, 1985) rules for seed testing and those which produced normal seedlings were considered to be vigorous (Baskin, 1987). The moisture content of the aged and normal seed was determined after drying for 1 hr at 130°C.

3.2.4 Trial description

A total of seven experiments were comprised of eleven legume species for four 1992 trials, and eight legume species for three 1993 trials (Table 3.2). Arrow leaf clover and birdsfoot trefoil were not available for Autumn A 1992 and Autumn 1993 sowings, respectively. Seed viability was calculated from the standard laboratory seed germination test (Table 3.7) and 500 viable seed of each species were sown by hand broadcasting, into 1 x 1 m plots, according to the time schedule given in Table 3.3. Seed was mixed with 200 g of dry vermiculite to facilitate an even distribution.

3.2.5 Management of trials

All the experimental sites were fenced to exclude animals. To reduce the competition from existing vegetation, Roundup (36% glyphosate) was blanket

Table 3.3 Time of sowing and herbage yield harvesting times at the Poukawa trial area for 1992 and 1993.

	Sowing season	Sowing date	Herbage yield harvested (DAS)
1	Autumn A 1992	27 March, 1992	258
2	Autumn B 1992	10 April, 1992	245
3	Winter A 1992	12 June, 1992	182
4	Winter B 1992	26 June, 1992	168
5	Autumn 1993	5 April, 1993	253
6	Winter 1993	22 June, 1993	175
7	Spring 1993	10 September, 1993	95

sprayed at the rate of 12 ℓ ha^{-1} (Jagschitz, 1978), 20 to 22 days before sowing. Prior to every sowing, dead material in the plots was removed by mowing. After sowing all the plots were trodden by 400 ewes ha^{-1} for 30 minutes. Blitzem antislug pellets (active ingredient metadehyde) were used at each trial. Rhizobium appropriate to each legume species was sprayed on plots within 15 days of sowing in each trial. The sites were grazed by sheep to 1000 kg DM ha^{-1} whenever the herbage mass grew exceeded 1500 kg DM ha^{-1}.

3.2.6 Measurements

3.2.6.1 Plant density

Visible seedlings were counted on 15, 30, 45, 60, 75, 90, 105 and 120 days after sowing (DAS). The 1 m^2 quadrat was sub-divided into 16 sections for easy and accurate counting. In the spring 1993 trial, the seedling density was only counted up to 90 DAS. Percentage of visible seedling survival was calculated by dividing number of seedlings by the 500 viable seed sown.

3.2.6.2 Herbage yield and botanical composition

Two 0.1 m^2 quadrats (0.5 x 0.2 m) were randomly placed in each plot and herbage cut to ground level with electric shears according to the schedule in Table 3.3. All the harvested material was separated into the components sown legume and other herbage material. The separated samples were washed in tap water and then dried at 80°C for 24 hours prior to calculation of dry matter yield.

3.2.6.3. Early plant development

After emergence, a sub sample of thirty seedlings was marked in each experimental unit with plastic pegs (100 mm long and 5 mm wide) to monitor the development pattern of individual species. Seedling development was recorded by counting cotyledons, spade leaves, and trifoliate leaves at 15, 30, 45, 60, 75, 90, 105 and 120 days after sowing (DAS) for the 1992 trials.

3.2.6.4 Micro-environment and environmental factors

A Digi-Sense Thermocouple thermometer (USA) was used (10 a.m.) to record soil temperature at 100 mm depth and gravimetric soil water content (g water/100 g soil) was measured from ten random soil cores per site from 30 to 50 mm depth at 15 day interval. Soil was weighed, then oven dried at 105°C for 24 hours (Painter, 1976). The 15 day average for these data was also calculated from the time of sowing of each trial.

Degree days were calculated using maximum and minimum temperature recorded at the nearby meterological station and a threshold temperature of 8°C. Evapotranspiration potential (ET_p) was calculated using the Priestley-Taylor equation (Priestley and Taylor, 1972). The Penman equation was also considered for calculation of evapotranspiration but the Priestly and Taylor equation was regarded as the most reliable for humid areas (Rosenberg *et al.*, 1983).

3.2.7 Statistical analysis

3.2.7.1 General Linear Model (GLM) and repeated time measurements

The field data were analysed by analysis of variance (ANOVA) using the general linear model (GLM) procedure of SAS (SAS,1989). Data for 1992 were analysed as a factorial arrangement of four sowing conditions and eleven legume species. Data for 1993 were analysed as a factorial arrangement of three sowing conditions and eight legume species. Net seedling survival was analysed using time as a repeated measurement (Mead *et al.*, 1993). There were seven legume species common to the 1992 and 1993 trials. These seven legume species and the seven sowing conditions of both the trial years were analysed using ANOVA on a pooled randomized complete block design (RCB) with four replicates (Dr. I.L. Gordon pers. comm.). The percentage contribution to the total herbage mass was also calculated using the pooled RCB design.

3.2.7.2 Standardized regression coefficients

To find the relationship between different environmental factors and seedling survival a PROC REG statement with a STB option was used to calculate the standard coefficients of linear regression (SAS, 1989). The STB option "prints standardized regression coefficients. A standardized regression coefficient is computed by dividing a parameter estimate by the ratio of the sample standard deviation of the dependent variable to the sample standard deviation of the regressor" (SAS, 1989). The eight environmental factors given in Table 3.6 were tested for their relationship to seedling survival over time, seedling survival over species and seedling survival over sowing conditions. Those factors that were significant at 5% level of probability were chosen for further analysis. The environmental factors chosen were GSWC, soil temperature, minimum air temperature and wind run.

3.2.7.3 Regression analysis

Standard linear regression analysis was used to relate seedling survival and the environmental factors. The environmental factors tested were those chosen in paragraph 3.2.7.2 above. The regression analyses using polynomial, sine, exponential functions to find the best fitted line were used. The best fit for each attribute after considering the coefficients of determination was found to be a linear function of $Y = a + b\,x$. The relationship was tested over time for all the species and sowing conditions. The relationship between the plant development rate and time was also examined by standard linear regression and the difference between the slope of two lines was also tested (Steel and Torrie, 1981).

3.3 Results

3.3.1 Climatic conditions

Rainfall for the trial years 1992 and 1993 was abnormal with total rainfall for the year 1992 being 933 mm and for 1993 being 563 mm. The 30 year average rainfall for the Poukawa research farm was 790 mm per annum. Daily air maximum, air minimum and soil temperature, wind run and rainfall were measured in 1992 and 1993 and monthly summaries are given in Tables 3.4 and 3.5, respectively. The average maximum and minimum air temperature and soil temperature were typical of the trial site except for winter 1993, which was cooler (-0.29°C in August) than winter 1992 (Tables 3.4 and 3.5). The daily average wind run was higher during 1992 (92 km / d) than 1993 (57 km / d).

3.3.2 Environmental factors

The data (average for each 15 DAS in each sowing condition) for different environmental factors are given in Table 3.6. The sowing conditions for winter sowings were wetter and colder than for autumn and spring sowings (Table 3.6). The average wind run was higher (approximately double) for the winter 1992 sowing as compared to the winter 1993 sowing.

3.3.3 Seed germination and vigour

The standard seed germination test, moisture content and accelerated aging test results of the legume species sown are given in Table 3.7. There was little difference between normal and aged seed germination for subterranean clover; it had a high seed vigour (Table 3.7). Persian clover, birdsfoot trefoil, strawberry and caucasian clovers had their germination reduced by about 35% when seed was aged (Table 3.7). White clover had the worst aged germination test with only 14% germination as compared to its standard germination of 81% showing very

Table 3.4 Climatic data (monthly average) recorded at Poukawa AgResearch daily at 0900 hours.

	1992											
Climatic factors	January	February	March	April	May	June	July	August	September	October	November	December
Maximum temperature (°C)	26.40	25.01	24.42	18.33	15.05	14.69	15.21	15.17	16.19	17.68	21.86	21.27
Minimum temperature (°C)	5.04	5.49	2.26	1.49	2.89	1.90	4.37	2.63	4.16	6.97	10.13	9.90
10 cm Soil temperature (°C)	21.68	20.32	17.92	13.09	9.56	7.62	8.36	8.48	10.94	13.90	17.82	18.22
Wind run (km/day)	74.12	64.82	68.80	73.08	88.05	68.26	95.86	123.49	98.55	141.76	102.01	104.56
Rainfall (mm)	22	70	28	53	86	63	157	33	80	166	88	87

Table 3.5 Climatic data (monthly average) recorded at Poukawa AgResearch daily 0900 hours.

	1993											
Climatic factors	January	February	March	April	May	June	July	August	September	October	November	December
Maximum temperature (°C)	25.39	23.02	21.67	19.01	18.00	16.40	14.03	15.05	15.73	23.91	20.55	24.61
Minimum temperature (°C)	9.60	11.45	8.44	5.44	2.54	2.04	-0.63	-0.29	1.19	4.50	5.22	7.85
10 cm Soil temperature (°C)	20.26	19.71	17.04	13.53	10.69	8.77	7.31	8.82	10.70	15.15	17.13	19.75
Wind run (km/day)	75.13	89.89	81.89	80.76	75.76	46.27	38.76	38.60	38.62	38.63	38.59	38.84
Rainfall (mm)	39	108	83	24	31	32	8	39	59	6	58	76

Table 3.6 Environmental data for seven trials encompassing 2 years and 3 seasons, at 15 d intervals after sowing.

AUTUMN A 1992

Days after sowing	GSWC (%)	Average 10 cm soil temp. (°C)	Total rainfall (mm)	Average maximum temp. (°C)	Average minimum temp. (°C)	Average wind run (km/day)	Total ET_p (mm)	Degree days (°C)
0	37.30	16.30	-	-	-	-	-	-
15	30.17	13.53	37	20.59	1.72	73.87	200.77	50
30	30.81	12.26	43	18.08	4.74	79.47	386.55	32
45	33.64	8.21	83	16.54	0.89	75.20	414.48	2
60	43.70	7.68	20	15.60	3.29	65.98	145.95	6
75	44.15	7.21	30	14.50	2.25	82.02	109.70	1
90	43.96	6.57	147	14.12	2.71	89.26	247.09	0

Table 3.6 cont'd

AUTUMN B 1992

Days after sowing	GSWC (%)	Average 10 cm soil temp. (°C)	Total rainfall (mm)	Average maximum temp. (°C)	Average minimum temp. (°C)	Average wind run (km/day)	Total ET_p (mm)	Degree days (°C)
0	29.41	18.2	-	-	-	-	-	-
15	32.29	14.33	53	19.21	0.34	83.16	231.87	76
30	30.80	10.53	7	16.59	1.64	46.13	209.19	43
45	33.49	9.43	24	15.71	5.02	80.16	435.82	32
60	38.15	8.62	54	14.78	0.78	76.65	388.47	3
75	43.25	8.01	75	14.11	2.53	69.12	111.87	6
90	43.28	7.25	20	13.31	2.10	81.65	104.33	0

Table 3.6 cont'd

WINTER A 1992

Days after sowing	GSWC (%)	Average 10 cm soil temp. (°C)	Total rainfall (mm)	Average maximum temp. (°C)	Average minimum temp. (°C)	Average wind run (km/day)	Total ET_p (mm)	Degree days (°C)
0	40.06	9.10	-	-	-	-	-	-
15	43.94	5.91	12	14.89	2.60	83.19	101.28	1
30	41.25	6.35	36	13.69	2.79	91.61	102.41	0
45	45.87	5.70	148	13.65	3.97	93.64	107.36	4
60	41.08	7.86	29	15.58	3.99	112.70	149.87	2
75	46.84	7.60	122	15.57	2.81	133.08	162.24	3
90	43.43	9.70	134	15.92	4.31	88.65	177.97	13

Table 3.6 cont'd

WINTER B 1992

Days after sowing	GSWC (%)	Average 10 cm soil temp. (°C)	Total rainfall (mm)	Average maximum temp. (°C)	Average minimum temp. (°C)	Average wind run (km/day)	Total ET_p (mm)	Degree days (°C)
0	46.32	7.10	-	-	-	-	-	-
15	43.14	5.82	134	14.06	2.51	98.66	102.41	0
30	47.17	6.10	30	13.72	3.99	90.86	107.36	4
45	42.92	6.93	20	15.69	3.82	118.21	139.21	2
60	48.79	7.37	82	15.30	2.79	144.16	162.25	4
75	44.33	9.45	134	15.57	2.18	96.63	169.23	15
90	47.84	11.20	114	16.54	6.07	119.71	277.92	36

Table 3.6 cont'd

AUTUMN 1993

Days after sowing	GSWC (%)	Average 10 cm soil temp. (°C)	Total rainfall (mm)	Average maximum temp. (°C)	Average minimum temp. (°C)	Average wind run (km/day)	Total ET_p (mm)	Degree days (°C)
0	46.26	19.2	-	-	-	-	-	-
15	36.49	18.5	19	20.61	5.40	73.63	212.32	69
30	41.82	11.3	17	17.00	4.27	83.81	156.58	37
45	42.64	9.3	16	16.78	3.92	80.49	124.02	42
60	41.54	8.9	13	16.76	0.50	70.74	112.48	2
75	42.29	8.3	10	17.47	2.07	40.93	96.71	10
90	41.97	7.3	13	14.53	0.01	39.94	98.22	0

Table 3.6 cont'd

WINTER 1993

Days after sowing	GSWC (%)	Average 10 cm soil temp. (°C)	Total rainfall (mm)	Average maximum temp. (°C)	Average minimum temp. (°C)	Average wind run (km/day)	Total ET_p (mm)	Degree days (°C)
0	42.29	8.3	-	-	-	-	-	-
15	41.97	7.3	15	13.12	0.29	42.05	91.22	2
30	40.63	5.2	10	14.02	-0.38	38.90	102.02	0
45	39.12	7.1	32	13.98	0.29	38.60	101.92	0
60	36.14	6.3	40	15.82	-0.14	38.60	152.06	0
75	40.23	9.6	15	15.59	-0.87	38.59	179.52	15
90	41.59	10.8	6	15.90	1.67	39.60	218.97	31

Table 3.6 cont'd

SPRING 1993

Days after sowing	GSWC (%)	Average 10 cm soil temp. (°C)	Total rainfall (mm)	Average maximum temp. (°C)	Average minimum temp. (°C)	Average wind run (km/day)	Total ET_p (mm)	Degree days (°C)
0	40.23	9.6	-	-	-	-	-	-
15	41.59	10.8	15	16.69	2.28	38.63	227.95	30
30	31.56	17.4	8	18.92	4.23	38.62	256.84	38
45	27.49	19.8	6	21.84	3.64	38.66	402.87	87
60	29.79	13.4	16	20.10	3.61	38.59	360.67	101
75	36.23	15.4	32	20.79	5.64	38.59	374.02	97
90	31.16	18.2	35	22.94	6.67	38.59	468.08	114

Table 3.7 cont'd

Species	Normal seed germination at 20°C (%)	Aged seed germination at 20°C (%)	Moisture content		Seed sown (number m^{-2})	Wt. of 100 seed (g)
			Standard (%)	Aged (%)		
Perennial legumes						
T. hybridum L. Alsike clover	79	-	-	-	633	0.0947
Lotus corniculatus L. Birdsfoot trefoil cv. Grasslands Goldie	82	57	13.4	56.5	610	0.1311
T. ambiguum Bieb. Caucasian clover cv. Grasslands Monaro	41	21	9.2	49.3	1220	0.2081
M. sativa L. Lucerne cv. Grasslands Oranga	87	-	-	-	575	0.2686
L. pedunculatus Cav. Maku lotus cv. Grasslands Maku	90	-	-	-	556	0.0851
T. fragiferum L. Strawberry clover cv. Grasslands Onward	75	57	11.3	52.9	667	0.1544
T. repens L. White clover cv. Grasslands Tahora	81	14	11.4	59.5	617	0.0654

low seed vigour (Table 3.7). The seed weight of subterranean clover was highest (0.7612 g / 100 seeds) and persian clover was lowest (0.0611 g / 100 seeds) among the sown legume species and the normal moisture content of the legume seed was from 8 to 13% (Table 3.7).

3.3.4 Plant density

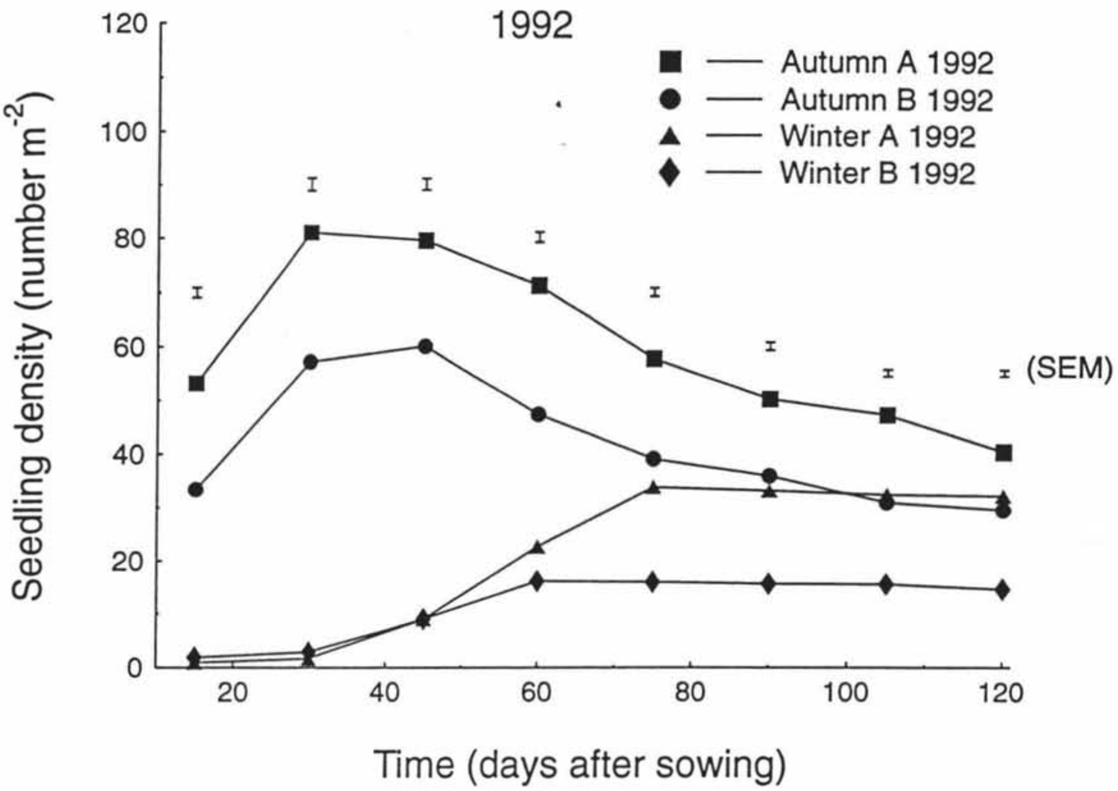
3.3.4.1 Response of seedling establishment over time

Initially the average seedling density of all species was higher ($P<0.05$) for autumn sowings than winter but by 40 DAS the difference had decreased (Figure 3.1). The winter sowings gave less seedlings at the start of the trial, as the maximum seedling density occurred later than for the other sowing conditions. The average maximum seedling density over all species for 1993 (mean 74 m^{-2}) was higher than for 1992 (mean 37 m^{-2}). The spring 1993 sowing had a lower seedling density than the autumn and winter 1993 sowings (Figure 3.1B). The maximum seedling density recorded was between 20 to 40 DAS for autumn season sowings and between 30 to 60 DAS for winter season sowings (Figure 3.1). The rate of net seedling appearance was greatest (3.23 seedling m^{-2} d^{-1}) in autumn and was least (0.75 seedling m^{-2} d^{-1}) in winter sowing conditions.

3.3.4.2 Response of legume species over time

The interaction between the legume species and sowing conditions was highly significant ($P<0.001$). Subterranean clover had the highest seedling density (>100 m^{-2}) at 30 DAS in all the autumn sowings but seedlings failed to appear by 30 DAS in all the winter sowings (Tables 3.8 to 3.14). Arrow leaf clover had the highest seedling density in winter A 1992, and winter and spring 1993 sowings (Tables 3.10, 3.13 and 3.14). Persian clover had the lowest seedling density in the autumn sowings (Tables 3.8, 3.9 and 3.12) whereas in winter sowings it had a higher seedling density than some species ($P<0.05$; Tables 3.10, 3.11 and

(A)



(B)

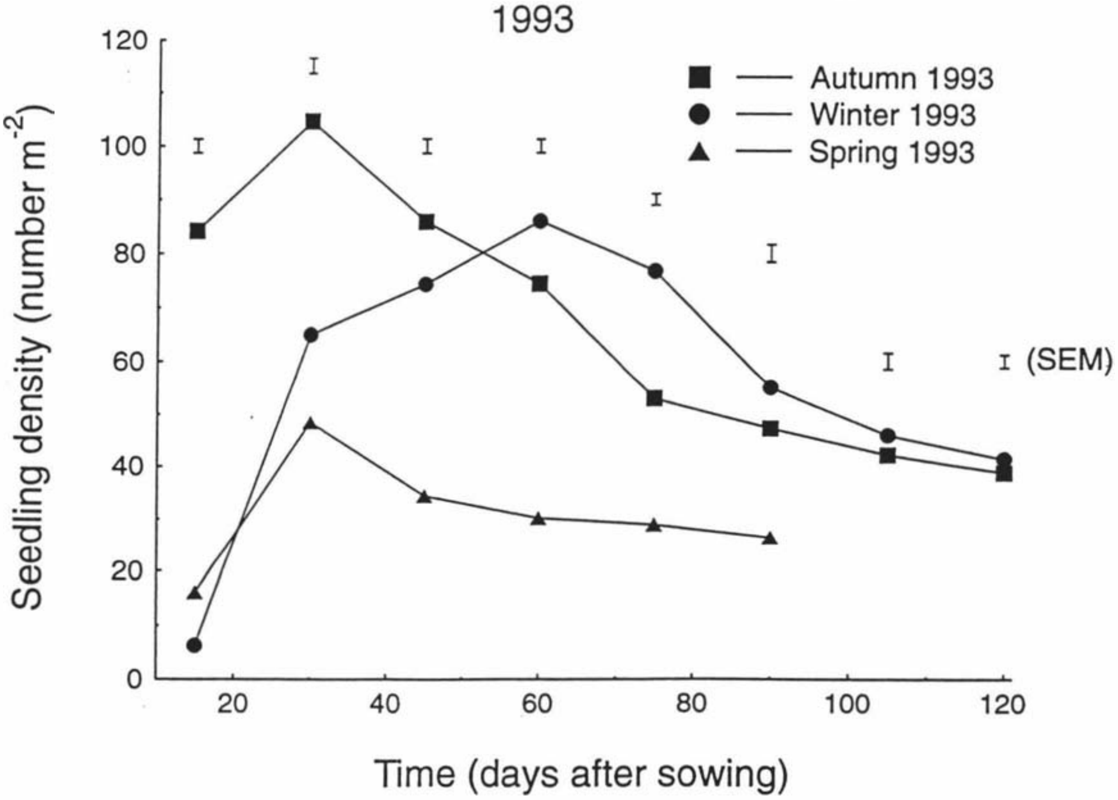


Figure 3.1 Seedling number per unit area for eleven legume species (A) under different sowing conditions in 1992 and for eight legume species (B) under different sowing conditions in 1993.

Table: 3.8 Responses of seedling density (plant m^{-2}) over time in **Autumn A 1992**.

Species	Days after sowing							
	15	30	45	60	75	90	105	120
Annual legumes								
Subterranean clover	149	152	160	125	76	74	70	55
Arrow leaf clover	-	-	-	-	-	-	-	-
Persian clover	18	28	38	41	36	32	30	26
Barrel medic	100	149	127	105	87	73	72	65
Murex medic	46	85	77	79	64	55	50	45
Perennial legumes								
Alsike clover	26	42	38	38	36	32	26	21
Birdsfoot trefoil	80	99	80	71	60	57	56	51
Caucasian clover	19	43	46	57	57	43	41	38
Lucerne	45	109	115	90	69	59	57	47
Maku lotus	13	56	62	53	49	40	34	29
Strawberry clover	38	49	54	54	45	38	36	28
SEM	8.4	7.7	7.2	6.1	5.0	4.7	4.5	4.1
Significance	***	***	***	***	***	***	***	***

***: P<0.001

Table: 3.9 Responses of seedling density (plant m^{-2}) over time in **Autumn B 1992**.

Species	Days after sowing							
	15	30	45	60	75	90	105	120
Annual legumes								
Subterranean clover	87	106	93	67	54	52	48	47
Arrow leaf clover	14	28	55	42	35	33	28	27
Persian clover	15	33	38	26	23	21	17	16
Barrel medic	81	113	108	87	76	67	57	53
Murex medic	62	91	89	66	54	52	44	43
Perennial legumes								
Alsike clover	11	37	40	35	28	26	22	21
Birdsfoot trefoil	26	56	60	45	37	36	32	31
Caucasian clover	2	13	28	37	28	22	20	19
Lucerne	35	70	72	51	44	40	35	33
Maku lotus	11	29	30	26	20	17	11	10
Strawberry clover	24	55	48	39	32	30	26	25
SEM	8.4	7.7	7.2	6.1	5.0	4.7	4.5	4.1
Significance	***	***	***	***	***	***	***	***

Table: 3.10 Responses of seedling density (plant m^{-2}) over time in **Winter A 1992**.

Species	Days after sowing							
	15	30	45	60	75	90	105	120
Annual legumes								
Subterranean clover	0	0	0	9	24	24	23	23
Arrow leaf clover	2	3	17	35	45	45	45	44
Persian clover	0	0	6	28	36	35	35	35
Barrel medic	4	8	16	24	30	29	29	29
Murex medic	3	4	10	17	23	23	22	21
Perennial legumes								
Alsike clover	0	0	8	26	37	37	35	35
Birdsfoot trefoil	1	3	24	43	67	66	64	63
Caucasian clover	0	0	0	22	31	30	28	28
Lucerne	0	0	5	11	20	19	19	19
Maku lotus	0	0	0	8	17	17	17	17
Strawberry clover	1	3	13	28	44	44	42	41
SEM	8.4	7.7	7.2	6.1	5.0	4.7	4.5	4.1
Significance	***	***	***	***	***	***	***	***

Table: 3.11 Responses of seedling density (plant m^{-2}) over time in **Winter B 1992.**

Species	Days after sowing							
	15	30	45	60	75	90	105	120
Annual legumes								
Subterranean clover	0	0	0	1	1	1	1	1
Arrow leaf clover	1	2	8	13	12	12	12	12
Persian clover	0	0	8	19	19	19	19	18
Barrel medic	0	0	0	1	1	1	1	1
Murex medic	0	0	1	2	2	2	2	2
Perennial legumes								
Alsike clover	9	12	18	28	27	26	26	23
Birdsfoot trefoil	11	17	33	54	53	52	51	47
Caucasian clover	0	0	6	11	11	11	11	11
Lucerne	1	1	3	8	8	8	8	6
Maku lotus	0	0	16	28	28	27	27	24
Strawberry clover	0	0	8	16	16	16	16	15
SEM	8.4	7.7	7.2	6.1	5.0	4.7	4.5	4.1
Significance	***	***	***	***	***	***	***	***

Table: 3.12 Responses of seedling density (plant m^{-2}) over time in **Autumn 1993**.

Species	Days after sowing							
	15	30	45	60	75	90	105	120
Annual legumes								
Subterranean clover	180	175	145	125	90	82	79	77
Arrow leaf clover	94	145	123	110	75	67	60	58
Persian clover	49	70	89	80	64	59	47	37
Perennial legumes								
Alsike clover	85	89	37	30	23	19	18	16
Birdsfoot trefoil	-	-	-	-	-	-	-	-
Caucasian clover	25	38	40	35	25	21	16	12
White clover	54	88	98	87	65	59	54	51
Strawberry clover	53	69	50	40	30	25	22	21
SEM	7.4	8.3	7.5	7.2	6.0	6.2	4.4	4.1
Significance	***	***	***	***	***	***	***	***

Table: 3.13 Responses of seedling density (plant m^{-2}) over time in **Winter 1993**.

Species	Days after sowing							
	15	30	45	60	75	90	105	120
Annual legumes								
Subterranean clover	0	4	6	5	5	4	3	3
Arrow leaf clover	45	145	151	138	128	89	76	71
Persian clover	6	45	52	72	67	57	51	44
Perennial legumes								
Alsike clover	0	94	106	124	105	65	52	47
Birdsfoot trefoil	0	72	89	116	104	80	77	74
Caucasian clover	0	51	65	76	58	21	14	11
White clover	0	44	51	60	58	57	36	33
Strawberry clover	0	66	76	98	91	68	57	48
SEM	7.4	8.3	7.5	7.2	6.0	6.2	4.4	4.1
Significance	***	***	***	***	***	***	***	***

Table: 3.14 Responses of seedling density (plant m^{-2}) over time in **Spring 1993**.

Species	Days after sowing					
	15	30	45	60	75	90
Annual legumes						
Subterranean clover	14	39	33	30	29	27
Arrow leaf clover	40	96	67	59	57	52
Persian clover	10	21	15	11	11	11
Perennial legumes						
Alsike clover	16	33	27	26	24	22
Birdsfoot trefoil	15	101	75	67	65	56
Caucasian clover	5	19	7	4	4	4
White clover	14	35	25	20	19	18
Strawberry clover	17	42	28	24	23	21
SEM	7.4	8.3	7.5	7.2	6.0	6.2
Significance	***	***	***	***	***	***

3.13). At 120 DAS subterranean clover, barrel medic, and arrow leaf and white clovers had the greatest seedling densities following the autumn sowings (Tables 3.8, 3.9 and 3.12), whereas birdsfoot trefoil, alsike and strawberry clovers had the greatest seedling densities following the winter sowings (Tables 3.10, 3.11 and 3.13). Over all the sowing conditions birdsfoot trefoil and strawberry clover had the highest seedling densities at 90 DAS among the perennial legume species. Among the perennials lucerne had the greatest seedling density (>110 seedlings m^{-2}) in both the sowings in autumn 1992 but by 120 DAS its seedling density had decreased to be not significantly different from birdsfoot trefoil and strawberry clover (Tables 3.8 and 3.9). White clover had the highest ($P<0.05$) seedling density of the perennials when sown in autumn 1993 but in winter and spring 1993 it had relatively lower seedling densities (Tables 3.12 to 3.14). Birdsfoot trefoil and arrow leaf clover had higher and significantly different seedling densities in the winter and spring sowings of 1993 (Tables 3.13 and 3.14). Initially the seedling density of caucasian clover was lower than other species but 60 DAS the seedling density was higher than for alsike and Maku lotus in autumn sowings (Tables 3.8, 3.9 and 3.12). The plant density from the spring 1993 sowing was for only 90 days because in December 1993 the herbage mass was cut for botanical composition measurements for all 1993 trials.

The interactions of time x sowing conditions, time x species and time x sowing conditions x species were all highly significant ($P<0.001$).

3.3.5 Seedling survival from viable seed over time

The average maximum seedling survival of seven legume species was 7 and 15% for the years 1992 and 1993, respectively (Figure 3.2) and occurred at 30 DAS for 1993 and 60 DAS for 1992. Among all the sowing conditions, autumn 1993 gave the maximum seedling survival (22%) 30 DAS, but at 90 DAS the winter 1993 seedling survival was higher ($P<0.05$; Figure 3.3). The lowest seedling survival was for the winter sowings of 1992 and spring sowings of 1993

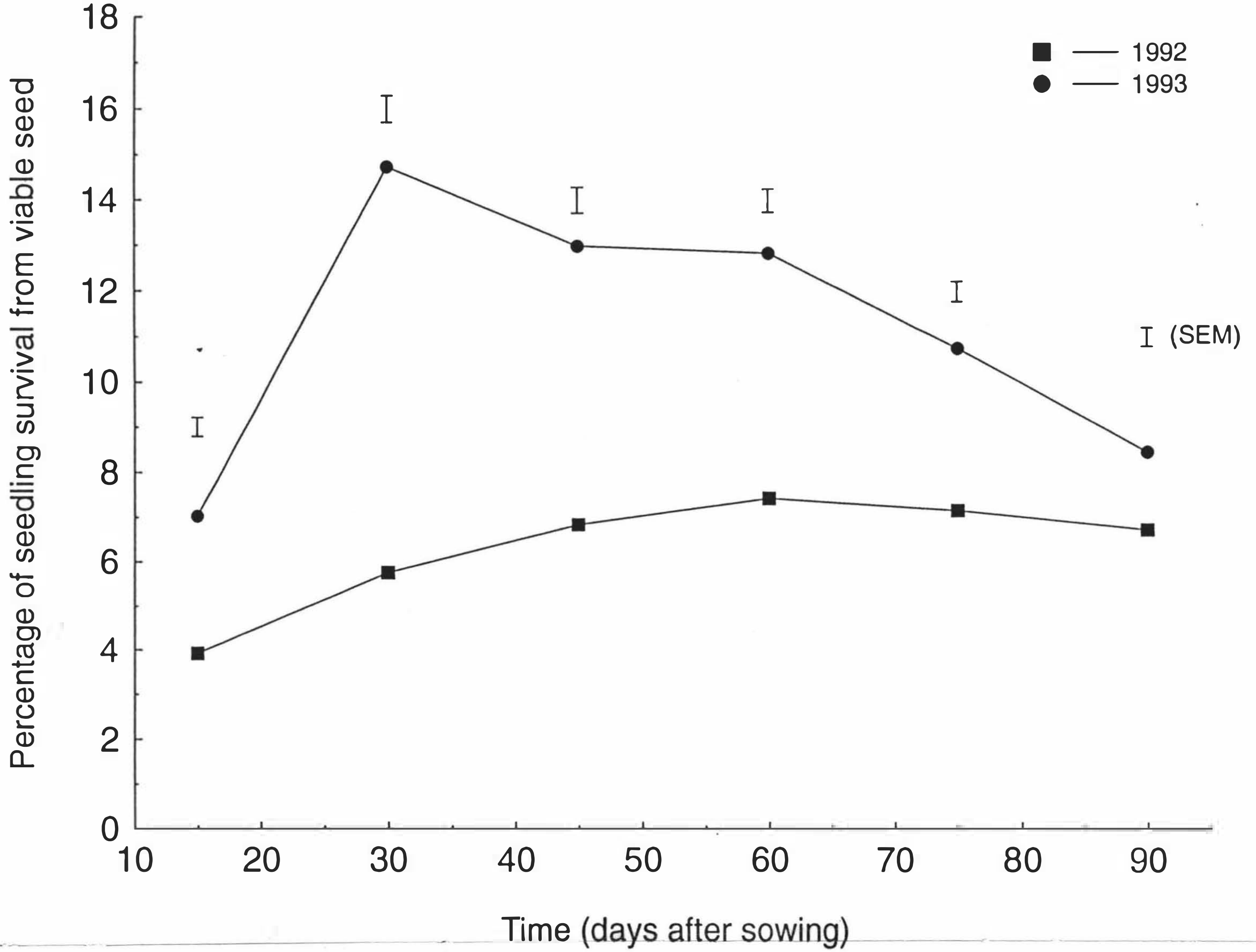


Figure 3.2 Percentage of seedling survival from viable seed sown of seven legume species and seven sowing conditions in 1992 and 1993.

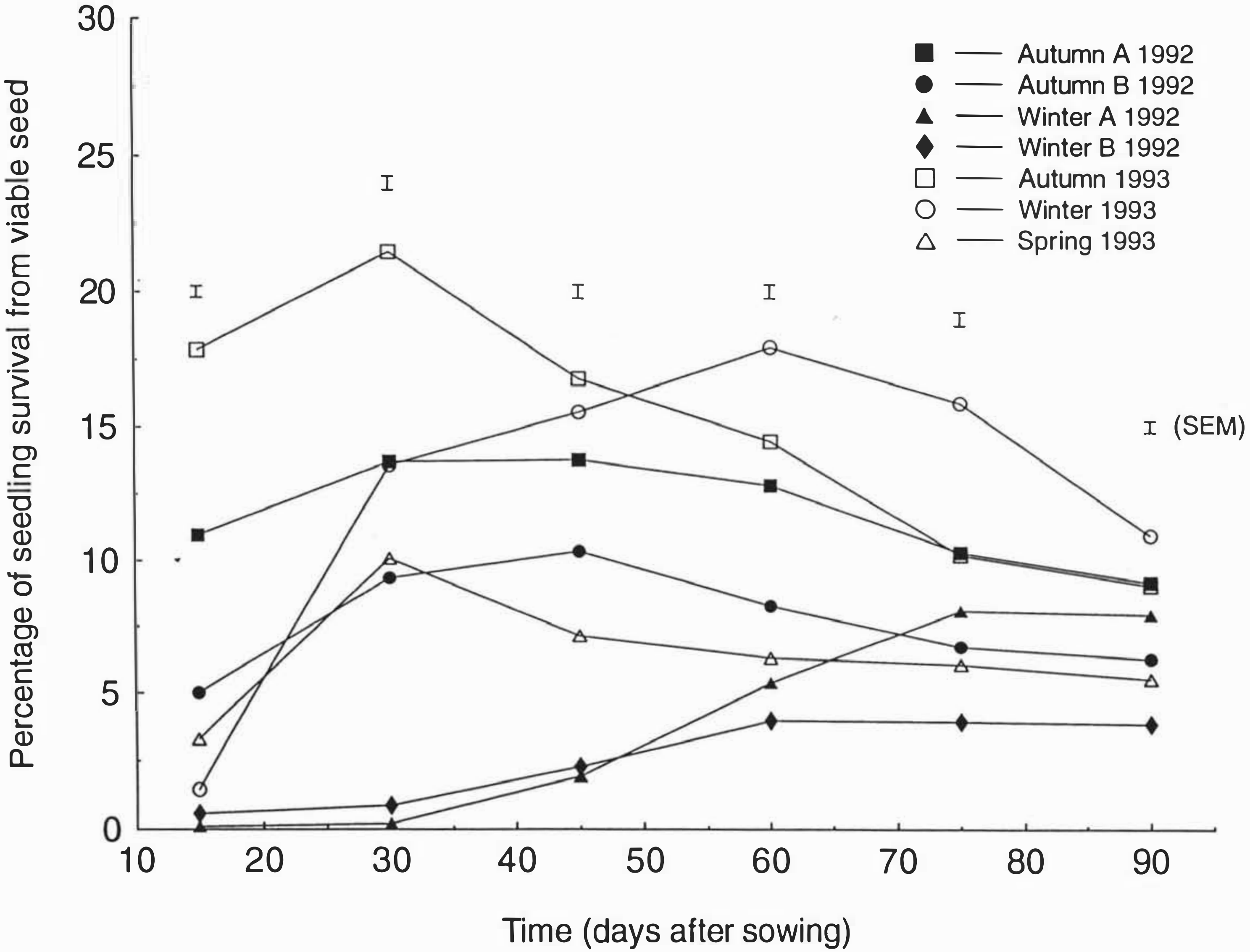


Figure 3.3 Percentage of seedling survival from viable seed sown of seven sowing conditions over legume species and time.

(Figure 3.3). Of the seven oversown legume species common to both 1992 and 1993, initially subterranean clover gave a significantly higher (P<0.05) percentage of seedling survival from viable seed, but at 90 DAS the highest survivals were for birdsfoot trefoil and arrow leaf clover (Figure 3.4). The lowest survival of seedlings from viable seed was for caucasian clover (P<0.05). There was no significant difference in the seedling survival of persian, alsike and strawberry clovers (6 - 7%; Figure 3.4).

3.3.6 Herbage yield and botanical composition

The contribution of oversown species to total dry matter was significantly different between sowing conditions (P<0.05; Figures 3.5 - 3.7). Herbage mass measurements were made 8, 6 and 3 months after sowing for autumn, winter and spring sowing conditions, respectively (Table 3.3). Herbage mass was measured in December following each sowing. Of the annual legume species sown in 1992, persian clover gave the highest December herbage contribution (9%) following winter A 1992 sowing, whereas all other species contributed less than 3% of December herbage mass following all other sowings (Figure 3.5). Among the perennial legumes, alsike and strawberry clovers produced more than 5% of the total herbage mass for the winter B 1992 sowing and birdsfoot trefoil produced nearly 3% for the winter sowing of 1992 (Figure 3.6). Lucerne and caucasian clover contributed less than 1% to December herbage mass during different sowing seasons of 1992 (Figure 3.6).

e contribution of the oversown legumes species to yield was higher following 3 sowings than 1992. All the legume species produced more than 14% of the erbage mass for the spring 1993 sowing, and the arrow leaf clover herbage vas particularly high (35%; P<0.05; Figure 3.7). Arrow leaf, persian, alsike ucasian clovers produced less than 1% of the total herbage mass for the 1993 sowing whereas arrow leaf, persian, white and strawberry clovers than 8% of the total herbage for the winter 1993 sowing (Figure 3.7).

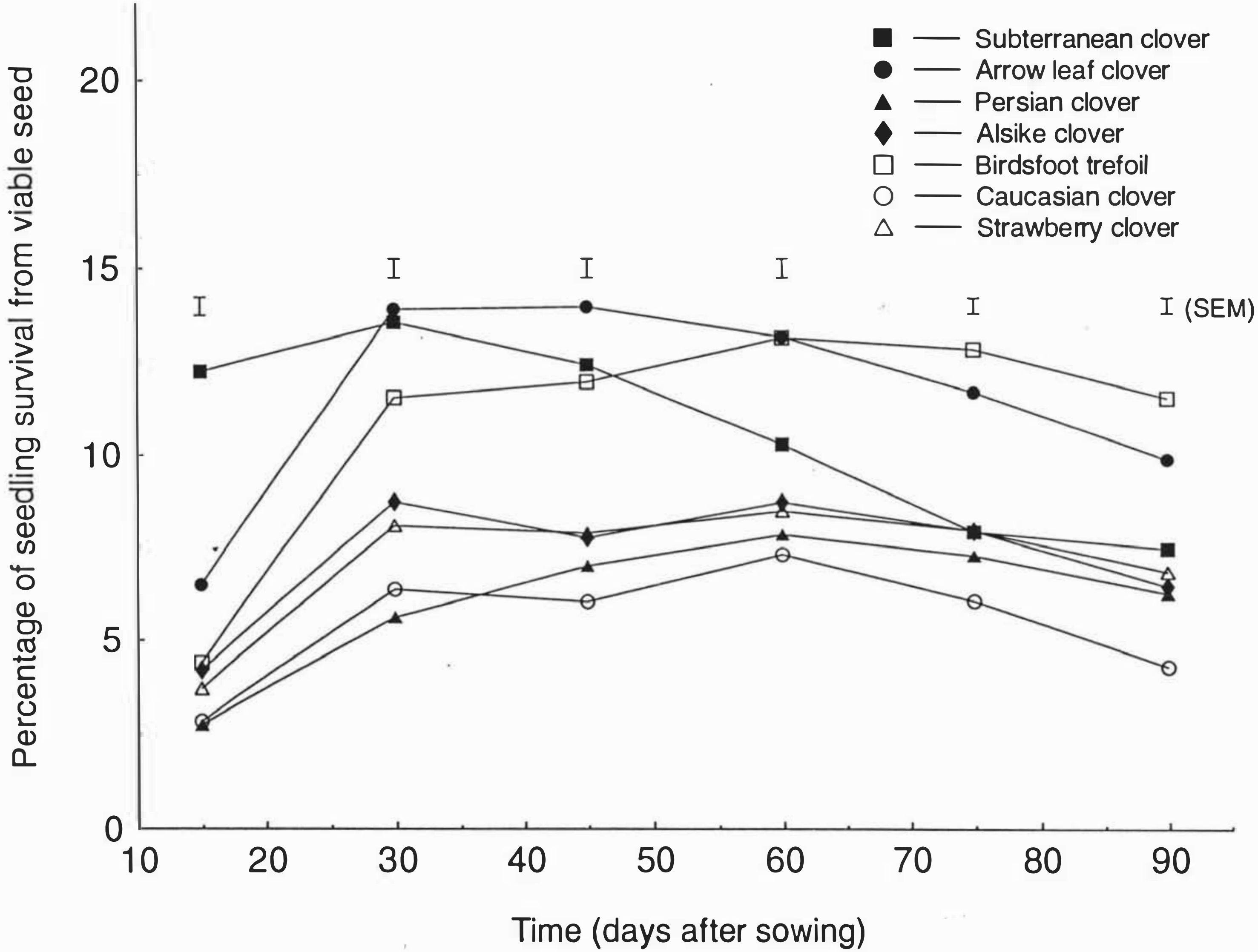


Figure 3.4 Percentage of seedling survival from viable seed sown of seven legume species over sowing conditions and time.

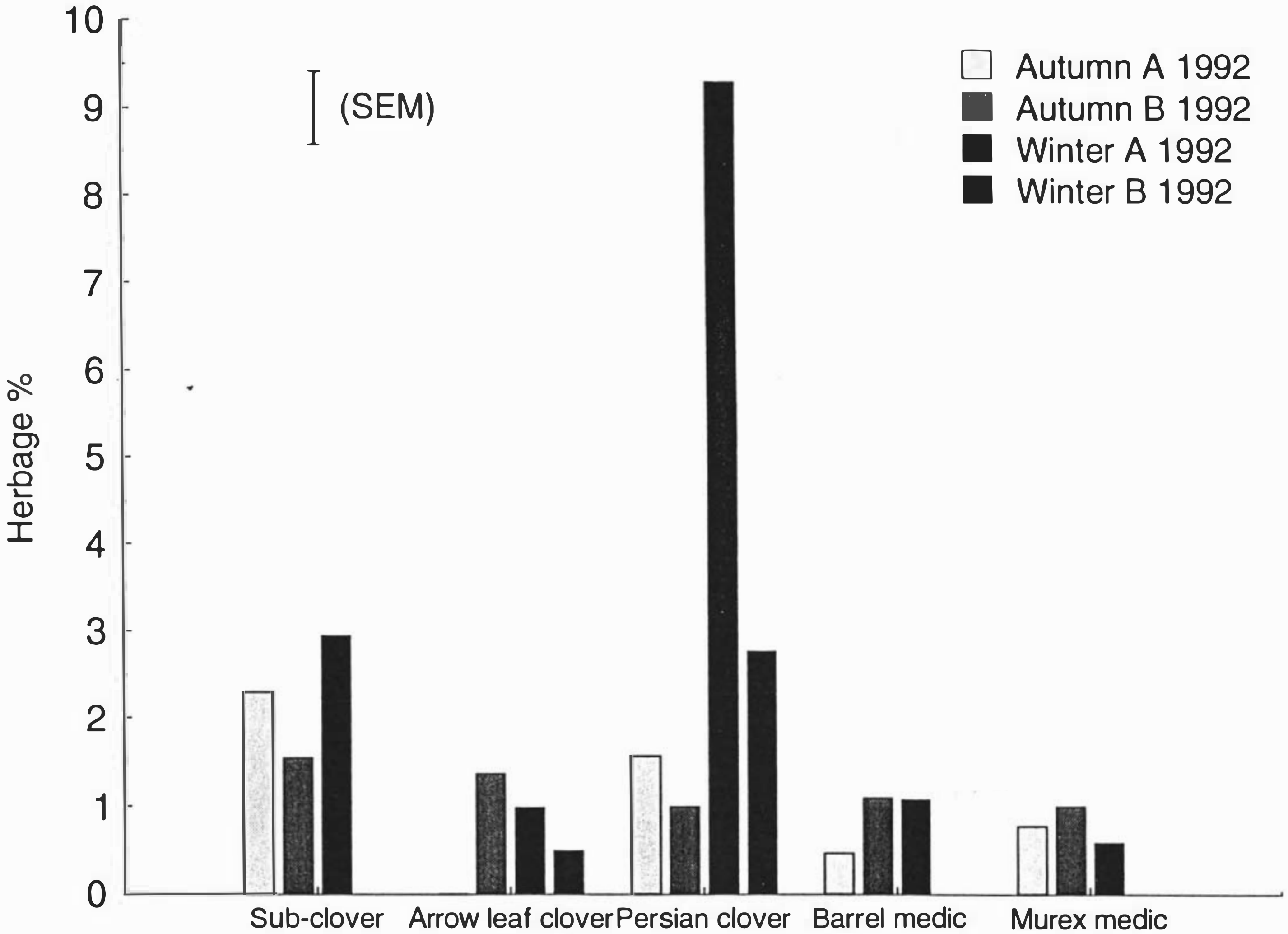


Figure 3.5 The percentage contribution to December herbage mass of five annual legume species under different sowing conditions in 1992.

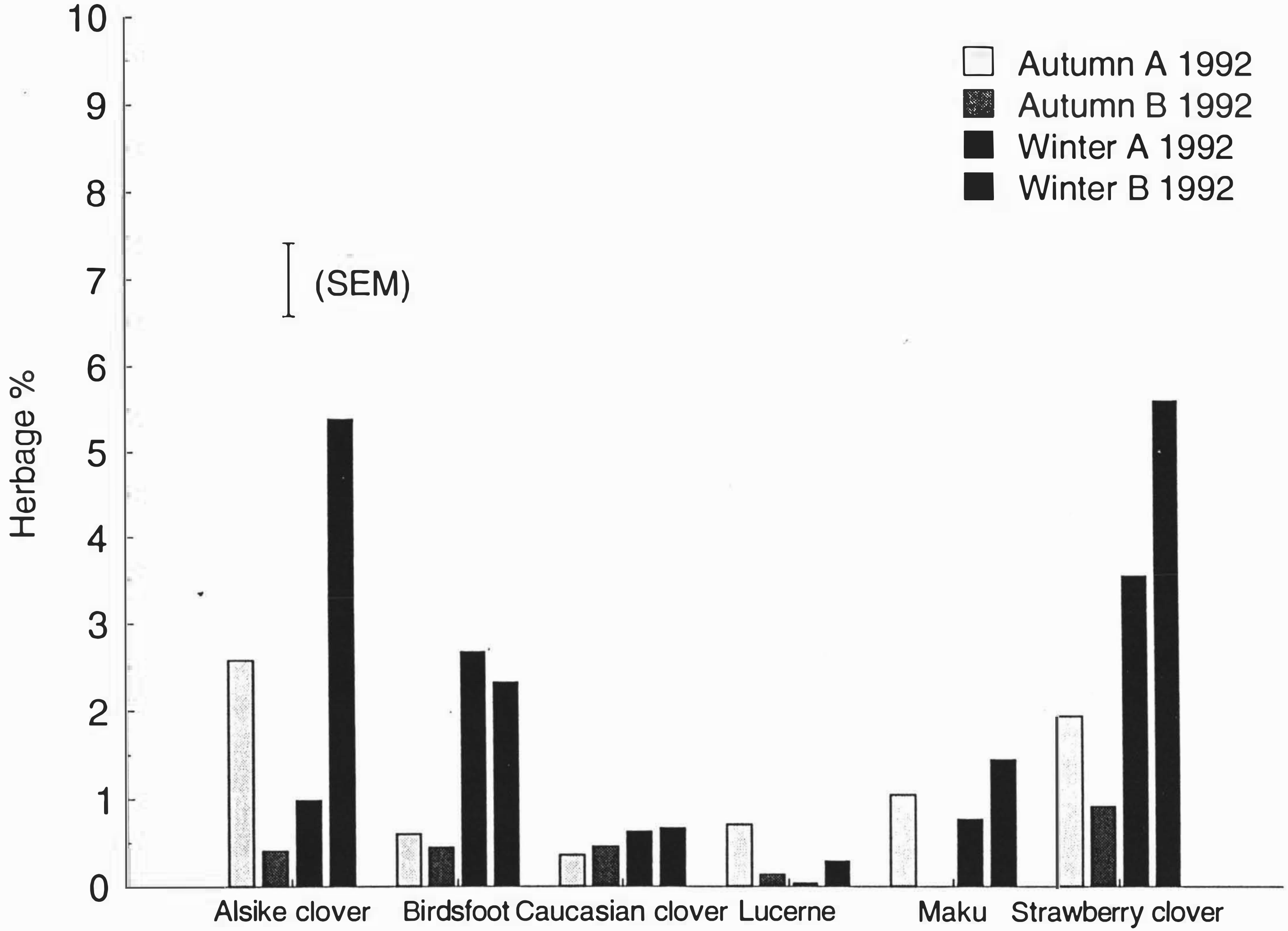


Figure 3.6 The percentage contribution to December herbage mass of six perennial legume species under different sowing conditions in 1992.

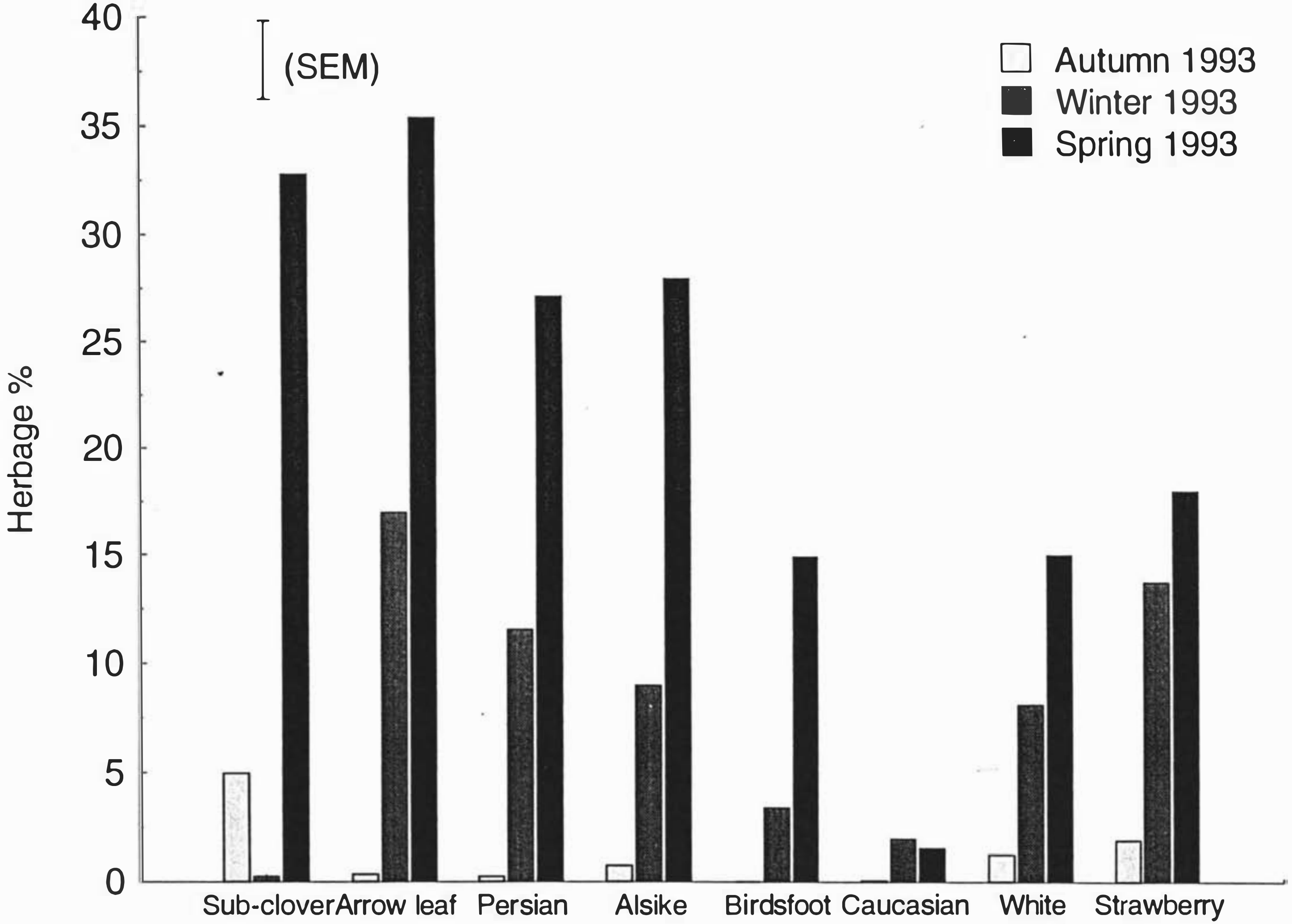


Figure 3.7 The percentage contribution to December herbage mass of eight legume species under different sowing conditions in 1993.

3.3.7 Early plant development

Plant development was approximately linear over time (R^2>98%) during the trials (Figure 3.8). Initial plant development following the autumn sowings was higher than the winter sowings but by 90 DAS this was reversed (Figure 3.8). There was a significant difference between the slopes of the two regression lines (P<0.001) showing the development rate for 90 DAS following winter sowing was greater than following autumn sowing. Of the annual legumes, murex and barrel medics and subterranean clover plant development rates were fastest following the autumn sowings (Figure 3.9), whereas subterranean clover development rate was the slowest of all the species following winter sowing. At 120 DAS persian clover development rate was slowest following autumn sowings but was similar to other annuals in winter sowing (Figures 3.9).

Among the perennial legumes, the plant development rate of birdsfoot trefoil was higher (P<0.05) and of caucasian clover was slower than all other species following both autumn and winter sowings (Figure 3.10). Initial plant development of Maku lotus, lucerne, strawberry and alsike clovers was similar (P<0.05) following autumn sowing, but by 120 DAS lucerne plant development rate was the fastest and Maku lotus was the slowest (Figure 3.10).

3.3.8 Influence of environmental factors

3.3.8.1 Response of environmental factors to sowing conditions over legume species and time

The four environmental factors, GSWC, soil temperature, minimum air temperature and wind run were significantly (P<0.05) related to seedling survival for all the sowing conditions except Autumn B 1992 (Table 3.15). Although the coefficient of determination (R^2) was not very high these factors were significantly related to seedling survival. Overall, the relationship of GSWC and soil

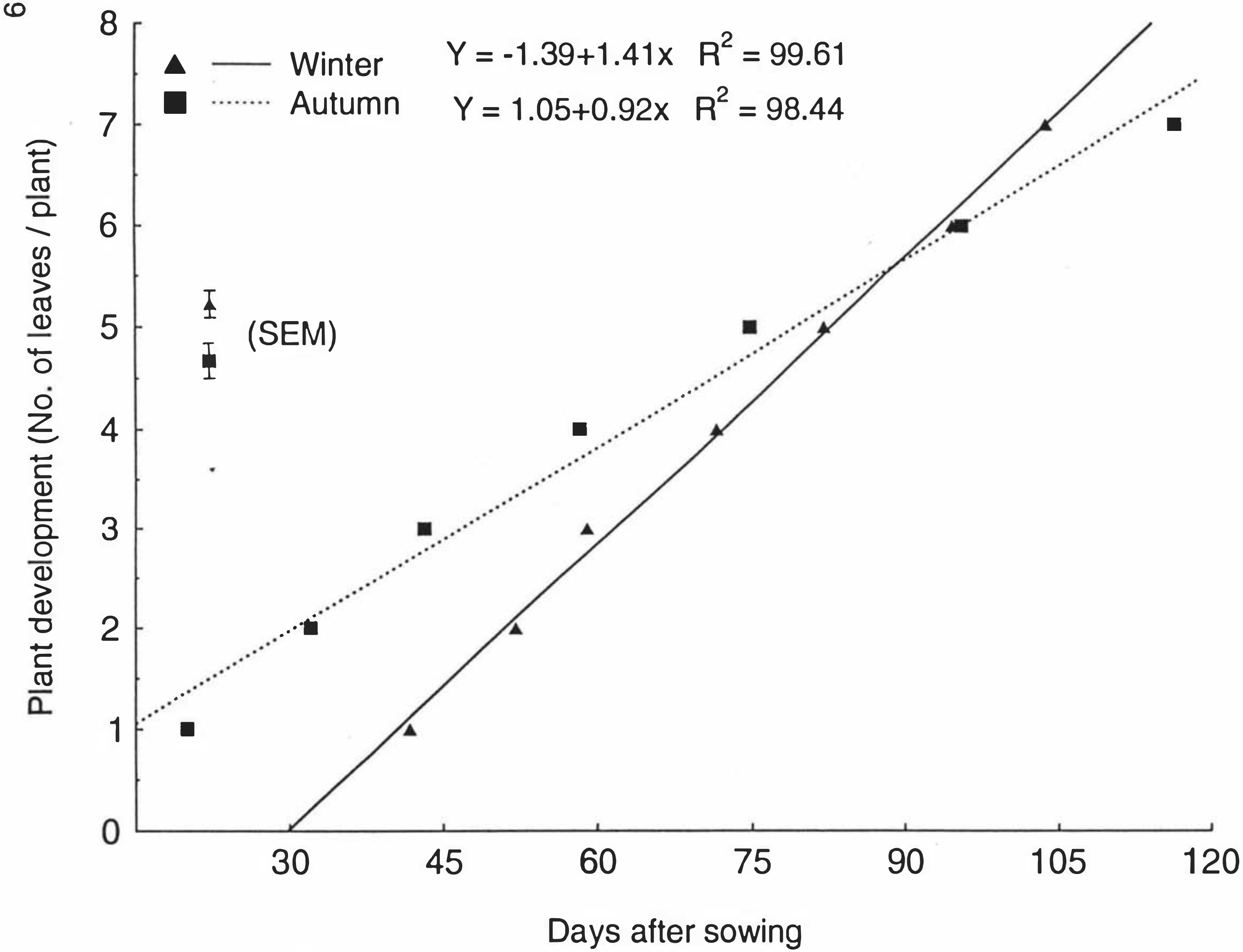


Figure 3.8 The relationship of plant development over time for eleven legume species during autumn and winter sowing conditions.
(1=cotyledon, 2=spade leaf, 3=1st trifoliate leaf, 4=2nd trifoliate leaf, etc.)

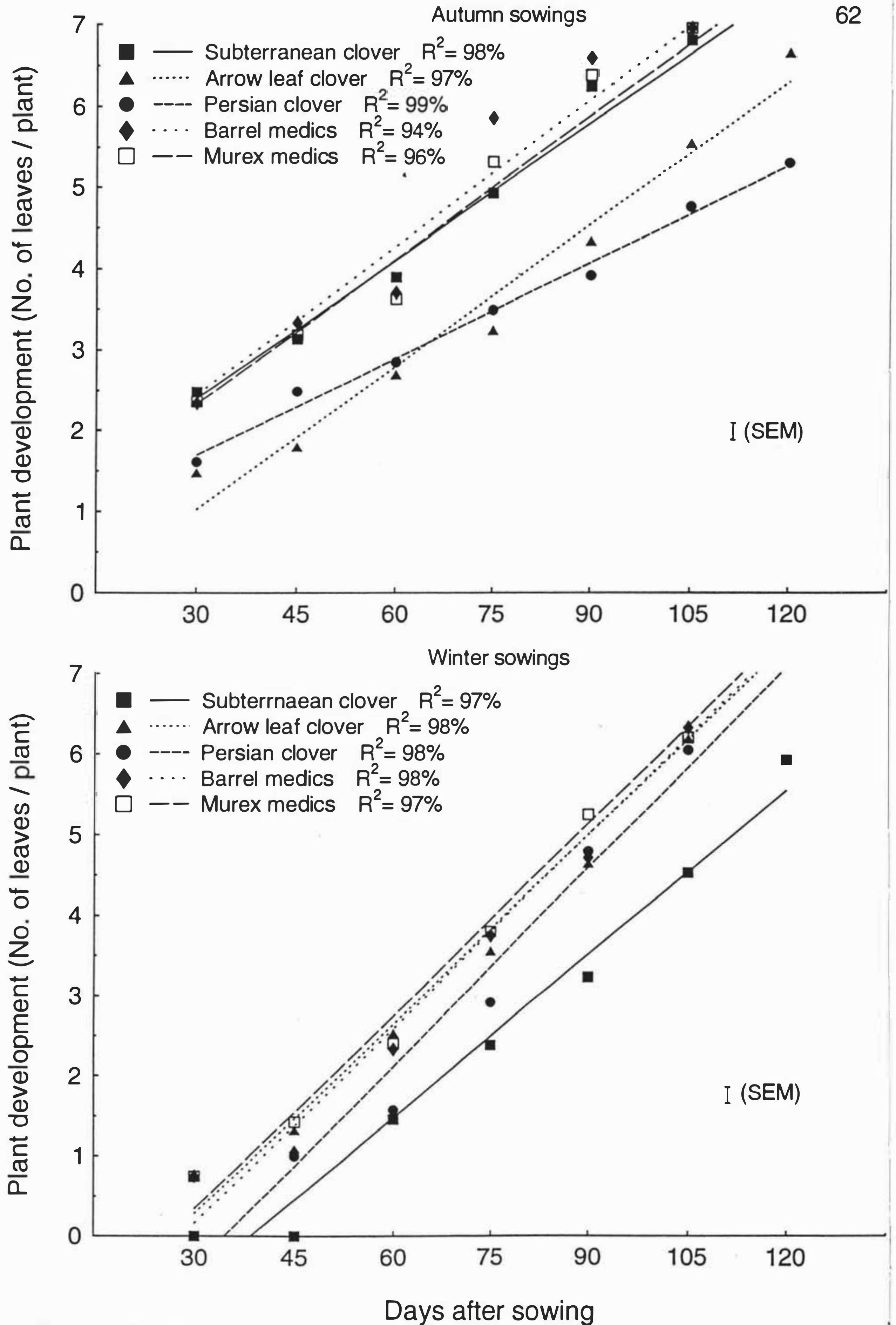


Figure 3.9 The relationship of plant development over time for five annual legume species following autumn and winter sowing.

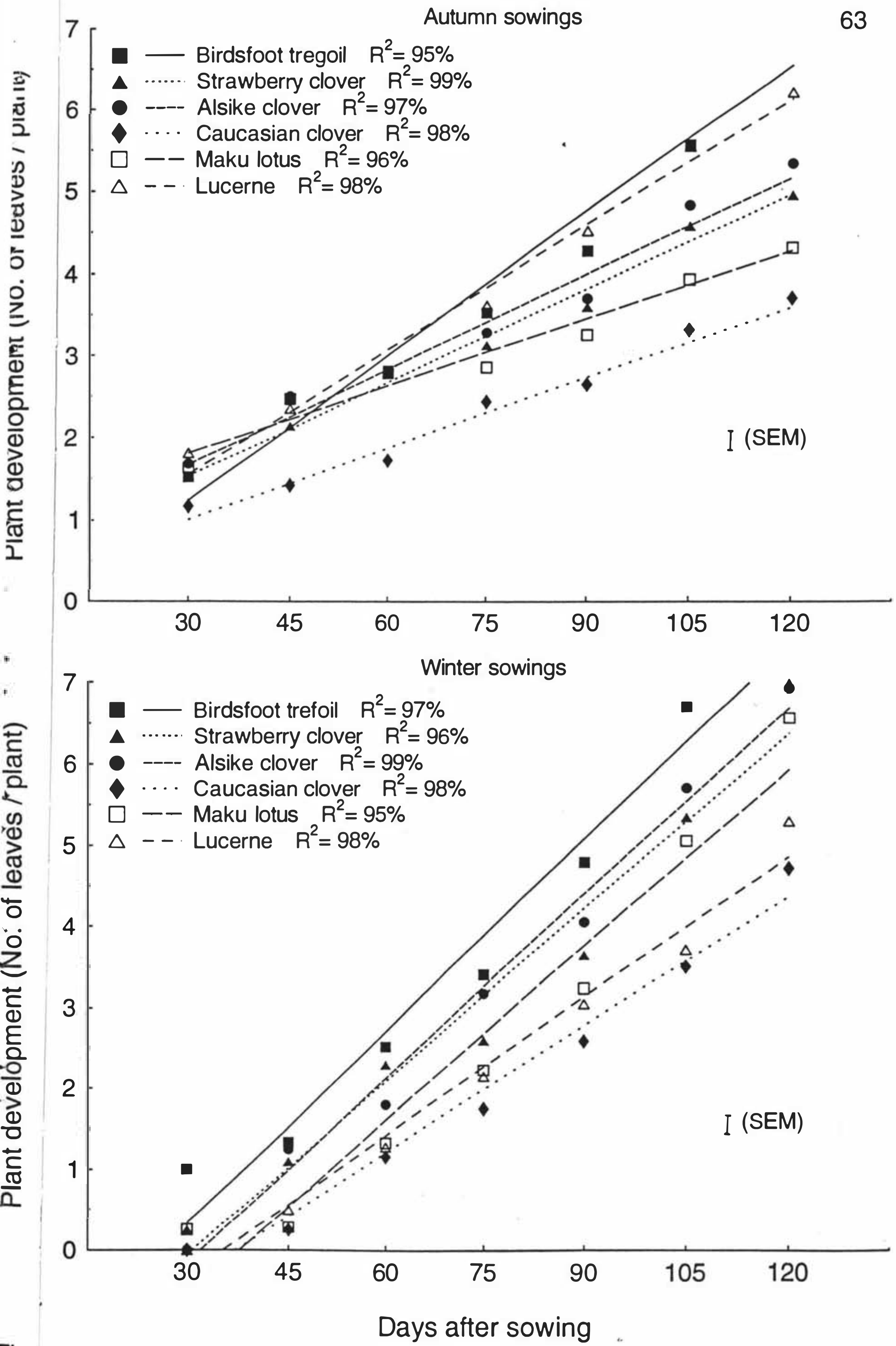


Figure 3.10 The relationship of plant development over time for six perennial legume species following autumn and winter sowing.

Table 3.15 The standardized regression coefficients, R^2 and significance levels of different environmental factors related to different sowing conditions.

Sowing conditions	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Autumn A 1992	-0.2696	-0.1593	0.1955	-0.0809	9	***
Autumn B 1992	0.0760	0.2557	-0.2617	01233	3	ns
Winter A 1992	0.5846	0.6877	-0.3489	0.5488	67	***
Winter B 1992	0.0466	0.4203	-0.1727	0.1956	20	***
Autumn 1993	0.9122	1.2384	-0.2568	0.2392	12	**
Winter 1993	-0.1250	-0.0002	-0.1291	-0.4591	33	***
Spring 1993	0.0255	0.4282	-0.2377	-0.2127	9	***

**:P<0.05; ***:P<0.001; ns: No significant difference temperature to seedling survival was higher than minimum temperature and wind run but all these factors varied in their relationship to seedling survival over sowing conditions.

3.3.8.2 Response of environmental factors to legume species over sowing conditions and time

The environmental factors were significantly related to seedling survival for all the legume species except caucasian clover ($P<0.01$; Table 3.16). Caucasian clover was least responsive to the environmental factors (Table 3.16) and its seedling survival was also very low compared to the other species (Tables 3.8 to 3.14).

3.3.8.3 Response of environmental factors to time over legume species and sowing conditions

All the environmental factors were significant over the time of the experiment ($P<0.05$; Table 3.17). Overall these environmental factors indicated strong general trends as they were significantly related to seedling survival over 90 days of the trial and also over seven species and seven sowing conditions.

The standardized coefficients of the slopes for the four environmental factors over time for the seven sowing conditions over seven species is given in Appendix 3.1 and for the individual species over time is given in Appendix 3.2.

3.3.8.4 Standard regression lines

The average relationship of the four environmental factors to seven sowing conditions and seven legume species showed that there was a high negative and positive relationship between seedling survival and GSWC and soil temperature, respectively, over the initial stages of the seed germination phase (i.e. 0 - 15 DAS; Figure 3.11). Minimum temperature and wind run showed a high negative

Table 3.16 The standardized regression coefficients, R^2 and significance levels of different environmental factors related to different legume species.

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	-0.3617	0.1169	-0.0488	0.1943	14	***
Arrow leaf clover	0.1334	0.2082	-0.2454	-0.4460	15	***
Persian clover	0.2079	0.1817	-0.3003	-0.1997	15	***
Alsike clover	0.0469	0.0857	-0.2301	-0.2358	13	***
Birdsfoot trefoil	-0.2078	0.1704	-0.1455	-0.1252	19	***
Caucasian clover	0.0814	0.0429	-0.1741	-0.0971	4	ns
Strawberry clover	-0.1412	0.0062	-0.2532	-0.2095	19	***

Table 3.17 The standardized regression coefficients, R^2 and significance levels of different environmental factors related over time after sowing the trials.

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	-0.2062	0.3304	-0.2669	-0.0744	38	***
30	0.1891	0.8438	-0.6334	0.2624	13	**
45	-0.2007	0.4144	-0.0007	-0.4658	18	***
60	0.3262	-1.0550	-0.4329	-0.1452	34	***
75	0.3715	0.1790	-0.3662	-0.5033	25	***
90	0.0018	0.3016	-0.5413	0.1022	9	***

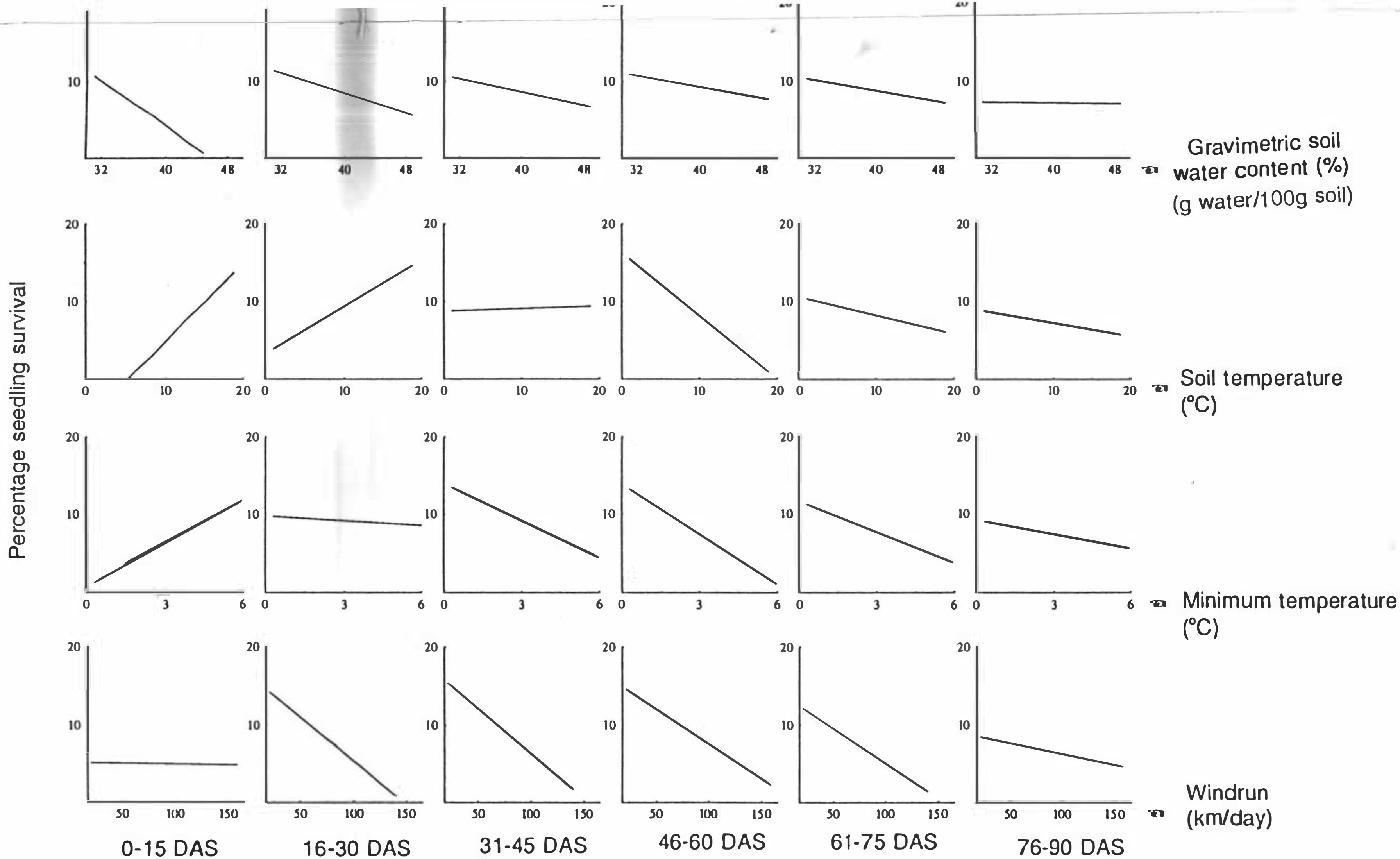


Figure 3.11 The average relationship of different environmental factors to percentage seedling survival from viable seed for seven sowing seasons and seven legume species over time.

relationship with seedling survival in the establishment phase from 30 to 90 DAS (Figure 3.11). Although the coefficient of determinations were not high (Table 3.18), they were significant (P<0.001). The intercept and slope of the regression line and coefficient of determination of GSWC, soil temperature, minimum air temperature and wind run over the time of the experiment are given in Table 3.18.

3.3.9 Survivorship pattern

The pattern of seedling density could be simplified to three phases of survivorship (Figure 3.12). A large set of data was used to determine this survivorship pattem which although having a low coefficient of determination (R^2) was significant (P<0.05). The three lines (Figure 3.12) showed a net increase in seedling density during the seedling appearance phase, a net decrease in density during the seedling survival phase and approximately constant density during the seedling establishment phase.

Table 3.18 The regression line intercept, slope and R^2 of environmental factors over time for seven sowing conditions and seven legume species.

	Environmental factors											
	Gravimetric Soil Water Content (%)			Soil temperature (°C)			Minimum temperature (°C)			Windrun (km/day)		
Days after sowing	Intercept	Slope	R^2 (%)	Intercept	Slope	R^2 (%)	Intercept	Slope	R^2 (%)	Intercept	Slope	R^2 (%)
0-15	33.1145	-0.7231	19.23	-5.3063	1.0073	34.29	0.6826	1.8601	14.80	5.2389	-0.0019	0.00
15-30	21.3358	-0.3222	4.24	3.2089	0.6045	6.49	9.8014	-0.2045	0.13	16.6675	-0.1130	7.07
30-45	17.1393	-0.2132	2.34	8.7438	0.0334	0.03	13.9394	-1.5845	9.34	17.4032	-0.1103	11.94
45-60	16.4773	-0.1795	1.80	16.3104	-0.8130	5.18	13.8684	-2.1452	19.64	16.0467	-0.0845	15.70
60-75	15.8903	-0.1783	0.84	10.5378	-0.2369	1.00	11.6099	-1.3153	16.20	13.6717	-0.0854	9.71
75-90	7.5900	-0.0104	0.01	8.8701	-0.1691	1.62	9.1867	-0.6044	7.62	9.0557	-0.0281	3.15

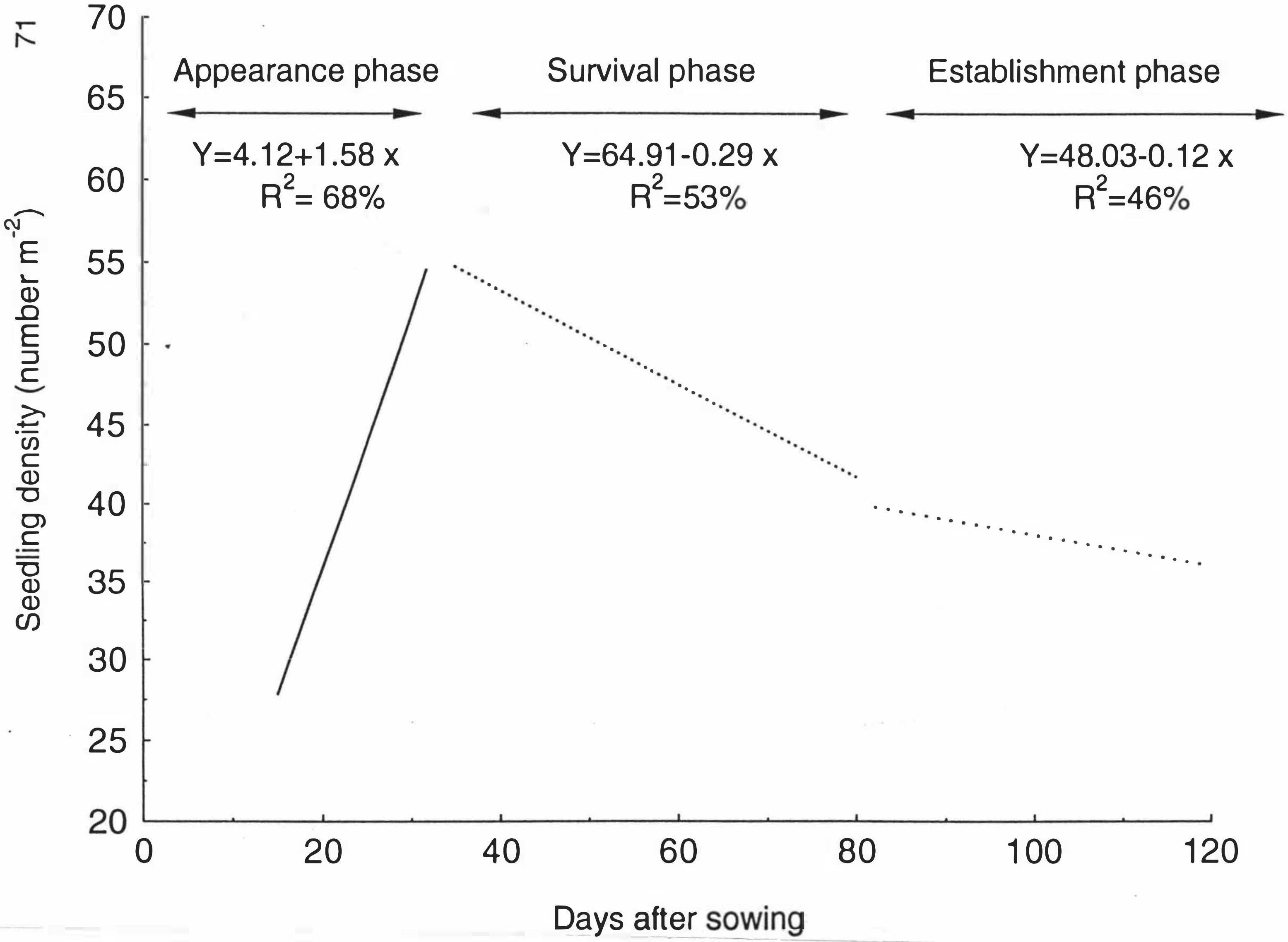


Figure 3.12 The average seedling survivorship pattern over time, for all legume species in all trials (for 500 viable seed sown).

3.4 Discussion

3.4.1 General

The oversowing method used in this series of trials was a small scale simulation of a procedure typically used by farmers when oversowing hill country. This method reduced vegetative competition for the first two to three months using a herbicide and provided an environment conducive to maximum seedling establishment (Wedderbum *et al.*, 1993). There were no major soil nutrient deficiencies (Table 3.1) although the site was of moderate fertility, and the general appearance of seedlings suggested rhizobia infection had occurred and nitrogen was being fixed.

3.4.2 Seedling survival and establishment

Overall, establishment of legume species by oversowing into existing pasture in drought-prone hill country was low but not dissimilar to other studies (Campbell, 1992; Wedderburn *et al.*, 1993; Awan and Kemp, 1994; Figure 3.2). By 30 DAS net seedling appearance (NSA; percent of viable seed sown) was less than 15% of the sown seed, and by 90 DAS, NSA of even the most productive species (subterranean clover) was less than 11% (Figure 3.4). The sowing conditions for autumn 1993 were better than for 1992, with maximum NSA of 20% of viable seed survival, but NSA at 90 DAS was similar for both years at less than 10% (Figure 3.3). The poor success from oversowing legume species into existing dryland hill pasture has also been previously reported by Madden *et al.* (1949); Suckling (1976); Charlton and Brock (1980); Lowther and Patrick (1992) and Campbell (1992). This contrasts with the performance of species established into a clean seedbed or established by transplanting (Woodman *et al.*, 1992). Chapman *et al.* (1985) noticed that most white clover seedlings were lost immediately after sowing and the main cause was environmental stress. Barker and Dymock (1993a) gave the possible reasons for the disappearance of seedlings as the failure of seeds to imbibe, the failure of imbibed seed to germinate, desiccation of fragile seedlings and insect, bird and mollusc predation.

Overall, the survival percentage in 1993 was twice ($P<0.05$) (maximum 15%) that of 1992 sowings (maximum 7%) (Figure 3.2). The rainfall in 1992 was 993 mm whereas 1993 was drier with 563 mm rainfall. The higher survival percentage of legume species during 1993 was probably due to the absence of excessive rainfall and less wind run. Large differences in seedling survival between years were reported by Chapman and Fletcher (1985).

3.4.2.1 Legume species

Subterranean and white clovers, and barrel and murex medics had a higher seedling density following autumn sowings than winter sowings at 120 DAS, confirming the results of Charlton and Giddens (1983) and Chapman and Fletcher (1985) for subterranean and white clovers. The other legume species, that is alsike, persian, strawberry and arrow leaf clovers, had highest seedling densities following winter sowings at 120 DAS. Birdsfoot trefoil was the only species that had a similar seedling density for all the sowing seasons at 120 DAS. Caucasian clover had low seedling density at all the sowing seasons as also noted by Williams and Caradus (1979); Spencer *et al.* (1975) and Stewart (1979). Although lucerne had its highest seedling density following autumn sowing at 30 DAS, it could not withstand competition from the resident pasture and had poor survival at 120 DAS (Groya and Scheaffer, 1981).

Among the annual species, subterranean clover averaged more than 12% of viable seed sown surviving to 120 DAS after autumn sowings. Similarly, Watkin and Vickery (1965) found this species had better establishment than many other legume species. The importance of seed size on the superior establishment of subterranean clover was suggested by Black (1958). The seed mass of 7.6 mg/seed for subterranean clover was the highest of all the 12 legume species oversown (Table 3.7). Watkin and Vickery (1965) found that subterranean clover establishment was higher than white clover, with the percentage establishment being 61 and 29, respectively, on hill country whereas in unploughable plains of Australia, Campbell (1963) found 9% establishment for subterranean clover. Suckling (1954) showed that the highest survival rate was for subterranean clover compared to white clover and lotus major, but subsequent grazing caused losses.

Of the perennial legumes sown in these trials, birdsfoot trefoil and white clover had the highest seedling densities. Similar results were found for white clover by Mitchell (1956) in hill country and by Charlton and Brock (1980) eight months after oversowing in hill country. Charlton (1977) reported white clover establishment of 7.6 - 7.9% of oversown seed after 4 months in North Island hill country. In South Island high country its performance was also superior to that of subterranean and white clover (Lowther, 1980).

In a hill country pasture, seedling appearance of Grasslands Tahora white clover 11 weeks after sowing was only 15% of the seed sown (Chapman *et al.*, 1993). Suckling (1976) recorded 9.0 -10.7% establishment of oversown G. Huia white clover 17 weeks after autumn oversowing whereas Chapman and Fletcher (1985) recorded 37.5 and 51.8% of seedling establishment for G. Tahora and G. Huia white clover, respectively 11 weeks after oversowing in late autumn. Dowling *et al.* (1971) undertook an experiment on establishment of pasture species in the Southern Tablelands, Australia and observed that the establishment percentage of viable seed of subterranean clover cv. Woogenellup and white clover cv. Ladino was 7.5 and 21.3% whereas the survival of seedlings was nil and 20.6%, respectively, 3 months after sowing.

Maku lotus and Caucasian clover seedling survival was lowest in these trials for all the sowing conditions ($P<0.05$). Despite adequate rainfall Maku lotus failed to establish on hill country (Ballantrae) because of strong competition from re-established resident grasses (Charlton and Brock, 1980). Alsike clover production and persistence was inferior to that of white clover in South Island (Allan and Chapman, 1987).

3.4.2.2 Seed vigour

Seed vigour differences between species were a poor determinant of establishment success. Subterranean clover with high seed vigour, white clover with low seed vigour and other species like birdsfoot trefoil, alsike and strawberry clovers with medium seed vigour had high seedling densities. Previously, Hampton and Hill (1990) had found that although the initial seed germination of white clover was 91%, after the accelerated aging (AA) treatment, germination reduced to only 15%. A similar result was found in this trial, 81 and 14%, respectively (Table 3.7). The AA method of testing seed vigour did not assist with prediction of seedling establishment. It was concluded that white clover had higher establishment from the autumn 1993 sowing than strawberry and persian clovers which had an average AA method seed germination of 48%.

The percentage of seedlings that emerge from a given sowing rate in the field cannot be predicted by the seed vigour test because establishment is also dependent on the soil, environment and weather after sowing (Perry, 1987). Perry (1987) also noted that in extremely adverse conditions few seedlings emerged regardless of the level of vigour and, conversely, in favourable conditions emergence may correlate with germination and a vigour test will show no advantage. Hampton and Hill (1990), however, found that vigour test results were more strongly related to field emergence than standard germination test results. Results from this study showed that seed vigour may not be relevant for oversown legume seeds in drought-prone areas particularly when comparing species. The moisture content of 8 to 13% for all the legume species used was normal (Wang and Hampton, 1989).

3.4.2.3 Temperature

Temperature is extremely important and variable in New Zealand hill country as there are extremes of not only latitude and altitude but also season and aspect (White, 1990). Germination of all legume species was clearly temperature dependent. Species sown in autumn started germination before 15 DAS whereas the same species did not germinate until 30 DAS from winter sowings (Tables 3.8 to 3.14). The GSWC was adequate (mean=40.3, std. dev.=5.8) for all the sowing conditions but overall the average 10 cm soil temperature for autumn and winter sowings was 15 and 8°C, respectively. Differences in legume seedling establishment were shown to be related directly to temperature variation (Charlton, 1977).

Subterranean and white clovers were the species most sensitive to cold temperature (Campbell *et al.*, 1987; White, 1990). Charlton *et al.* (1986) observed that subterranean clover germinated poorly at 5°C and white clover was less temperature dependent than subterranean clover (Hampton *et al.*, 1987). Adequate surface soil moisture followed by a moist humid period of 4 to 6 weeks in conjunction with a suitable temperature were essential for seedling growth (Hampton *et al.*, 1987). Birdsfoot trefoil germination and survival was high compared to the other species in both autumn and winter sowings which suggested less effect of low temperature on this species. Overall, subterranean and white clovers established well when sown in warmer seasons and birdsfoot trefoil, persian and strawberry clovers established well when sown in cooler seasons (Tables 3.8 to 3.14).

3.4.2.4 Moisture

Soil moisture (100 mm depth) was probably adequate during the germination point of the soil at the trial site were 35 and 15% GSWC, respectively (Scotter *et al.,* 1979). The soil conditions for winter B 1992, autumn 1993 and winter 1993 were too wet (GSWC >42%) at the time of sowing (Table 3.6). It also sometimes became excessively wet during seedling growth under different sowing conditions (Table 3.6). While a high frequency of rainfall after oversowing of pasture legume species on unploughable areas in Australia was beneficial to germination (Campbell *et al.*, 1987). Barker *et al.* (1988) analysed 14 oversowing experiments in New Zealand hill country and found Grasslands Tahora white clover germination was insensitive to rainfall in the week before or 3 weeks after sowing, but was significantly related to rainfall 1 and 2 weeks after sowing. Dowling *et al.* (1971) noted that the surface soil moisture conditions were the key factor in the establishment of aerial sown pastures (see Chapter 5). Little research has previously noted the potentially negative effect of wet soil conditions on seed germination and survival.

Soil water and the vapour pressure deficit in the micro-environment above the soil surface are the dominant factors affecting the germination and establishment of exposed seed under field conditions (McWilliam and Dowling, 1970). Also, water loss from an imbibed seed is an important factor limiting the germination of exposed seeds in the field (McWilliam and Dowling, 1970). Bellotti (1984) observed a high seedling emergence from a cultivated seedbed despite low soil water content, and low emergence from an aerial sown grass species despite favourable soil moisture potential.

3.4.2.5 Early plant development

Initially, plants developed leaves more rapidly after autumn sowings but by 90 DAS the development rate following winter sowing was greater (Figure 3.8). These measurements were carried out only for the development of leaves and not for stolons or roots. Chapman and Fletcher (1985) observed the effect of autumn and spring season sowings on the development rate of white clover not for stolons or roots. Chapman and Fletcher (1985) observed the effect of autumn and spring season sowings on the development rate of white clover cvs. G. Huia, G. Tahora and Kent. They found seedling development rate after spring sowing was more rapid than after autumn sowing and 25% of the seedlings in autumn had stolons 157 DAS as compared to 89 - 90 DAS for the spring sowing. Suckling (1950) observed better initial survival and growth of legume species following autumn sowing. Chapman and Fletcher (1985) suggested the affect on seedling survival rate was probably related to the intensity of competition from the surrounding vegetation. Nevertheless, season had little effect on seedling survival despite probable differences in the intensity of competition from surrounding vegetation (Chapman and Fletcher, 1985).

Establishment was better for species with high development rates. In this study subterranean clover development rate was highest following autumn sowing whereas persian clover development rate was highest following winter sowing. Plant development rate for caucasian clover was low, in both the autumn and winter sowing seasons. Paljor (1973) reported that caucasian clover had a slow relative growth rate and low net assimilation rate. Some of the legume species like subterranean and white clovers have high seedling relative growth rate (Grime and Hunt, 1975).

3.4.2.6 Survivorship pattern

A summary of seedling density over time for all legume species, season and years found three broad phases of development (Tables 3.8 to 3.14; Figure 3.12). These were similar to those identified by Bellotti (1984), and are:-

- Phase A — Radicle emergence and seedling appearance (0-30 DAS);
- Phase B — Net seedling survival (30-90 DAS), resulting from the conflicting processes of seedling death and new seedlings appearing;

Phase C Seedling establishment (90 DAS and onwards).

Winter sowing delayed the radicle emergence and seedling appearance phase to more than 30 DAS, but the general line for this phase in Figure 3.12 came from a large data set of all the species and all the sowing conditions from 0-30 DAS. A more stable seedling density was reached at 90 DAS (Figure 3.12). Harper (1977) identified that the dominant factors controlling seedling survival were fairly constant and suggested that it was useful to consider the sum of all the constraints controlling seedling survival as a filter. This filter reduced the seedling density from the start of the seedling survival phase to the end of the seedling establishment phase (Bellotti, 1984). The maximum seedling density occurred at 30 DAS for all species and sowing conditions (Figure 3.12), but with the passage of time the seedling survival filter reduced the seedling density until a stable condition was reached. From 30 to 90 DAS most of the seedlings probably died from competition induced stress (Bellotti, 1984).

3.4.3 Botanical composition

Overall, the contribution of sown legume to herbage mass was greatest following the spring 1993 sowing (Figure 3.7). This was partly due to the lower total herbage mass compared with other sowing conditions as the growing season for this sowing was only for 95 DAS. Winter sowing gave higher herbage mass than autumn sowing probably due to the less competition from the regrowth of existing vegetation created by the lower minimum temperature.

Campbell (1968) and Bellotti (1984) observed that the dry matter yield of all grasses and clovers was greater for treatments in which herbicide was applied. Although in all of these trials a high rate of herbicide was used, the percentage herbage mass in sown legumes was not increased to more than 15% for the autumn and winter sowings, as is typical when legumes are oversown in hill country (Levy, 1970).

Among all the legume species, persian clover had the greatest contribution to herbage mass (< 10%) following autumn sowing and strawberry, white and arrow leaf clovers had the greatest contribution to herbage mass (< 15%) following the 1993 winter sowing. Chapman *et al.* (1985) found the contribution of white clover cv. G. Huia to total herbage accumulation was 1.1 and 1.4% during the years 1982-83 and 1983-84, respectively. The low contribution of legume species to total herbage mass emphasised the importance of improving the establishment rate from oversowing into drought-prone areas of hill country if legume species are to make a useful contribution to pasture productivity.

3.4.3 Environmental vs plant factors

The results of these trials showed that GSWC, soil temperature, minimum air temperature and wind run were more important environmental factors than maximum air temperature, rainfall, degree days and potential evapotranspiration. Soil temperature and GSWC were more important during the seedling appearance phase and minimum air temperature and wind run were more important for seedling survival and seedling establishment phases (Figure 3.11). Considerable research has shown the effects of soil moisture (Dowling *et al.*, 1971) and soil temperature (McWilliam *et al.*, 1970) on establishment, however there is little information on the effect of minimum temperature and wind run. Minimum temperature probably reduced the competition from the resident pasture and wind run dried the surface soil moisture which certainly affected seedling survival.

3.4.3.1 Soil moisture

The overall responses to the key environmental variables were similar for all species, but the rate and range of responses were species dependent. The soil moisture during early stages of seed imbibition and germination (0 -15 DAS) showed a negative relationship with seedling survival (Figure 3.11). The availability of soil water and vapour pressure deficit in the micro-environment above the soil surface exercises the dominant control over the germination and seedling survival of oversown seed under field conditions (McWilliam and Dowling, 1970). Contact between the seed and soil was very important for water uptake as observed by Campbell and Swain (1973b) and Dowling and Robinson (1976). Oversowing legume seed on the soil surface cannot be successful every time because germination is affected by the seed soil contact (Harper and Benton, 1966). Rapid drying of the soil surface has also been suggested as a major cause of seedling mortality (Suckling, 1949) probably due to root desiccation (Cocks and Donald, 1973).

Adequate soil moisture is necessary during emergence to reduce soil strength and to permit fast root growth (Barley, 1976; Campbell *et al.*, 1987). The moisture loss from the germinating seed is also important with surface sowing where a drying environment is present even when the soil is wet (Leach *et al.*, 1976). Excessive soil moisture creates waterlogging conditions which reduces the germination of surface sown seed. High soil moisture also provides a good environment for fungal diseases (Stovold *et al.*, 1980).

3.4.3.2 Soil temperature

In a moist environment, soil temperature is the main limiting factor for germination (White *et al.*, 1972; Radcliffe and Lefever, 1981). Up to 30 DAS all the sown legume species showed a positive relationship between seedling survival and soil temperature. The average minimum and maximum soil temperature in all the trials was between 5 and 18°C. Soil temperature was less important after seed germination and initial seedling survival (Figure 3.11). Benjamin (1990) observed an approximately linear increase in seedling survival with increasing soil temperature from threshold to maximum. Muendel (1986) found a relationship between seedling emergence and soil temperature only when surface soil was moist.

3.4.3.3 Minimum temperature

Minimum temperature was probably an index for the growth of the existing vegetation and therefore for competition. Although minimum temperature probably slowed seedling growth, this was apparently preferable to severe competition from recovery of the resident vegetation that would occur at a higher temperature. There was shoot as well as root competition for the survival of sown legumes. Dowling *et al.* (1971) found the competition from the resident vegetation was a major cause of seedling mortality and Seager *et al.* (1992) also observed the severe effect of below ground competition. The minimum temperature recorded during all the trials showed a negative relationship with seedling survival by 30 DAS. Research on seedling survival in relation to minimum air temperature has not been reported previously.

One of the main problems in the establishment of surface sown pasture is the control of competition during the first two years of establishment in Australia (Campbell *et al.*, 1987). The main reason for the failure of seedlings was competition from resident grasses that re-established after spraying with herbicide (Dowling *et al.*, 1971). Campbell and Swain (1973b) observed that most seedling death occurred during early summer from aerial winter sown pasture due to competition induced stress. In tropical legumes, the main cause of poor establishment was competition from resident vegetation (Cook and Dolby, 1981).

3.4.3.4 Wind run

Wind run is a well known factor affecting water loss but its influence on ersowing success does not appear to have been previously recognized. nd run aids evaporation and transpiration and it can be an indicator of likely ersowing success. High wind run decreased the seedling survival even 15 \S (Figure 3.11). Soil moisture is also an indicator of soil strength or pedance, and through the action of wind, these factors are most extreme at e soil surface. Charlton and Brock (1980) concluded that low rainfall (35% normal) combined with high wind run (810 km /day) may have caused high edling mortality during the survival phase of white clover seedlings. A tailed study on wind is presented in Chapter 5.

is likely that these four environmental factors could be used to model edling survival of oversown legume species. A simple model will be veloped in Chapter 8 using GSWC, soil temperature, minimum air mperature and wind run as key determining factors to predict the optimum ne for oversowing.

3.5 Summary

Not all the legume species used were suitable for oversowing in dry North Island hill country. The annual species subterranean, arrow leaf and persian clovers and barrel medic, and the perennial species birdsfoot trefoil, strawberry and white clovers, and luceme were suitable for oversowing in drought-prone hill country. Table 3.19 summarises the suitability of different legume species under different sowing conditions; the species whose survival percentage was 10% or more were probably more suitable for oversowing. Subterranean and white clovers and luceme were more suitable for autumn sowing whereas birdsfoot trefoil, strawberry, alsike and persian clovers were more suitable for winter sowing (Table 3.19). Only arrow leaf clover and birdsfoot trefoil showed promising establishment following spring sowing. In this series of trial however, only the initial legume establishment was studied, not the long term persistence.

The greatest loss of potential plants after oversowing was non-appearance of seedlings, accounting for approximately 80% of viable seed. The rate of germination was clearly temperature dependent, and seed germination was poor when the soil was too wet. Seedling survival was greatest when daily minimum air temperature was low and, therefore, there was less competition. Although there were some general trends for all the legume species, there were also some large differences between species related to the effect of temperature on germination, the minimum temperature for germination and rate of germination. There is a need to select legume species with characteristics that enable establishment by oversowing and to refine the oversowing technique. This large set of data will be used to develop a simple model of the relationship between environmental variables and establishment of seedlings by oversowing (see Chapter 8).

Although these trials indicated the importance of environmental factors on

Table 3.19 Optimum time for oversowing of different legume species as observed under different trials at Poukawa

Species	Autumn season	Winter season	Spring season	Survival % 90 DAS	Suitable for oversowing
Annual legumes					
Subterranean clover	✓	x	x	14	yes
Arrow leaf clover	✓	✓	✓	10	yes
Persian clover	x	✓	x	7	no
Barrel medic	✓	x	x	14	yes
Murex medic	✓	x	x	11	yes
Perennial legumes					
Alsike clover	x	✓	x	9	no
Birdsfoot trefoil	x	✓	✓	13	yes
Caucasian clover	✓	x	x	6	no
Lucerne	✓	x	x	10	yes
Maku lotus	✓	x	x	6	no
Strawberry clover	x	✓	x	10	yes
White clover	✓	x	x	12	yes

establishment, there are also other variables like seed coating, use of insecticide and fungicide, allelopathic and soil texture affect, seed size and seed rate which affect the fate of viable seed sown and these will be investigated in Chapters 4, 6 and 7. These results also indicated that more detailed study of the effect of wind run on surface soil moisture is required (Chapter 5).

Chapter 4

Chapter 4

SEED RATE AND SEED SIZE EFFECTS ON SEEDLING DENSITY AND HERBAGE YIELD[1]

4.1 Introduction

The legume contribution of most hill country pasture in New Zealand is less than 20% of total herbage mass (White, 1990). Legumes are important for providing high quality feed and improving the soil nitrogen status (Levy, 1970). A range of new legume species and cultivars has been evaluated for this purpose (Charlton, 1991; Woodman *et al.*, 1992; Awan *et al.*, 1993), and more than 20 legume cultivars have been certified in New Zealand (Rumball, 1983). The availability of these new legume species / cultivars has created a need for appropriate evaluation of their potential for establishment by oversowing into hill country pasture.

Traditional sowing rates recommended for the establishment of legume species may be suitable for full cultivation and undersowing, that is, 5 kg ha^{-1} (about 100 seeds m^{-2}) for subterranean clover and 3 to 4 kg ha^{-1} (about 500 seeds m^{-2}) for white clover (Scott *et al.*, 1985; White, 1990), but oversowing in hill country may benefit from higher sowing rates than those recommended (Suckling, 1954). Higher seed rates for oversowing legumes have been suggested but not defined (White, 1990) although Chapman *et al.* (1993) used a seed rate of 8 kg ha^{-1} for white clover oversowing in hill country.

An increased sowing rate might be a cheap and simple method to increase the percentage of legume in hill country pastures, but there is little information on the optimum sowing rate for oversowing legume species in hill and high country pastures, especially for new species and cultivars. Higher sowing rates for

[1]Part of this work will be published in the Proceedings of the Agronomy Society of New Zealand; 24 (in press; Awan *et al.*, 1994).

oversowing have been suggested by different researchers but not properly defined. Further information on the effect of different sowing rates on the establishment of new legume species / cultivars, especially when oversowing, is required.

Seed size is usually defined by the seed weight (Shipley and Peters, 1990) and average seed weight is one of the least variable parts of the plant (Harper *et al.*, 1970). Seed size is a relatively easily determined parameter that is assumed to be a sound predictor of the success of establishment and the vigour of a crop (Naylor, 1980). Under field conditions, the size of the endosperm is also an important factor in determining the potential ability of a species to establish itself (Beveridge and Wilsie, 1959; Mazer, 1989).

Jurado and Westoby (1992) evaluated 32 species in arid central Australia, and concluded that species from environments where seedlings were prone to drought during establishment tend to have larger seeds. The plants with larger seeds might be able to allocate a larger proportion of carbon to root mass rather than shoot mass during early growth (Jurado and Westoby, 1992). The seedlings of heavier-seeded species tend to survive longer than seedlings from lighter-seeded species (Jurado and Westoby, 1992). Atkinson (1973) found that seedlings from larger seeded monocotyledonous species remained independent of mineral nutrients in the soil for longer than did lighter seeded species.

Selection of larger seeds within a species to get higher legume establishment has been used for a long time. Kidd and West (1919) worked with seed size within lucerne and sainfoin, Findlay (1919) within red clover, Schmidt (1921) within crimson clover and Black (1956) within subterranean clover. Black (1956) found that the large seed size (8 mg/seed) of subterranean clover gave 200 mg dry weight per plant compared to the small seed size (3 mg/seed) which gave 60 mg dry weight per plant 30 DAS. There have however been few studies on the influence of seed size on the growth of pasture legumes and there is still a paucity of information on the effect of seed size on pasture legume establishment and its contribution to herbage yield.

Accordingly, the aims of these studies (for oversowing into a hill country sward) were to:-

- determine the effect of different sowing rates on the total herbage mass of different legume species, and
- compare the seed size effect on legume establishment and total herbage mass within and between legume species,

4.2 MATERIALS AND METHODS

4.2.1 Site

Two adjacent trials were conducted during 1993 at the AgResearch Hill Country Research Station, Ballantrae, in the Ruahine Range of the lower North Island. The resident pasture comprised 25% chewings fescue (*Festuca rubra* spp. *commutata* L.), 25% browntop (*Agrostis capillaris* L.), 15% sweet vernal (*Anthoxanthum odoratum* L.), 15% perennial ryegrass (*Lolium perenne* L.), 5% crested dogstail (*Cynosurus cristatus* L.), 8% white clover (*Trifolium repens* L.) and 7% other species (Barker and Dymock, 1993b). The research site was at 310 m altitude on a north-west (sunny) aspect, the soil was Ngamoka silt loam with a pH of 5.4 and moderate soil fertility (Olsen P 10 µgP/g soil). No fertilizer has been applied for ten years. Rainfall and air temperature were measured about 1 km from the trial site.

4.2.2 Materials and trial description

4.2.2.1 Experiment 1. Seed rate trial

The legume species used were subterranean clover cv. Karridale, persian clover cv. Kyambro, white clover cv. Grasslands Tahora and strawberry clover cv. Grasslands Onward. The percentage of viable seed was obtained from a germination test at room temperature (20°C), and was 90, 78, 81 and 75% for subterranean, persian, white and strawberry clovers, respectively. The seed of persian clover was scarified with sand paper prior to the germination test. The seed rates used for the four legume species were 100, 500, 1000 and 2000 viable seeds per m^2. The equivalent sowing rates for the four legume species are given in Table 4.1.

Table 4.1 Species and sowing rates of viable seed

Species	No. of seed sown m^{-2}			
	100	500	1000	2000
Annual species	kg seed ha^{-1}			
1. Subterranean clover cv. Karridale	5.0	25.0	50.0	100.0
2. Persian clover cv. Kyambro	0.7	3.5	7.0	14.0
Perennial species				
3. Strawberry clover cv. Grasslands Onward	1.25	6.25	12.5	25.0
4. White clover cv. Grasslands Tahora	0.7	3.5	7.0	14.0

4.2.2.2 Experiment 2. Seed size trial

The legume species used were subterranean clover cv. Karridale, strawberry clover cv. Grasslands Onward and crimson clover (*Trifolium incamatum* L.). The seed was manually separated into three sizes with sieves. From each species subsamples of the large and small seed size were chosen and the medium size was discarded. The diameter and weight of the seed and standard seed germination test are given in Table 4.2. For each seed size 500 viable seed were sown in 1 m^2 plots.

4.2.3 Management

The sites were blanket sprayed with 4.32 kg a.i. ha^{-1} of glyphosate which was equivalent to 12 ℓ ha^{-1} of Roundup (Jagschitz, 1978), 21 days before sowing (Awan *et al.*, 1993). Seed was hand broadcast during autumn (19 April 1993) for both trials. Inoculum was not used since it was assumed that seedlings of all species would be infected by resident rhizobia. All seedlings were green and healthy. After oversowing, plots were trodden by sheep at 400 ewes ha^{-1} for 30 min. The trial areas were fenced to exclude animals, and were grazed by sheep as required.

4.2.4 Statistical analysis

A factorial combinations of four legume species and four seed rates with three replicates for the seed rate trial, and three legume species and two seed sizes with four replicates for the seed size trial, were arranged in complete block designs. Statistical analyses were by analysis of variance (ANOVA) using the GLM model of the SAS package (SAS, 1989).

Table 4.2 Species and seed size, normal seed germination test and seed diameter and weight

Species	Seed size	Seed diameter (mm)	Sieve used to separate seed (#)	Normal seed germination (%)	Wt. of 100 seed (g)
1. Subterranean clover cv. Karridale	Large	> 2.9	9.5	92	0.8419
	Small	< 2.0	7.5	98	0.3825
2. Strawberry clover cv. Grasslands Onward	Large	> 1.6	6.5	80	0.1790
	Small	< 1.3	5.75	76	0.1275
3. Crimson clover	Large	> 2.0	7.5	88	0.5692
	Small	< 1.6	6.5	80	0.2831

4.2.5 Measurements

4.2.5.1 Plant density

Seedling density was determined at 15, 30, 60, 90 and 120 DAS for both trials. A 1 m^2 quadrat was sub-divided into four divisions for accurate counting.

4.2.5.2 Yield and botanical composition

For both trials the herbage yield was determined 7 months after sowing from two 0.1 m^2 quadrats per plot cut at ground level, and whole samples were separated into resident species and sown legumes. To calculate dry matter (DM) the samples were washed, then dried at 80°C for 24 hours. The plots were then grazed by sheep to a residual herbage mass of approximately 1000 kg DM ha^{-1} and one month later the herbage yield was again measured as previously but only for the seed rate trial.

4.2.5.3 Plant dry weight

Ten months after sowing five plants from each plot were randomly selected from the seed rate trial site, cut to ground level, and dry weight (24 hours at 80°C) recorded.

4.3 Results

4.3.1 Weather

The annual rainfall for the trial year was 1150 mm and the average for the previous 24 years was 1200 mm. Monthly rainfall for March, April, May and June, 1993 was 135, 111 ,76 and 155 mm, respectively. The air temperature was also typical for the site and during the trial year average daily minimum and maximum temperature was 11.2 and 21.7 °C for January and 7.5 and 14.7 °C for August 1993, respectively.

4.3.2 Seedling survival

4.3.2.1 Seed rate trial

There was a significant interaction between the sowing rate and legume species for seedling density ($P<0.05$). On average, the highest sowing rate produced the highest seedling density for all four legume species, but there was no significant difference between 1000 and 2000 seeds m^{-2} for white clover ($P<0.05$, Figure 4.1). The highest sowing rate treatment (2000 seeds m^{-2}) produced the maximum seedling density of 702, 659, 572 and 345 for subterranean clover, strawberry clover, persian clover and white clover, respectively. The establishment (% of viable seed sown) of white clover was lower than for the other species particularly at the highest sowing rate (2000 seeds m^{-2}; Figure 4.1). Overall, seedling establishment was 24, 19%, 37 and 26% for strawberry, white, subterranean and persian clovers, respectively at 120 days after sowing.

4.3.2.2 Seed size trial

There was a significant difference between the seedling density of large and small seed sizes during the early stages of seedling survival, upto 90 DAS

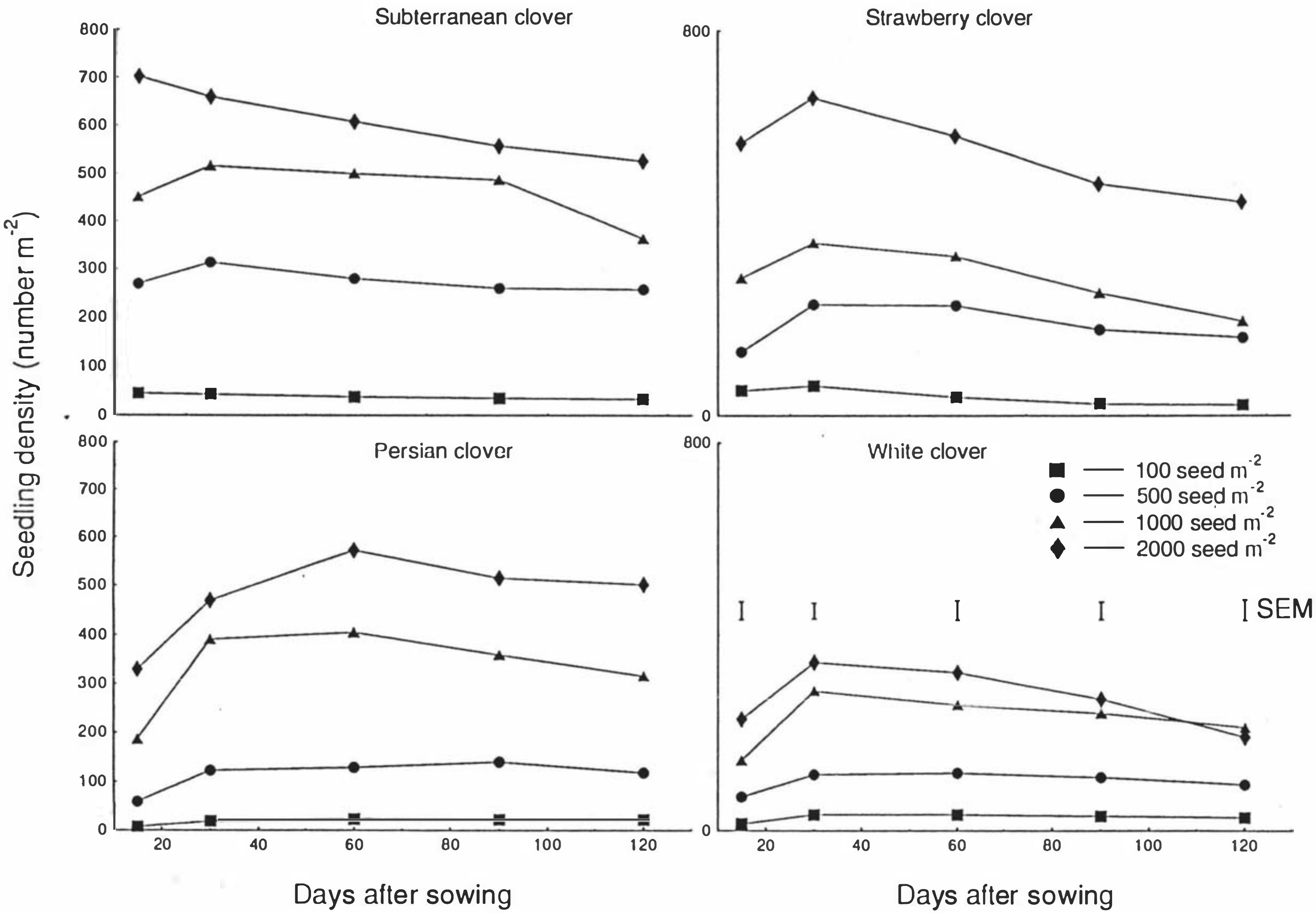


Figure 4.1 The average response of seedling density to four legume species and four seed rates over time (Vertical bars show all species SEM).

(P<0.05; Figure 4.2). The seedling density was similar at 90 DAS for both seed sizes ($\overline{x}$=105). Overall, the maximum seedling density for the large seed size was higher than for the small seed size. The main effect of legume species was significant (P<0.05; Figure 4.3). On average, subterranean clover had a higher seedling density than the other species, and strawberry clover had the lowest seedling density (Figure 4.3). At 15 DAS subterranean clover had its maximum seedling density whereas crimson and strawberry clovers had their maximum seedling densities at 30 DAS (Figure 4.3).

The interaction of legume species with seed size was not significant (see Appendix 4.1).

4.3.3 Herbage mass and botanical composition

4.3.3.1 Seed rate trial

The two annual legume species had a significantly higher contribution to herbage mass than the two perennial legumes in absolute terms and as a percentage of total herbage mass (P<0.05, Figure 4.4).

The maximum contribution of the annual legumes to total herbage mass, seven months after sowing (before grazing), was 71% for subterranean clover at 1000 seed sown m^{-2}, and 32% for persian clover at 2000 seed sown m^{-2} (Figure 4.4). Strawberry and white clovers contributed less than 8% of the total herbage mass before grazing at all the sowing rates. The average total herbage mass for all the sowing rates and legume species combined was 2288 kg DM ha^{-1} before and 1455 kg DM ha^{-1} one month after grazing the site.

The herbage contribution of the two perennial legumes increased in the month following the first grazing (Figure 4.4), with white clover providing more than 10% of the herbage mass at 1000 seed sown m^{-2}. The maximum percentage of

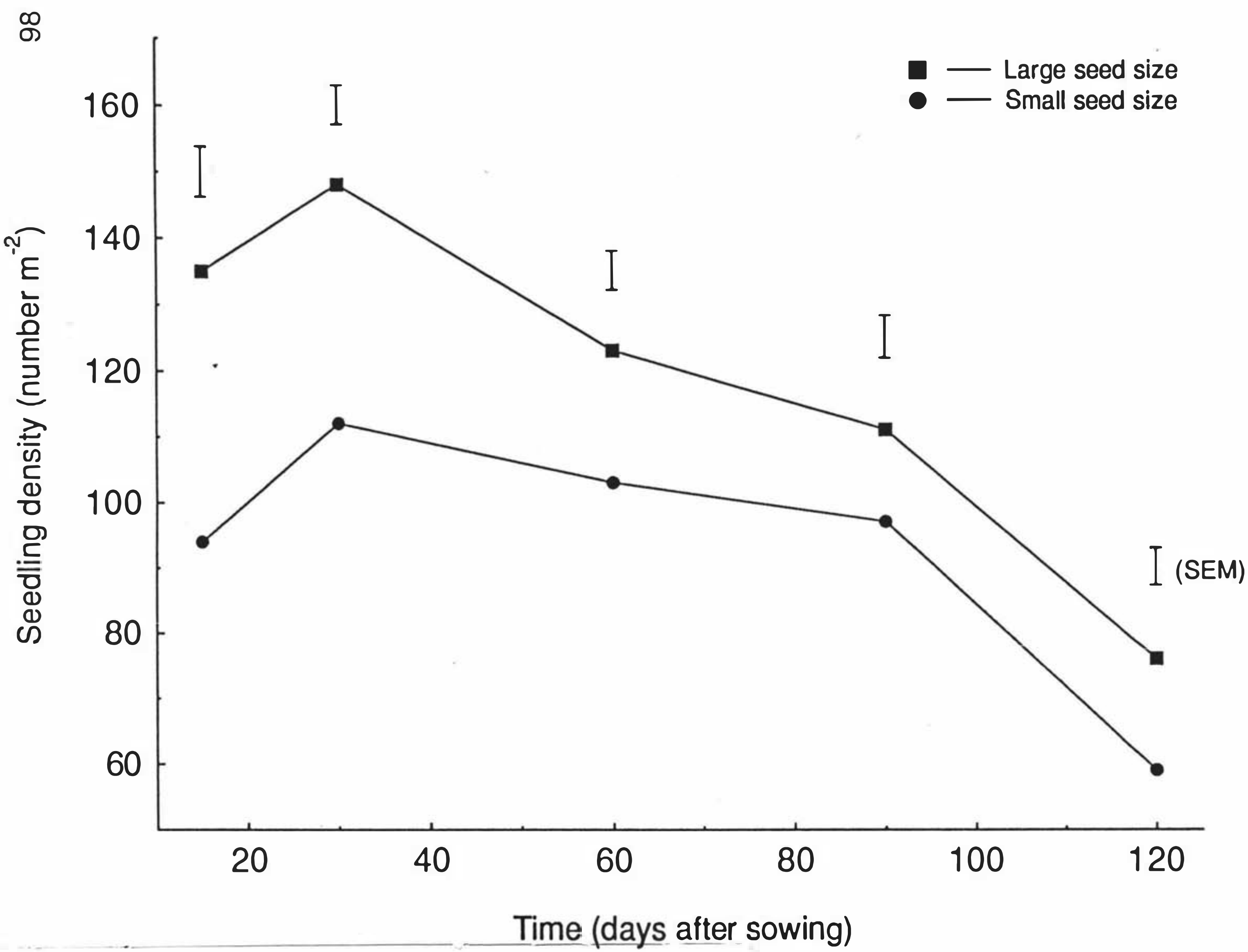


Figure 4.2 The average response of seedling density to two seed sizes for three

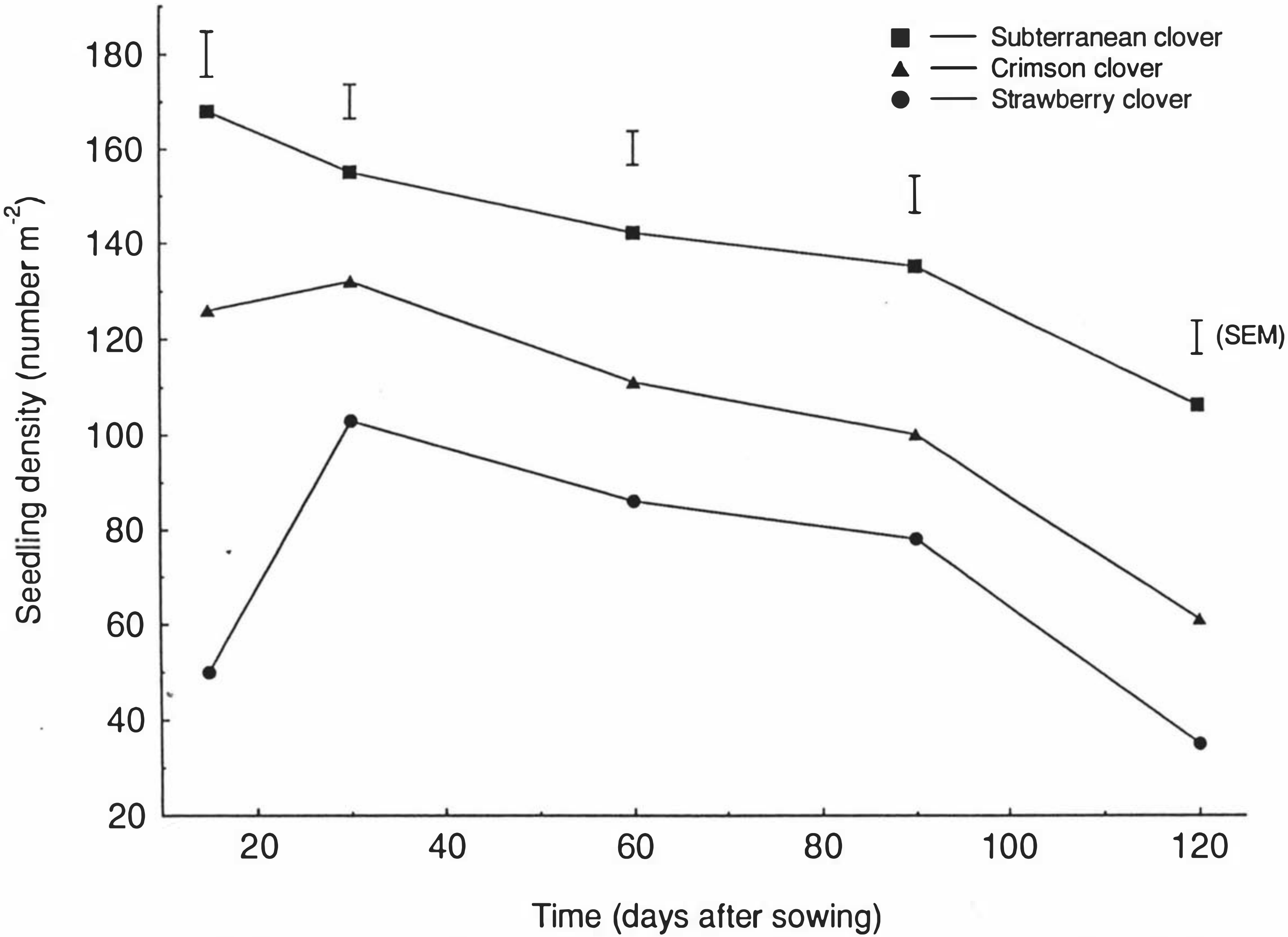


Figure 4.3 The average response of seedling density to three legume species over time.

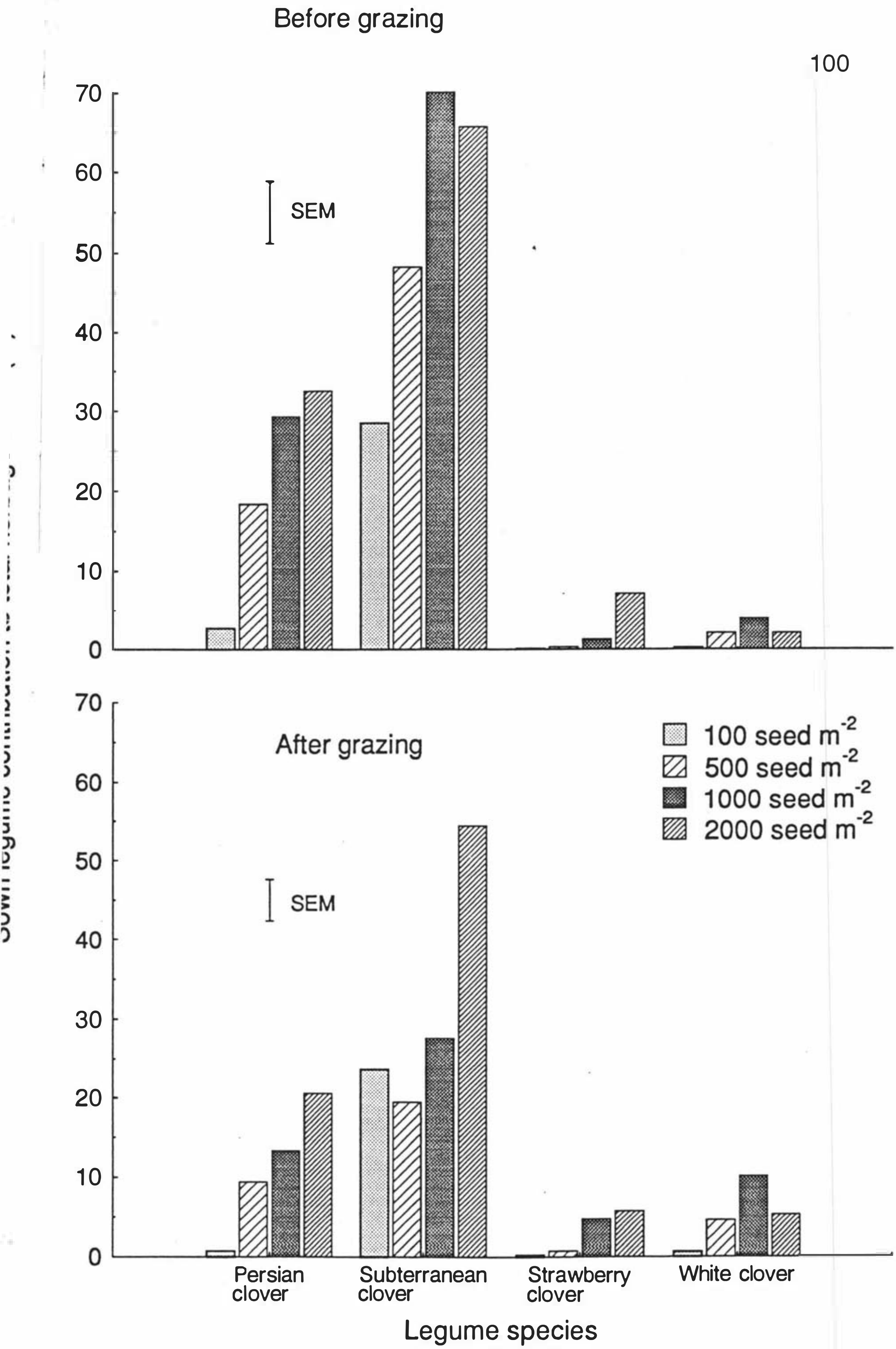


Figure 4.4 The contribution of four legume species to herbage mass before grazing at 7 months after oversowing and after grazing,8 months after oversowing for different sowing rates.

herbage mass from subterranean clover had decreased from 71% before initial grazing to 28% at the second grazing, and for persian clover the decrease was from 32% to 20% (Figure 4.4). Overall, the maximum contribution of the sown legume species towards total herbage mass was at either 1000 or 2000 seeds sown m^{-2} (Figure 4.4). The relative performance of 2000 seeds sown m^{-2} was stable, since the legume species contribution towards total herbage mass at this sowing rate was 65 and 55% at the first and second grazing, respectively.

4.3.3.2 Seed size trial

Subterranean clover had a significantly higher herbage mass than crimson and strawberry clovers at both seed sizes ($P<0.05$; Figure 4.5). The large seed size gave a significantly higher herbage mass than the small seed size for subterranean clover but not for the other two species. The maximum contribution of subterranean clover to the total herbage mass was 78% for large seeds and 50% for small seeds (Figure 4.5). Both seed sizes for strawberry clover gave less than 1% of the total herbage mass. The interaction of legume species with seed size was not significant ($P>0.05$).

4.3.4 Plant dry weight (sowing rate trial)

The main effects of legume species and sowing rate were significant ($P<0.05$) for dry weight per plant whereas their interaction was not statistically significant ($P=0.34$; Table 4.3). Persian clover had a significantly higher plant weight than white, subterranean and strawberry clovers at 300 DAS (Table 4.3). The average plant dry weight for the annual legume species was 161 mg, and for the perennial legumes only 56 mg. Plant dry weight for the 100 seeds m^{-2} treatment was approximately half that of the remaining three treatments which were not significantly different (Table 4.3)

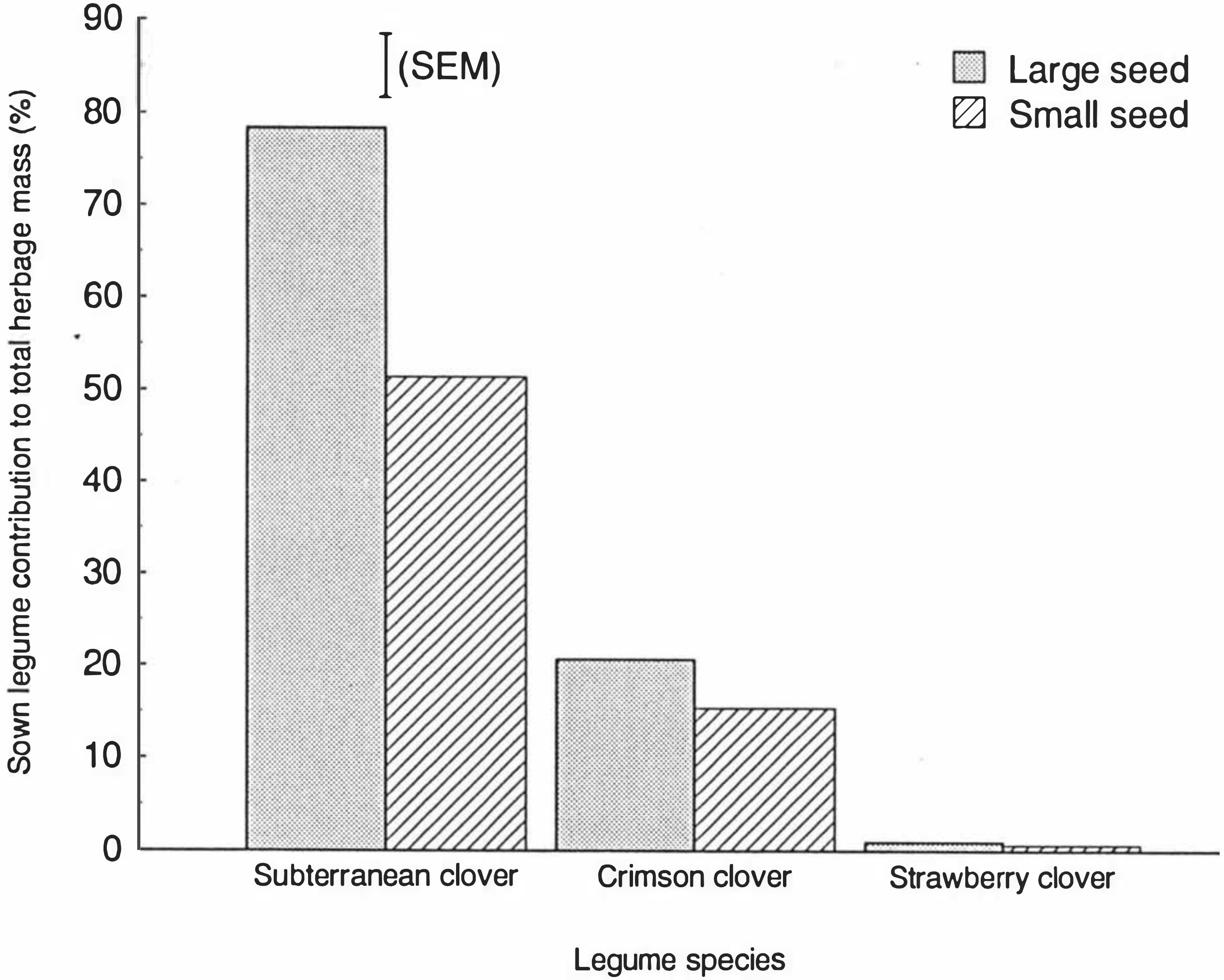


Figure 4.5 The contribution of two seed sizes of three legume species to herbage mass 7 months after oversowing.

Table 4.3 Dry weight (mg) per plant for four sowing rates and four legume species at 300 DAS.

Treatments	Plant weight (mg / plant)
Species	
Subterranean clover	135
Persian clover	187
Strawberry clover	65
White clover	47
SEM	17
Seeds m^{-2}	
100	63
500	116
1000	119
2000	135
SEM	23
F - test	
Species	***
Seed rate	**
Species x Seed rate	ns

**: P<0.01; ***: P<0.001
ns: no significant difference

4.4 DISCUSSION

4.4.1 Seed rate

The greatest legume contribution to herbage mass was at either 1000 or 2000 viable seeds m^{-2} for all four species, which was two to three times the usual recommended oversowing rate (Scott *et al.,* 1985; White, 1990). Although the percentage of the perennial legumes in the pasture can increase after oversowing sowing, the percentage of annual legumes derived directly from sowing represents the maximum number of plants for that season. It is suggested that higher sowing rates of at least 1000 seed m^{-2} are warranted when oversowing hill country pastures, even if the existing sward is suppressed or defoliated by herbicide prior to sowing.

The increased contribution of the legume species to herbage mass at the higher sowing rates was mainly due to increased plant density, as individual plant weight was similar for all sowing rates except 100 seeds m^{-2} (Table 4.3). Presumably the increased number of plants enabled the sown legume species to be more competitive against regrowth from the existing sward. In the first 120 DAS there was only a small decline in plant numbers suggesting that there was little within species competition for the sown legume species, even at the highest sowing rate. The similarity in the percentage contribution to herbage mass at 1000 and 2000 seeds m^{-2} by persian clover, subterranean clover and white clover suggested that either within or between species competition affected later legume growth. One month after grazing, herbage mass decreased by about 65% which enabled the perennial legumes to grow with less competition resulting in a higher herbage mass. In contrast, the annual legumes had already completed their vegetative growth cycle and their herbage mass decreased.

Although proportionally fewer seeds produced a seedling at higher sowing rates, the total number of seedlings established increased progressively and these seedlings improved the competitiveness of the sown legume species within the existing sward. Seedling establishment of white clover was lower (19% of total viable seed sown) at 120 DAS than for the other sown legumes. In a hill pasture oversowing trial, seedling appearance of Grasslands Tahora white clover 11 weeks after oversowing was only 15% of the 8 kg bare seed ha^{-1} sown (1413 seed m^{-2}; Chapman *et al.*, 1993).

Overall, subterranean clover had the highest net seedling survival (i.e. 35%) of the total seed sown. The better establishment of the annual legume species showed that seed and seedling characteristics such as large seed size (Jurado and Westoby, 1992) and high seedling relative growth rate (Grime and Hunt, 1975) give better establishment independent of the sowing rate, and when oversowing conditions are good. That is, technology can improve successful establishment from oversowing, but establishment is ultimately dependent on the characteristics of the legume species. Seed coating is one way of increasing germination rate (Scott, 1989) but the success of using the increased sowing rate demonstrated this was an alternative option to seed coating. The choice between higher sowing rates and seed coating would be dictated by relative cost.

4.4.2 Seed size

Seed size within species affected seedling density between 0 and 90 DAS but not from 90 DAS onwards. Black (1957) found similar results for subterranean clover in Australia. He used different cultivars of subterranean clover to differentiate large and small seeds whereas in this trial the different seed size was from the same cultivar of Karridale. The larger seed size probably produced higher seedling numbers due to its higher food reserve.

Lawson and Rossiter (1958) contradicted Black (1957) and reported that seed size did not affect the growth of subterranean clover, provided equal weight of viable seeds were sown per unit area. Evans *et al.* (1992) concluded that seed size was poorly correlated (r = -0.09) with winter production for 30 subterranean clover cultivars and accessions, although there were significant differences between seed size. Shipley and Peters (1990) reported that the relative growth rate of 204 species, mainly herbaceous plants, decreased with increasing seed size.

Kneebone and Cremer (1955) showed that although average seed size of a species had little effect on the final percentage of germination, there were significant differences in seedling vigour both between and within species. They also concluded that samples of large seed from some grass species had more vigorous seedlings that emerged sooner and subsequently grew faster. Hunt (1954) found a correlation of 0.80 between perennial ryegrass seed weight and number of established seedlings per 100 seeds planted. Beveridge and Wilsie (1959) showed that seedling vigour was correlated with seed size, but there was no consistent relationship between seed size and emergence. McKell (1972) also found that size and weight were characteristics associated with seedling vigour. Within a species, selection of larger seeds for sowing could be advantageous as root growth has been related to seed size (Asher and Ozanne, 1966; McWilliam *et al.*, 1970). Evans (1973) showed that in white and red clovers, plant size and root length were related to seed size.

The seed size effect was only noticed for the herbage contribution of subterranean clover whereas for the other two species the different seed sizes had the same herbage mass contribution. As the subterranean clover had a higher seed density compared to the other two species, competition from the resident pasture growth probably did not affect its herbage contribution as much as for the other two species. Seed size may or may not have an impact on establishment for all the legume species because some species are not suitable for oversowing irrespective of seed size.

4.5 Summary

The sowing rate trial showed that if seed density was increased from 500 to 1000 seeds per m^2, then the initial contribution of oversown legume species to the total herbage mass in hill country will be increased. That is, sowing rates although greater than those recommended would increase the legume contribution to the total herbage mass. The higher legume percentage would produce more seed which would be helpful in subsequent years.

The seed size data suggested that some consideration should be given to seed size or weight but there are also some other important factors. It is important to have heavier seed when oversowing into hill country pasture to improve the likelihood of the seed contacting the soil as the heavier seeds get to the soil surface better than lighter seeds. Some species however, may not be suitable for oversowing even though they have heavy seed.

Chapter 5

Chapter 5

SOIL SURFACE MOISTURE, ITS MEASUREMENT AND INFLUENCE ON EARLY SEEDLING SURVIVAL AND FATE OF SOWN SEED OF THREE OVERSOWN LEGUME SPECIES[1]

5.1 Introduction

The environment for seed following oversowing is more severe than for seed sown following cultivation (Meeklah, 1958; Evans *et al.,* 1966). Failure of the radicle to enter the soil has been identified in field studies as one of the major causes of seedling mortality for aerial sown temperate legumes (Dowling *et al.*, 1971). Seedling emergence was always higher from a cultivated seed bed despite low soil water content, and lower from surface sowing despite favourable soil moisture (Bellotti, 1984). Grass radicles enter the soil surface more easily than legumes because they are often anchored to the soil surface by hairs, have a more acute angle of entry, a smaller root diameter and seminal roots (Campbell and Swain, 1973b). The thicker radicle of legumes compared to grasses also makes entry into the soil more difficult (McWilliam and Dowling, 1970).

Legume radicles tend to grow along the soil surface until they encounter a suitable entry site, whereas grass radicles tend to enter more directly with less exposure to desiccation (Campbell *et al.*, 1987). For a root to transmit maximum pressure to the resting soil it must have adequate anchorage (Taylor and Gardner, 1963). Radicle entry can be improved artificially by anchoring seed to the soil surface with a bent pin or nylon mesh (Dowling *et al.*, 1971), or naturally by sowing species with root hairs that provide anchorage. Breeding pasture species with large number of hairs on the radicle could improve anchorage and the establishment of surface sown pasture. A rough soil surface favours radicle entry compared to a smooth surface (Campbell, 1992). Adequate contact

[1]Part of these results have been accepted for publication in the Journal of Agricultural Science, Cambridge. (Awan *et al.*, 1995).

between the seed coat and soil moisture during imbibition is also important, even in relatively moist conditions (Sedgley, 1963; Choudhary and Baker, 1981).

Germination of oversown seed is sensitive to rapid fluctuations in moisture and humidity in the micro-environment of the seed at the soil-air interface (Dowling *et al.*, 1971). Although equipment such as the Time Domain Reflectrometer (TDR) (Topp and Davis, 1985), the Neutron Moisture Meter (NMM) (Grant, 1975) and the hydrogenously shielded neutron probe (Chanasyk and Naeth, 1988) are presently used to measure soil moisture at depth, they are not suitable for measurement of moisture at the soil surface. A noninvasive TDR probe has been modified to measure soil moisture in the upper 10 mm soil surface (Selker *et al.*, 1993) but is an expensive equipment. Neutron techniques have difficulty in measuring moisture near the soil surface because large and unpredictable proportions of fast neutrons escape into the atmosphere (Noble, 1973).

Although expensive methods for measuring soil moisture are available like the aircraft scatterometer (Jackson and O'Neill, 1985), the multibeam passive microwave radiometer system for remote sensing (Jackson *et al.*, 1984) and passive microwave sensors (Schmugge, 1989), these are not economical for farms or practical for small experimental plots. Gravimetric soil water content determination at the surface is time consuming, and conditions could change while oven-drying takes place.

Studies in Chapter 3 showed that wind run was also one of the factors that influenced early seedling emergence and survival because the wind run is an indicator of both the soil surface moisture and strength.

The purpose of this study was to:-

- develop a fast and easy method to measure the soil surface moisture,
- determine the effect of fluctuating soil surface moisture on seedling survival of three oversown legume species with different seed sizes,
- determine the effect of wind run on early seedling survival, and
- determine the fate of oversown seed.

5.2 Materials and methods

5.2.1 Glasshouse experiments

Two glasshouse experiments were conducted at Massey University, Palmerston North, between 1 October and 30 November 1993. Perennial ryegrass-white clover pasture growing on a Tokomaru silt loam soil (pH 5.9) was blanket sprayed with 12 ℓ of Roundup (36% glyphosate) per ha, and after 20 days intact sods (420 x 300 x 50 mm) were placed in plastic trays and transferred to the glasshouse. These trays were perforated at the base and sides to facilitate drainage. The 0 to 50 mm depth of the Tokomaru silt loam soil had field capacity and permanent wilting point at 38 and 15% GSWC, respectively (Scotter *et al.*, 1979). The GSWC at the time of sowing was 30, 59 and 80% for low, medium and high soil moisture treatments, respectively and these treatments were maintained before the start of the trial.

The following three soil surface moisture treatments were imposed:

1) a high moisture treatment was maintained by misting 200 mℓ water on the soil surface per tray every 3 hr during daylight,
2) a medium moisture treatment was maintained by misting 200 mℓ water on the soil surface per tray once each day at 0900 hours,
and
3) a low moisture treatment was misted similarly to the medium moisture treatment, but oscillating fans blew air (3 m s^{-1} or 97 km d^{-1}) across the soil surface for 9 hr each day during daylight hours.

The average maximum and minimum air temperature was 23.0 and 12.2°C, respectively for October and 24.3 and 14.3°C, respectively for November. Daylength (approx 12 hr) was not artificially controlled.

The three legume species used in both experiments were;

1) subterranean clover cv. Karridale,
2) strawberry clover cv. Grasslands Onward, and
3) caucasian clover cv. Grasslands Monaro.

Seeds of each species were sown on the surface of the sods with one seed per 20 x 20 mm square in a grid (total 216 seeds) on 3-6 October, 1993. The seed was identical to that in Chapter 3 with standard germination test given in Table 3.7. Subsequently seed was pressed into the surface of the soil with a studded roller that applied average pressure of 1.28 Mg m^{-3} to the soil surface, which simulated the sheep treading that usually follows oversowing (Lancashire, 1961). Each tray was "trodden" 5 to 10 passes of the roller.

Experiment 1 had 36 plastic trays (3 species, 3 soil moisture levels and 4 replications) and measured the relationship between surface soil moisture and early seedling survival. Experiment 2 had the same treatment structure (36 trays) but monitored the fate of oversown seed.

5.2.2 Field experiment

A field experiment was located within the same herbicide-sprayed pasture as used for the sods in the glasshouse experiments. Intact sods were placed in plastic trays as previously described, except the trays were buried to 30 mm depth, in contact with the sub-soil. The same three legume species as used in the glasshouse experiments were sown on 2 October 1993 using the same technique for oversowing and simulated treading as for the glasshouse experiment. No irrigation was applied, the sown seeds received only natural rainfall and wind. The daily average wind speed was 222 and 273 km d^{-1} (approximately 3 m s^{-1} over 24 hr) for September and October, 1993, respectively. The mean maximum and minimum air temperatures were 17.1 and

8.7°C for October and 16.9 and 7.6°C for November, respectively. Rainfall recorded near the field site was 60 mm for October and 138 mm for November.

5.2.3 Measurements

5.2.3.1 Soil surface moisture test

Paper strips (20 x 5 mm) were dipped into near saturated cobalt chloride (4 mol ℓ^{-1} $CoCl_2$) solution and then dried at 60°C. A sub-sample of strips was found to have an average of 7.82 (0.76 SEM) mg $CoCl_2$ per strip. The blue colour strips were placed on a smoothed area of the soil surface and the time for the colour to change to a uniform pink was recorded. Measurements began on 4 October and continued at 2-4 day intervals during each experiment. After the surface measurement in the glasshouse experiment, soil to 5 mm depth was scraped from the sample area with corer, oven dried (105°C, 24 h) and the surface gravimetric soil water content (GSWC; g water / g soil, %) calculated. The GSWC to 30 mm depth was also measured for the plastic trays in the glasshouse.

5.2.3.2 Plant density

All visible seedlings from Experiment 1 in the glasshouse and the field trial were counted 2, 4, 6, 9, 12, 15, 20, 30 and 40 days after sowing.

5.2.3.3 Fate of sown seed

Sods (46 x 300 x 50 mm) of soil were removed from each tray every 2, 4, 6, 9, 12, 15, 20, 30 and 40 days after sowing. The total number of seeds, radicles and seedlings (alive or dead) visible on the soil surface were counted and radicle lengths measured. After these measurements, soil sods were put in a sieve and washed with water to remove all the soil to enable counting of the buried seed and radicles. The seeds which were present on top of the soil and buried in the soil were recorded separately.

5.2.4 Statistical analysis

In the glasshouse experiments there was a factorial combination of three surface moisture treatments, three legume species, and four replicates in a randomised complete block design (RCBD). Total and net seedling survival for the second experiment was analysed using time as a repeated measurement. In the field trial, three legume species with four replications in a RCBD was used. Data were analysed by analysis of variance using the GLM procedure of SAS (SAS, 1989).

The calibration curve for the relationship between surface GSWC and the time for colour change by the $CoCl_2$ paper was calculated using the exponential equation $Y = ae^{-bx}$. The relationship of surface GSWC over time for the three levels of soil moisture was determined using simple linear regression and the relationship of total seedlings plus radicles over time in Experiment 2 in the glasshouse was determined with the polynomial (cubic) equation $Y = a + bx + cx^2 + dx^3$.

5.3 Results

5.3.1 $CoCl_2$ technique

The time for the colour change of the $CoCl_2$ paper strips was exponentially related to direct determination of surface GSWC (R^2 = 78%; Figure 5.1). The 7.82 mg $CoCl_2$ per strip was calculated to absorb 6.52 mg H_2O (6 mols H_2O per mol $CoCl_2$) in the transition from the blue to pink colour. For a moist soil (transition time 3 minutes, GSWC 54%) this equated to a water flux of 31.25 $mm^3/mm^2/24$ hr.

5.3.2 Gravimetric soil water content (GSWC)

Frequent spraying of water in the high surface moisture treatment resulted in a higher surface GSWC (48 to 80%) than the other treatments (Figure 5.1). The medium surface moisture content was 30 to 59% and the low surface moisture content was 17 to 30% (Figure 5.1). The average soil surface moisture for low, medium and high was 22, 41 and 61% GSWC, respectively (Table 5.1).

In the glasshouse the surface (5 mm) GSWC was always lower than the total (30 mm) GSWC for all the treatments (Table 5.1). In the field the absence of significant rain resulted in drying of the soil surface to 12% GSWC, predicted by $CoCl_2$ test. Ten mm rainfall on 12 and 20 October and again on 1 and 9 November increased surface GSWC to an average of 35% (Figure 5.2). The GSWC fluctuated more under field conditions. In the glasshouse the medium and high soil surface moisture tended to decline over the time of the trial (Figure 5.2).

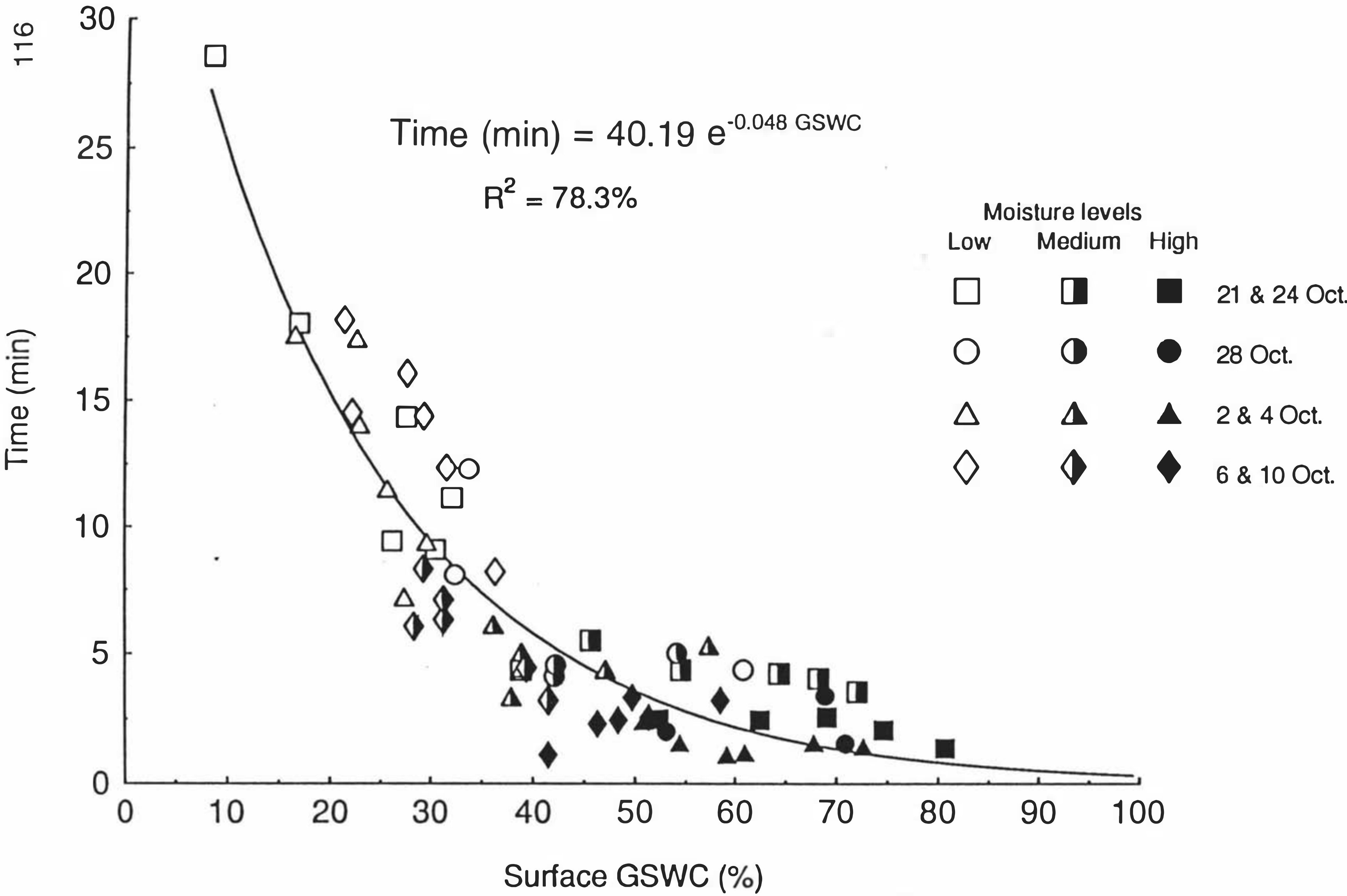


Figure. 5.1 The relationship between the time for $CoCl_2$ paper strips to change from blue to pink and the gravimetric soil water content (GSWC) of the surface 5 mm of the soil in the glasshouse experiment.

Table 5.1 Surface (5 mm depth) and total (30 mm depth) gravimetric soil moisture content (%) for three moisture treatments imposed on natural sods in the glasshouse.

Date	Low moisture		Normal moisture		High moisture	
	Surface	Total	Surface	Total	Surface	Total
4 October	30.34	-	59.48	-	80.01	-
8 October	17.52	36.21	46.11	61.94	63.95	82.62
12 October	21.72	49.62	40.70	51.32	62.64	89.23
16 October	23.21	51.32	31.45	59.89	48.36	76.62
20 October	24.31	52.58	30.21	62.39	53.17	81.32
24 October	16.89	31.89	38.83	71.32	60.44	92.85
Mean	22.33	44.32	41.13	61.37	61.43	84.53
SEM	2.0	4.3	4.4	3.2	4.5	2.9

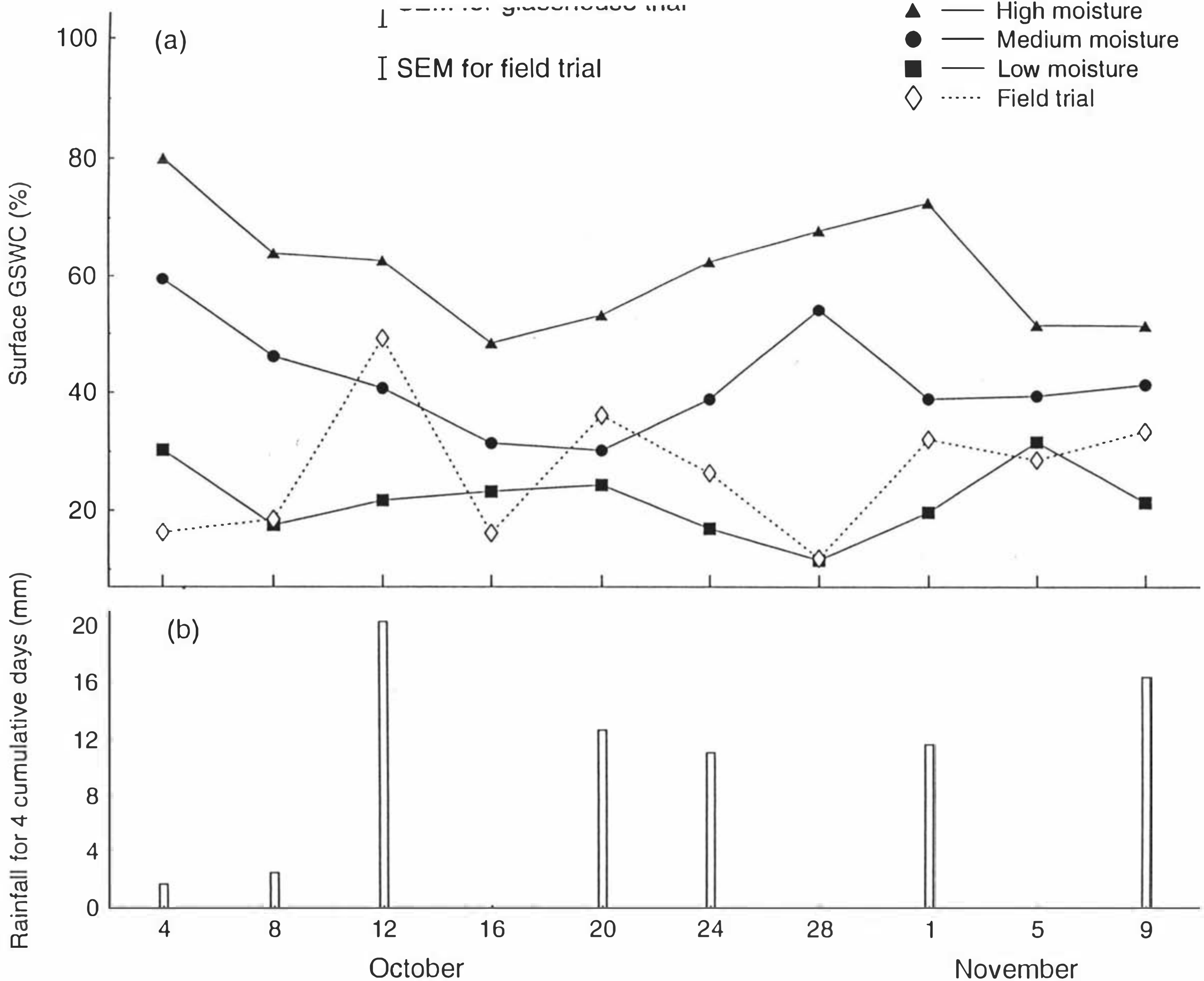


Figure. 5.2 a) Average response of surface gravimetric soil water content (GSWC) using the $CoCl_2$ technique to three levels of soil moisture in the glasshouse and one in the field conditions, and b) the occurrence of rain in the field experiment.

5.3.3 Early seedling survival and response of legume species

5.3.3.1 Experiment 1

Net seedling density was similar for the medium and high soil surface moisture treatments except for subterranean clover which was higher for the medium moisture treatment at 18 days after sowing (DAS) (Figure 5.3). The medium and high surface soil moisture gave higher seedling densities than the low moisture treatment for all the species from 4 to 18 DAS (Figure 5.3). For all the moisture levels the order of seedling survival was subterranean clover > strawberry clover > caucasian clover. The field trial gave a higher seedling density for subterranean clover and a lower density for the other two species than the low moisture treatment in the glasshouse. Germination was delayed for strawberry, subterranean and caucasian clovers in the field than to in the glasshouse (Figure 5.3).

Figure 5.4 shows that at 10 DAS the seedling density at all surface GSWC levels was greatest for subterranean clover and least for caucasian clover. Seedling density of all three species increased when the soil surface GSWC increased from 23 to 45% (Figure 5.4). The percentage increase in the number of seedlings per tray was 164, 197 and 172% for caucasian, strawberry and subterranean clovers, respectively between 23 and 45% soil surface GSWC (Figure 5.4).

5.3.3.2 Experiment 2

The total seedlings and net number of seedlings and radicles that survived and died from 0 - 40 DAS are shown in Figure 5.5. The main effects of legume species and soil moisture and their interaction were significant ($P<0.05$). There were more radicle deaths in the low moisture treatment and more seedling deaths in the high moisture treatment (Figure 5.5; Table 5.2). Overall, at all moisture levels the total seedling number was higher (> 130 per tray) for subterranean

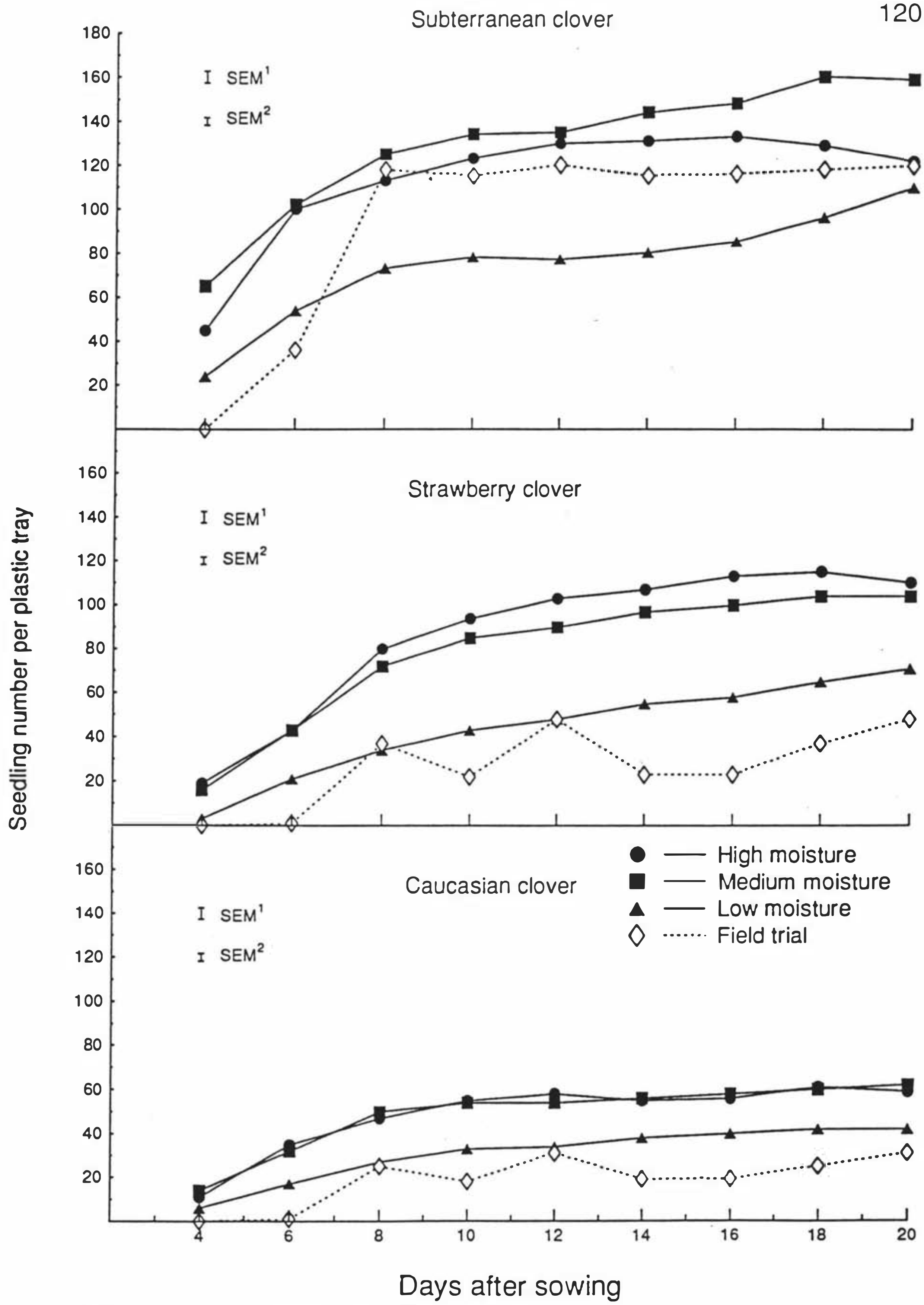


Figure. 5.3 Average response of seedling number per tray of three legume species to three surface moisture treatments in the glasshouse and one in the field.
[1]SEM for glasshouse trial; [2]SEM for field trial.

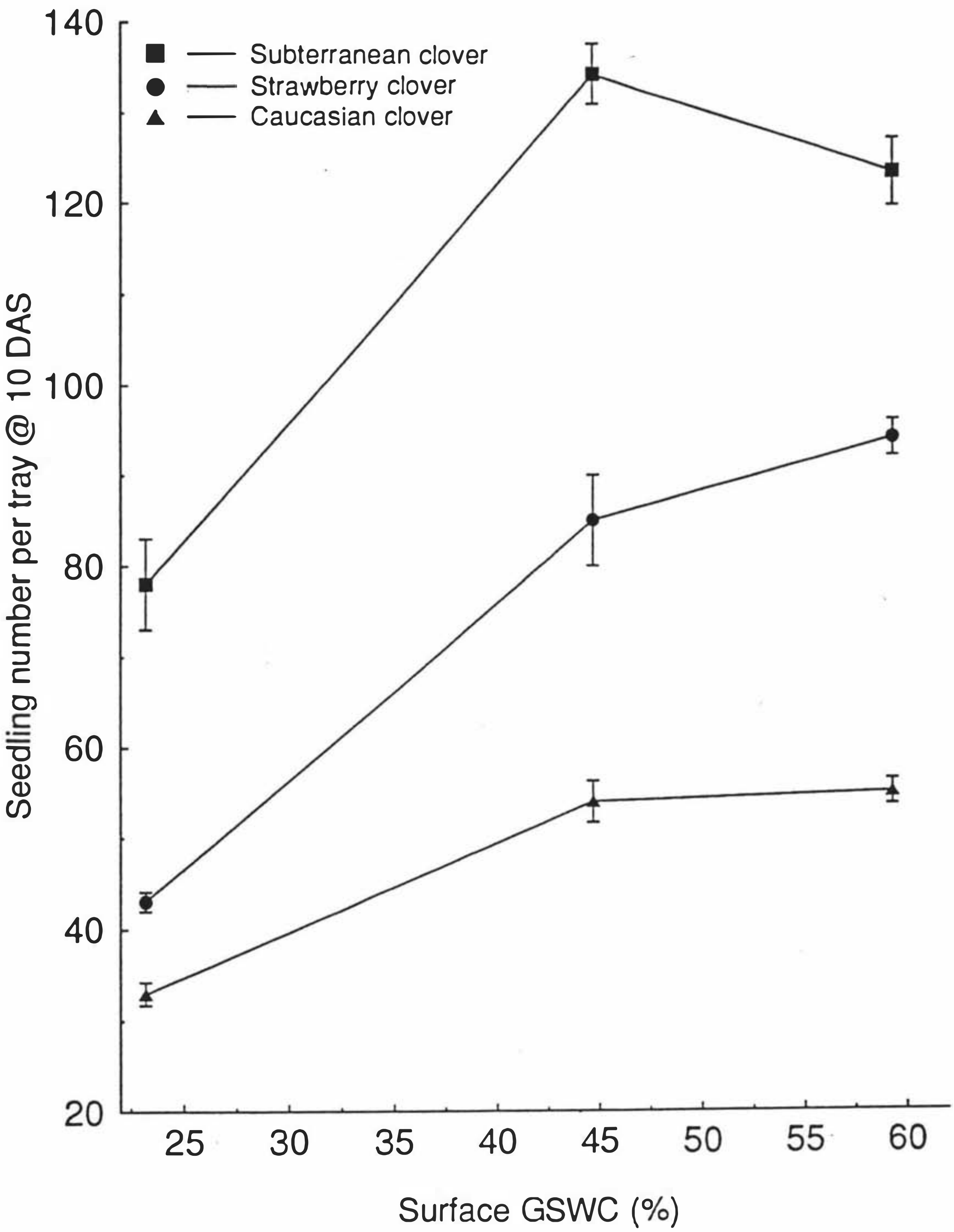


Figure. 5.4 The response of seedling number per tray at 10 DAS to the GSWC of the soil surface measured by $CoCl_2$ test for three legume species in the glasshouse.

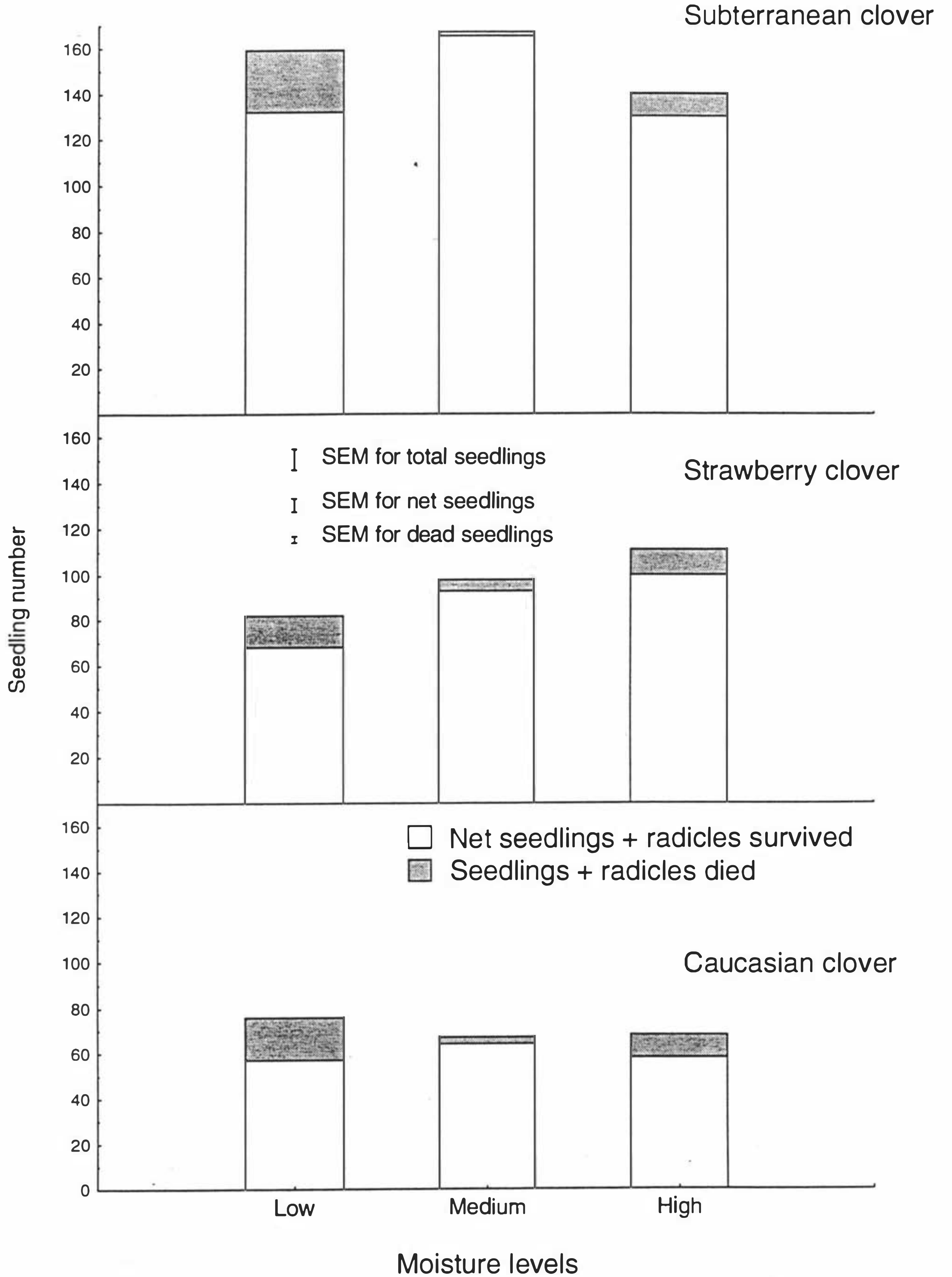


Figure. 5.5 The total and net number of seedlings plus radicles that survived and died for three legume species at three surface moisture levels in the glasshouse after 40 DAS.

Table 5.2 Fate of 216 seed oversown in plastic trays from 0 - 40 DAS in Experiment 2 (Figures in parentheses are the percentage of total number of seed that were accounted for).

Species	Moisture	Visible seedlings	Seedling died	Radicles alive	Radicles died	Ungerminated and hard seed (6 -40 DAS)	Imbibed but ungerminated seed (0 - 4 DAS)	Total (% of seed sown)	Seed not found (unaccounted)
Subterranean clover	Low	95 (44)	0	37 (17)	27 (13)	49 (23)	9 (3)	216 (100)	0
	Medium	141 (67)	2 (1)	24 (11)	0	36 (17)	9 (4)	211 (98)	5
	High	104 (57)	10 (5)	26 (14)	0	32 (17)	11 (7)	182 (84)	34
Strawberry clover	Low	49 (32)	0	19 (12)	14 (9)	59 (39)	13 (8)	153 (71)	63
	Medium	82 (49)	5 (3)	11 (7)	0	58 (30)	11 (7)	167 (77)	49
	High	90 (51)	11 (6)	10 (6)	0	54 (30)	12 (7)	177 (82)	39
Caucasian clover	Low	33 (17)	2 (1)	24 (12)	17 (9)	94 (48)	24 (13)	192 (89)	24
	Medium	55 (29)	3 (2)	9 (5)	0	106 (56)	18 (8)	190 (88)	26
	High	47 (25)	10 (5)	11 (6)	0	101 (54)	19 (10)	188 (87)	28
SEM		3.8	1.4	2.5	2.1	3.6	1.2		
Significance									
Species		***	ns	***	**	***	**		
Moisture		***	***	***	***	*	**		
Species x Moisture		***	ns	ns	*	ns	*		

clover than strawberry clover (< 110) and caucasian clover (< 80). At the high moisture treatment strawberry clover produced a greater number of seedlings than at the low and medium moisture treatments (Figure 5.5).

For seedling survival there was a significant interaction between the three species and time, using repeated measurement analysis ($P<0.001$). The comparison of percentage of seedlings that survived from viable seed and total seed oversown is given in Table 5.3. The number of viable seed sown was calculated by subtracting the hard and ungerminated seed from the total seed present in each tray (Table 5.2). The total number of ungerminated and hard seeds that were present in the soil sods is given in Table 5.2. On the basis of the number of viable seeds sown, subterranean clover still had significantly higher seedling survival than to the other two legume species ($P<0.05$; Table 5.2). The medium moisture level always had a higher number of visible seedlings (average > 85%) for each legume species compared to the low and high moisture levels (average < 75%).

Almost all the legume species at the different moisture levels could be fitted to a cubic response for seedling survival over 0 to 30 DAS except at the high moisture level for subterranean and strawberry clovers (Figure 5.6). These functions were used to estimate the time taken to half the maximum seed germination (Table 5.4). The species reached their maximum number of seedlings at different times and they also differed in the time taken for half of the seed to germinate (Table 5.4). Half of the maximum seed germination occurred in less than two days for subterranean clover at all the moisture levels. For strawberry and caucasian clovers half of the maximum seed germination occurred after 5 days or more except for the medium moisture level of caucasian clover at 2 DAS (Table 5.4).

Table 5.3 Seedling survival (visible seedlings plus alive radicles) of three legume species under three moisture regimes from total seed sown and viable seed sown (standard germination test).

Species	Moisture	% survival from total seed accounted for	% survival from viable seed sown
Subterranean clover	Low	61	79
	Medium	78	94
	High	71	87
Strawberry clover	Low	44	72
	Medium	56	85
	High	57	81
Caucasian clover	Low	29	58
	Medium	34	76
	High	31	67
SEM		3.1	4.1
Significance			
Species		***	***
Moisture		**	**
Species x moisture		**	**

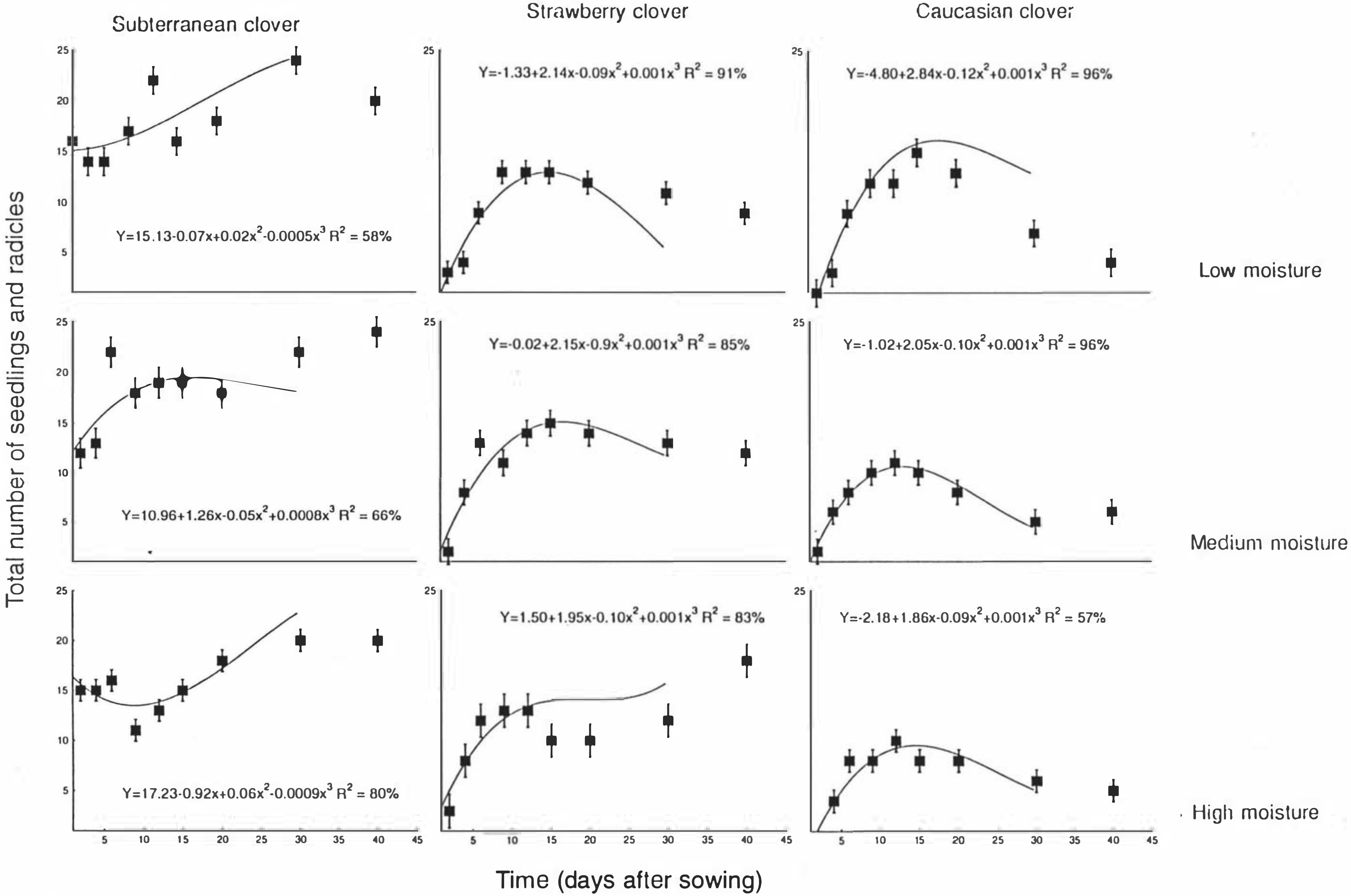


Figure. 5.6 Relationship of total number of seedlings and radicles over time for different legume species and different soil surface moistures.

Table 5.4 Days to half of the maximum seedling germination for three species and three moisture levels.

Species	Moisture level	Predicted time for half of the seed to germinate (days after sowing)
Subterranean clover	Low	< 2
	Medium	< 2
	High	< 2
Strawberry clover	Low	5
	Medium	5
	High	7
Caucasian clover	Low	6
	Medium	2
	High	5

5.3.4 Fate of oversown seed

The total number of visible and dead seedlings, visible and dead radicles, and ungerminated and unaccounted for seeds is given in Table 5.2 of the 216 seed sown. A greater number was unaccounted for in strawberry clover, which had the smallest seed, than in subterranean and caucasian clovers. Overall, there were 100 seeds (47% of total) of caucasian clover that did not germinate even at the high soil moisture level, and there were 39 (18% of total) and 57 ungerminated seeds (26% of total) for subterranean and strawberry clovers, respectively (Table 5.2). There were no radicle deaths for any legume species in the high and medium moisture levels and there were more seedling deaths ($\leq$ 5%) at the high moisture level (Table 5.2). In the low moisture level the overall average radicle death was more than 10% of seed sown.

Overall for subterranean, strawberry and caucasian clovers seed produced 70, 52 and 31%, respectively of a visible seedling or radicle (Table 5.2). All the species and moisture levels had less than 6% of seed produced a radicle or seedling that died. The fate of seed at the last harvest (40 DAS) represented the success of seedling establishment to that time (Table 5.5). Subterranean clover had approximately 2.5 and 6 times as many seedlings under the low moisture treatment as strawberry and caucasian clovers, respectively. There were significantly more hard and ungerminated seed for caucasian clover than for the other two species (Table 5.5).

The simulated treading technique with the studded roller resulted in about 51% of oversown seed buried (i.e. not visible on top of the soil surface) with the remainder on the soil surface (Table 5.6). This data excluded the germinated seeds and was an estimate based on the ungerminated seed that were found during the trial period. As a consequence of the greater proportion of

Table 5.5 Fate of sown seed at the last harvest (40 DAS)

Species	Moisture	Visible seedlings	Seedling died	Radicle alive	Radicle died	Ungerminated seed
Subterranean clover	Low	20	0	0	1	1
	Medium	24	0	0	0	0
	High	18	2	0	0	0
Strawberry clover	Low	8	0	0	0	2
	Medium	10	2	0	0	2
	High	18	3	0	0	2
Caucasian clover	Low	3	1	0	2	5
	Medium	6	1	0	0	4
	High	4	2	0	0	7
SEM		1.7	0.8	-	0.6	1.1
Significance						
Species		***	ns		ns	***
Moisture		*	**		*	ns
Species x moisture		**	ns		ns	**

Table 5.6 Percentage of oversown seed of the three legume species after simulated sheep treading. Seed was regarded as on the surface of the soil if visible and buried in soil if not visible during the whole trial period.

Species	Moisture	Seed on the soil surface (%)	Seed burried in the soil (%)
Subterranean clover	Low	43	44
	Medium	30	38
	High	27	45
Strawberry clover	Low	41	40
	Medium	31	40
	High	31	36
Caucasian clover	Low	70	59
	Medium	59	59
	High	55	57
SEM		5.0	3.2
Significance			
Species		***	***
Moisture		**	*
Species x moisture		*	*

ungerminated seed in caucasian clover there was more seed found in this treatment during the experiment but there was no evidence of any difference between the species in terms of the proportion of the seed buried by treading (Table 5.6).

5.3.5 Ungerminated / hard seed

The average percentage of ungerminated seed from the standard germination test, the accelerated aging (AA) test (for detail see Chapter 3) and the seed oversown are compared in Table 5.7 for subterranean, strawberry and caucasian clovers. The standard laboratory seed germination test gave a lower percentage of ungerminated and hard seed compared to the AA test and oversown seed (Table 5.7). The seed oversown in plastic trays had 19, 35 and 53 percent of ungerminated and hard seed for subterranean, strawberry and caucasian clovers, respectively.

5.3.6 Radicle length

There was a significant interaction between the different legume species and soil moisture levels on radicle length on the soil surface ($P<0.05$). The radicle length was greatest at the low moisture level in all the species ($P<0.05$; Figure 5.7). The subterranean clover radicle length was higher (> 8 mm) at the low moisture level than for the other two species but the radicle length at the low moisture level for strawberry and caucasian clovers increased relatively more, compared with the other moisture levels than for subterranean clover ($P<0.05$; Figure 5.7).

Table 5.7 Percentage (average) of hard and ungerminated seed present in standard seed germination test, accelerated aging test and oversown on soil surface and then trodden.

Species	Standard seed germination test	Accelerated aging test	Seed oversown on soil surface
Subterranean clover cv. Karridale	6	10	19
Strawberry clover cv. G. Onward	15	19	35
Caucasian clover cv. G. Monaro	27	49	53
SEM	6.08	11.08	9.82

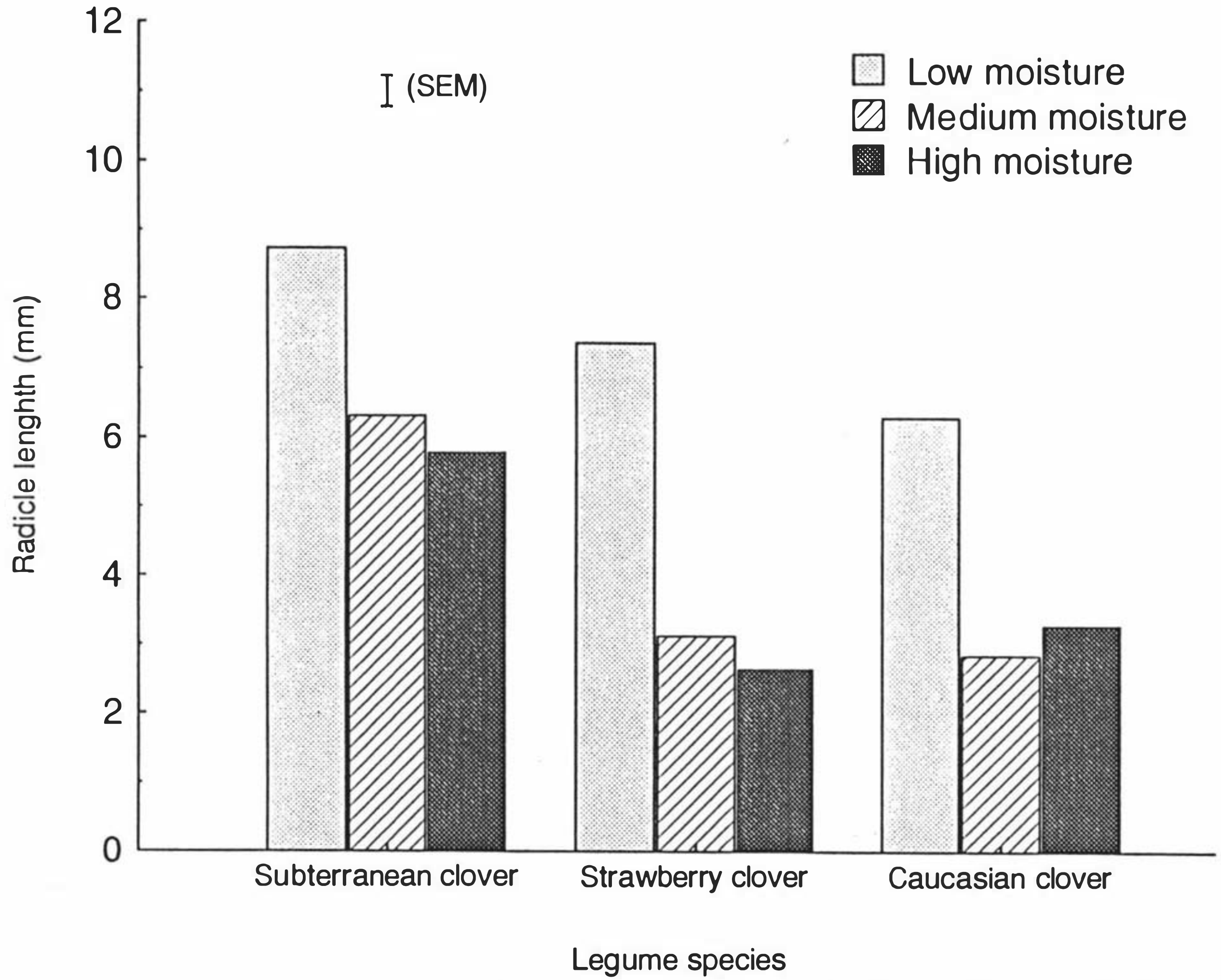


Figure. 5.7 The average length of radicles present on the soil surface from oversown seeds of three legume species at three moisture levels from 0 to 20 DAS.

5.4 Discussion

5.4.1 Surface soil moisture and its measurement

Soil surface moisture has been shown to be vital for successful oversowing of legumes because of seed imbibition and radicle entry (McWilliam *et al.,* 1970; Campbell and Swain, 1973b; Campbell, 1992), but the best moisture conditions for germination and seedling survival for surface sown pasture have been difficult to predict using indirect methods like probability of rainfall (Campbell, 1968), timing of rainfall (Barker *et al.,* 1988), and soil moisture budgets (Dowling and Smith, 1976). Direct measurement of soil surface moisture offers a way of defining the relationship between seedling survival from oversowing and soil surface moisture for use in predictive models.

The $CoCl_2$ technique was a cheap, rapid and sensitive technique for measuring changes in the soil surface moisture. The good relationship between soil surface moisture and legume seedling survival demonstrated that the $CoCl_2$ technique has a role in determining the best soil surface moisture conditions for oversowing of different pasture legume species. It is difficult to predict or measure soil surface moisture indirectly, especially from rainfall, whereas the $CoCl_2$ test can directly measure it. $CoCl_2$ strips can possibly be used as a predictor tool for suitable conditions for oversowing as they directly measure a key parameter of seed germination. Nevertheless, the technique requires further testing for the possible effects of factors like different soil textures and storage of $CoCl_2$ strips on the calibration.

The calculated water flux for a transition time of 3 min (31 mm day^{-1}) was considerably greater than the calculated potential evapotranspiration of 3 mm day^{-1} in the prevailing field conditions. This suggested that water movement into the $CoCl_2$ strip was largely due to capillary action rather than evaporation. Therefore, contact between the soil surface and the $CoCl_2$ strip was critical to the consistency of the technique. Although it was not investigated, the $CoCl_2$ strips might provide a useful indication of potential evaporation from the soil surface.

Large differences between surface GSWC and total GSWC occurred in the glasshouse, even for the low moisture level where surface GSWC was less than half of the total GSWC (Table 5.1). These results suggested that soil moisture at depth was a poor indicator of seed germination and seedling survival compared with the surface soil moisture. The difficulty in predicting successful oversowing from rainfall or soil moisture budgets (Campbell, 1968; Dowling and Smith, 1976; Barker *et al.,* 1988) was presumably due to poor correlation between these measurements and soil surface moisture. The influence of the wind on soil surface moisture was shown by the lower surface GSWC in the low moisture treatment relative to the medium moisture treatment (Table 5.1).

5.4.2 Response of legume species to soil moisture

The $CoCl_2$ technique showed that the field soil surface moisture was too low for germination of strawberry and caucasian clovers, even though the total GSWC was near to field capacity soon after sowing (Figure 5.2). Soil surface moisture needed to be around 45% GSWC for the maximum germination of seed, but this was species dependent as observed in the glasshouse trial (Figure 5.4). Compared to this GSWC (45%) for higher germination, the GSWC for field capacity was 38% and only the low moisture treatment had lower GSWC values than field capacity. The slow germinating species were more sensitive to soil surface moisture (Figures. 5.2 and 5.3). The faster germination and larger radicle of subterranean clover contributed to its superior seedling survival compared with caucasian and strawberry clovers.

There was greater seed germination and seedling survival in the medium moisture treatment than for other moisture treatments for all the legume species. The high and, particularly, the low moisture treatments reduced seedling survival.

Dowling *et al.* (1971) observed that the surface soil moisture condition was the key factor in seedling survival and establishment. McWilliam and Dowling (1970) noted that water loss from an imbibed seed was also an important factor. However, there were more seedling deaths for all species under the high moisture than the low moisture conditions (Table 5.2), probably due to anaerobic conditions. Campbell and Swain (1973b) also noted that seedling mortality during germination and emergence of aerial sown seed was mainly related to low soil moisture. Bellotti (1984) concluded that the factors affecting seedling survival were first reduction or cessation of germination at low water potentials, and second radicle entry into hard soil, which was also a function of soil moisture.

5.4.3 Radicle survival and death

The low moisture treatment had a significant effect on radicle death (Table 5.2) and radicle length (Figure 5.8) of all three legume species. Radicle death probably occurred due to insufficient soil surface moisture resulting in radicle elongation but failure into the hard soil surface. Campbell *et al.* (1987) observed that legume radicles tended to grow along the soil surface until they encountered a suitable entry site and radicles of surface sown seed may not penetrate a compacted surface (Campbell and Swain, 1973b).

Wind has both direct and indirect effects on radicle and seedling survival from oversown seed. Wind directly affects imbibition by the seed through its effect on surface soil moisture (Sedgely, 1963; McWilliam and Dowling, 1970), and indirectly by drying out the radicle while it is exposed to the air and by increasing soil impedance to radicle penetration (Campbell and Swain, 1973a; McWilliam *et al.*, 1970). The radicle tips of slower germinating species were observed to desiccate and all species suffered radicle death in the low moisture treatment and in the field experiment. Once the tip of the radicle is killed, the remainder of the radicle though healthy has no chance of entry into the soil and desiccation of the radicle tip during attempted entry into the soil was reported as a major problem by Campbell and Swain (1973b).

Similarly, Tadmor and Cohen (1968) observed that the ability of the radicle to enter the soil, and the rate of its extension during early seedling development was especially important under conditions where the surface soil moisture dried rapidly after a period of rain. McWilliam *et al.* (1970) found radicles of legume species could not survive five hours of drying, indicating that the radicle must enter the soil quickly to escape desiccation, even in controlled environmental conditions.

5.4.4 Seed germination tests

The percentage of ungerminated or hard seed after oversowing was greater than that in the standard germination test for subterranean, strawberry and caucasian clovers. Presumably some of the viable seed became dormant or non-viable, although glasshouse conditions were good for germination. A similar result was recorded by Chapman *et al.* (1985) under hill country oversowing for white clover cv. G. Huia. The cause of this greater proportion of ungerminated seed was unknown. The number of viable seed is usually calculated from the standard seed germination test or accelerated aging test but these clearly do not equate to the germination of legume species when oversown. The large number of ungerminated seed affected the seedling survival by producing less seedlings. Greater losses of seedlings from the pre-appearance phase and few losses from the post-appearance phase were also noted by Chapman *et al.* (1985) for white clover cv. G. Huia, whereas Barker and Zhang (1988) observed that seedling losses for G. Tahora were split equally between pre- and post-appearance phases.

5.4.5 Fate of oversown seed

Location of seed after oversowing and simulated treading was difficult, even in the glasshouse experiment where seed was oversown precisely with the a quadrat and carefully applied, simulated treading. The unaccounted or disappearance rate of seed was higher for small seeded strawberry clover than for the large seeded species. Most of the ungerminated seed was on the soil surface and in the low moisture condition.

5.5 Summary

Soil surface moisture is a dominant factor influencing the early survival and establishment of surface sown seed, but its measurement is difficult. A cobalt chloride ($CoCl_2$) saturated paper strip technique was a useful, fast and cheap method to measure the soil surface moisture. Although this technique was not measuring actual soil evaporation, it could be a useful indicator of potential evaporation.

The soil moisture at depth was a poor indicator of seed germination and seedling survival compared with the surface soil moisture. Germination and seedling survival was lower at 25% surface GSWC than at 45 and 62% GSWC. The low soil moisture treatment and the field trial gave the lowest seedling survival. Subterranean clover was less susceptible to low soil surface moisture and had better net seedling survival at all the moisture levels compared with the other two legume species. The rate of germination was clearly species dependent.

The main cause of low surface soil moisture was wind, which quickly dried and hardened the soil surface, and either killed radicle tips or increased radicle length prior to entry into the soil.

Standard seed germination tests did not adequately predict the proportion of viable seed in the seed sown. The percentage of ungerminated or hard seed was higher under oversown conditions compared to the standard seed germination test.

Chapter 6

Chapter 6

EFFECT OF GLYPHOSATE RESIDUE, AND LITTER PHYTOTOXICITY ON OVERSOWN LEGUMES

6.1 Introduction

To reliably establish pasture by oversowing in grass dominant pasture it is essential that the resident species be killed or retarded by herbicide (Campbell, 1968). Chemical renovation of pasture has been used world-wide. In practice, establishment of surface sown species is less successful on un-sprayed than on herbicide sprayed pastures (Campbell, 1968; Dowling *et al.*, 1971; Campbell, 1976). Glyphosate (N-phosphonomethyl glycine) is commonly used to kill pasture before oversowing (Blackmore, 1957; Naylor *et al.*, 1983; Kim *et al.*, 1985; Lambert *et al.*, 1985; Malik and Waddington, 1990; Campbell, 1992), since it controls a wide range of grasses, some legumes and broadleaf weeds (Campbell, 1974a).

Glyphosate has been used in pasture renovation for suppressing or killing the existing vegetation because 1) it is non-selective, 2) it is translocated readily throughout the treated plants, 3) it is inactivated rapidly within most soils and 4) it is non-toxic to humans (Moshier and Penner, 1978). Nevertheless, Campbell (1976) concluded there is a chance the residual sprayed plant material might impair the germination and emergence of oversown seed. Davies and Davies (1981) found that a 21 days interval between applying glyphosate and sowing was significantly better ($P<0.05$) for the direct drilling of white clover than 14, 7 and 0 day intervals. Awan (1989) found a residual effect of glyphosate on seedling survival of white and subterranean clovers and Maku lotus when herbicide was sprayed two days before sowing. Overall this effect reduced the number of seedlings by 70% at 30 DAS compared to the paraquat treatment.

The results in Chapter 3 showed that in seven trials the establishment of legume species by oversowing into existing pasture was less than 15%. As, a high rate of glyphosate (12 ℓ ha^{-1}) was blanket sprayed on the resident pasture 20 to 22 days before oversowing each trial, there was a need to examine the possibility of phytotoxicity of glyphosate sprayed pasture on seed germination and seedling survival.

6.2 Materials and method

The trial was conducted in a glasshouse of the Department of Plant Science, Massey University between August and September, 1993. There were two soil surface treatments and three legume species.

The two surface treatments were:

1. Intact sods sprayed with glyphosate at the rate of 12 ℓ ha^{-1} 20 days before sowing were placed in plastic trays (420 x 300 x 50 mm). The dead plant material that resulted from the herbicide used on the sods was not removed. The sods were described in Section 5.2.1.

2. Fine river sand of grade 3 in identical plastic trays.

The three legume species used were:

1. Subterranean clover cv. Karridale
2. Strawberry clover cv. Grasslands Onward
3. Birdsfoot trefoil cv. Grasslands Goldie.

Bare seed of each species were sown on the surface of the sod and the sand with one seed per 20 x 20 mm square in a grid (total 216) on 1 August 1993. The standard seed germination test is given in Table 3.7. The seed oversown on the sods was subsequently pressed into the soil surface with a studded roller as previously described (Section 5.2.1). Seeds oversown on the sand surface were pressed down with the palm of the hand as it was unsatisfactory to use the studded roller on the sand. An automatic irrigation system was used to irrigate the trays from the base every day at 0900 hours.

The average maximum and minimum temperature in the glasshouse was 27.3 and 10.6°C for August and 29.2 and 11.1°C for September, respectively. Daylength (approximately 10 hr) and humidity were not artificially controlled.

All the visible seedlings were counted at 4, 6, 8, 10, 12, 14, 16, 18, 20, 30 and 40 days after sowing. At the end of trial (40 DAS) dry weight (24 h at 80°C) per plant was recorded for all the species and treatments.

There was a factorial combination of three legume species, two surface treatments and four replicates in a randomized complete block design. Data were analysed by analysis of variance using the GLM procedure of SAS (SAS, 1989).

6.3 Results

6.3.1 Seedling number

The number of seedlings per tray for all three species was significantly greater for the herbicide-sprayed soil than the ordinary sand up to 8 DAS, but from 12 DAS there was no significant difference between the two surface treatments ($P<0.05$; Figure 6.1). The final seedling number for both surface treatments was approximately 120, 56% of the seed sown.

6.3.2 Dry weight per plant

An interaction between legume species and surface treatment significantly affected the dry weight per plant ($P<0.05$; Figure 6.2). The dry weight of subterranean clover was higher for the herbicide sprayed surface treatment whereas for birdsfoot trefoil and strawberry clover there was no significant difference in dry weight per plant between the two surface treatments ($P<0.05$; Figure 6.2).

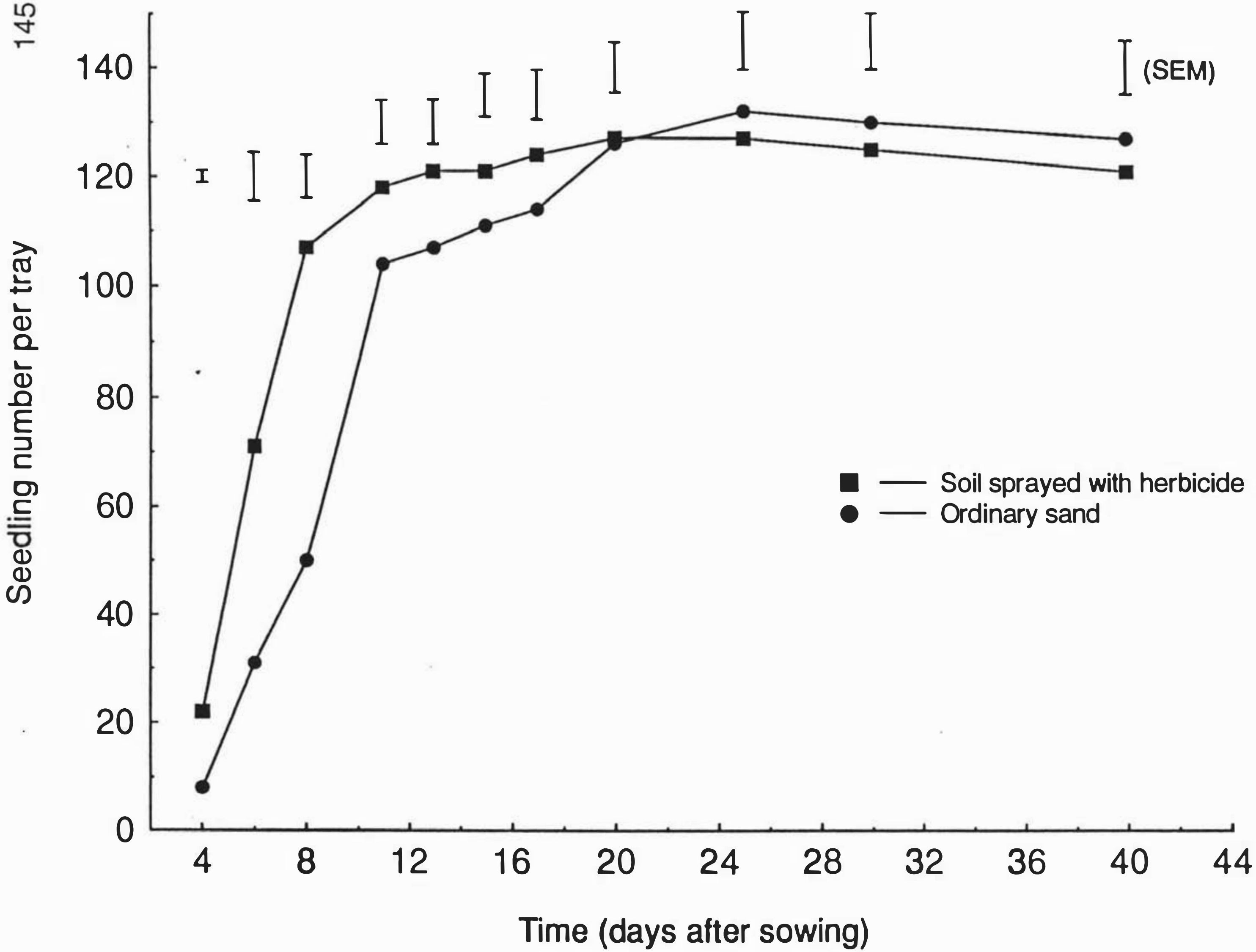


Figure 6.1 The average response of seedling number per tray of three legume species to two surface treatments in the glasshouse.

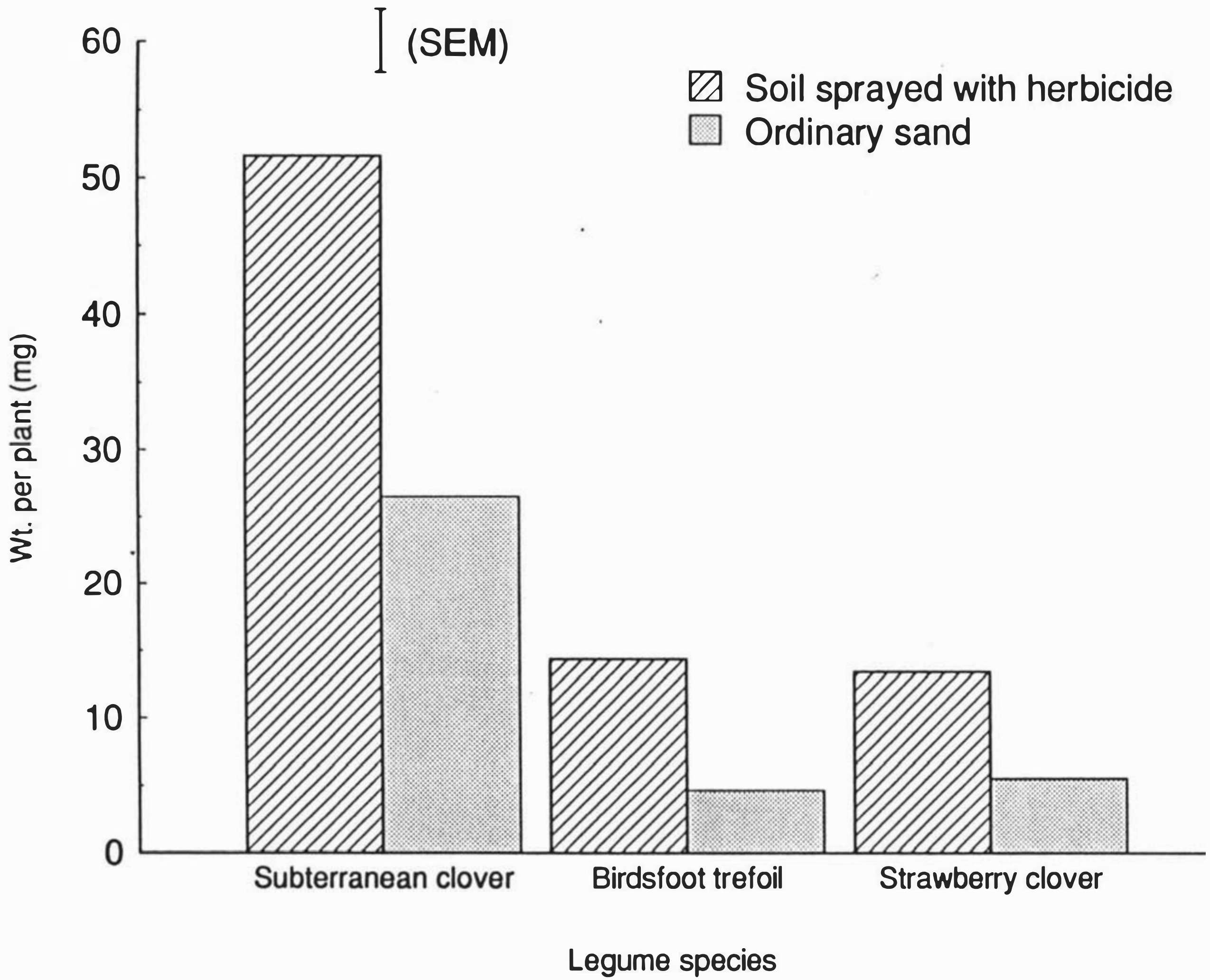


Figure 6.2 The dry weight per plant of three legume species 40 DAS for two surface treatments in the glasshouse.

6.4 Discussion

There was no evidence that the plant residue from glyphosate sprayed pasture was phytotoxic to seed germination and seedling survival. Initially the seedling numbers were higher for the herbicide sprayed soil compared to un-sprayed sand (Figure 6.1). Glyphosate sprayed 20 days before sowing did not have any residual affect on seedling survival and similar results were observed by Davies and Davies (1981) for white clover establishment. A 14 day interval between spraying glyphosate and sod seeding of alfalfa and alsike clover was also found to be adequate for their establishment (Welty *et al.*, 1981). The time between spraying and sowing was dependent on temperature and moisture, and high temperature (30°C) was a cause of greater toxin formation (Moshier and Penner, 1978). Campbell (1976) concluded that a time interval of 10 days for grasses and 20 days for alfalfa between glyphosate spraying and surface sowing was needed for adequate establishment. Glyphosate was rapidly in-activated when it contacted the soil (Sprankle *et al.*, 1975) so its application should not affect germination and seedling growth of grasses and clovers if sprayed a short period before sowing (Segura *et al.*, 1978).

Segura *et al.* (1978) observed phytotoxic effects of a low rate (2 kg ha^{-1}) of glyphosate when it was directly applied on oversown seed of red clover. The glyphosate affected primary emergence of clover seedlings from seed with symptoms including acute chlorosis, wilting, stunting and necrosis of the seedling (Segura *et al.*, 1978), and also reduced length of shoot (Moshier and Penner, 1978).

In this trial the plant material killed by glyphosate was not removed from the sod, in order to observe the effect of toxicity of either the herbicide or the plant residue. No toxic effect of the dead plant material was observed on seedling survival (Figure 6.1). However, Toai and Linscott (1979) demonstrated that alfalfa seedling development and growth was inhibited by toxins released from dead quackgrass (*Agropyron repens* (L.) Beauv.) leaves.

Dry weight per plant was significantly higher for soil sprayed with herbicide for subterranean clover. The silt loam soil used in this trial had a greater water holding capacity and greater nutrient content compared to sand, which probably helped to increase the dry weight per plant. Sand was used to avoid any phytotoxic effects but it was probably an inferior growth medium than the soil and may have had nutrient deficiency. The surface of the sand might have dried during each day and possibly decreased germination.

The results of this trial suggested that the high rate of glyphosate unlikely to cause poor legume establishment (Chapters 3, 4 and 5), and there was also no apparent phytotoxic effect on seed germination and seedling survival. However, the trial was not a fool-proof experiment as the sand was an inferior medium compared with the soil. It was possible that the 56% seedling survival in both the treatments was coincidental and other factors may have reduced seedling survival in sand treatment.

6.5 Summary

The glyphosate did not cause any major phytotoxic effect on seed germination and seedling survival if sprayed 20 days before sowing. The dead material of the resident pasture killed by herbicide did not show any residual effect of toxicity on the seedling survival compared to sand.

Chapter 7

Chapter 7

EFFECT OF SEED COATING AND SEED DRESSING ON LEGUME SEEDLING SURVIVAL

7.1 Introduction

Chapter 3 reported a series of trials in which bare seed of twelve legume species was oversown in drought-prone hill country. In these trials the net seedling survival from viable seed was less than 15%. Bare seed was used so that direct comparison could be made between species, and so that the environment by species interactions could be observed. Seed coating or pelleting is one way in which the germination and survival of seedlings can be improved (Scott, 1989). Reports on the effectiveness and ineffectiveness of seed coating are relatively common especially in Australia and New Zealand. Brockwell (1962) and Scott (1989) from Australia and Lowther and McDonald (1973); Vartha and Clifford (1973); Scott and Hay (1974); and Scott (1975) from New Zealand emphasised the importance of seed coating and concluded that there was a marked increase in pasture establishment when seed coating was used whereas Cullen and Ludecke (1966); Cullen *et al*. (1965); Adams and Lowther (1970); Douglas (1970) and Dowling (1978) agreed that seed coating did not increase the establishment of legumes in oversowing trials, even when the seed was inoculated.

The constituents of seed coating vary considerably and therefore the mechanisms by which they might improve establishment also varies. Constituents of seed coatings include nutrients, fungicides, insecticides, rhizobia, organic compounds, enzymes, lime, fertiliser, dyes, bird repellants and "innert" matter for seed ballistics. For example Musgrave (1975) concluded that seed coating had no consistent effect on seedling establishment, but the benefits of coating on nodulation and plant survival offer one of the most practical ways of increasing the establishment of oversown legumes. Because of the severe environmental conditions in tussock grasslands high country Lowther (1977) recommended oversowing of inoculated coated seed of legumes.

Campbell *et al.* (1987) proposed that seed coating could assist establishment from surface sowings by protecting the radicle tip against desiccation but no suitable pellet has been designed for this purpose because the radicle tip grows away from seed during attempted entry into the soil (Dowling *et al.*, 1971). For example, subterranean clover radicles grew along the soil for more than 8 mm in low moisture conditions (Chapter 6). Suckling (1949) indicated that coating of seeds could be beneficial during spring when seedling survival is usually very low in moist hill country, possibly due to a high slug population. Cameron (1993a) showed the need for fungicide seed treatment to control seedling diseases of tall fescue and red clover.

In New Zealand the use of seed coating has been controversial and its recommendation has received varied support (Lowther, 1977). However, commercially coated legume seed has been widely used in New Zealand (MacKinnon *et al.*, 1977) but mainly to increase nodulation of oversown legumes through improved survival of rhizobia on the seed after inoculation (Lowther, 1975).

Due to the conflicting reports on seed coating it was decided to test the effect of seed coating and seed dressing on oversown legume seedling survival at the Poukawa site used for the oversowing trials in Chapter 3. Therefore, the purpose of this study was to:-

- test the effect of commercial seed coatings on legume seedling survival;
- test the effect of seed dressing with fungicide and insecticide on seedling survival of legume species.

7.2 Materials and Methods

The trial was situated at AgResearch, Poukawa Research Farm (Section 3.2.1). There were five seed treatments (Table 7.1) and two legume species. The legume species used were subterranean clover cv. Karridale and white clover cv. Grasslands Tahora. These species were chosen for their common usage in hill country pasture. The seed coating and dressing of the legume species was carried out by Hodder and Tolley Limited (commercial seed coating company), Palmerston North on seed from the same seed line. The 500 viable seed of each species and treatment was sown by hand broadcasting in 1 x 1 m plots on 30 March, 1994. The number of viable seed was calculated according to the viable seed percentage measured by the accelerated aging test method (Table 3.7). The seed coating increased the seed size so for uniformity bare, dressed and coated seed was mixed with 200 g of dry vermiculate to ensure an even distribution during broadcasting. The management of the trial was similar to that described in Section 3.2.5.

All viable seedlings within the 1 m^2 plots were counted at 15, 30, 45 and 60 days after sowing. Meterological data were collected 500 m from the trial site.

There was a factorial combination of two legume species, five seed treatments and four replicates in a randomized complete block design. Data were analysed by analysis of variance and to make particular comparisons between groups of seed treatments, the contrast statement of the GLM procedure of SAS was used (SAS, 1989).

Table 7.1 Seed treatments of legume species

Treatments	Description
Bare seed	No seed treatment
Fungicide	Seed dressing of Apron 35 fungicide at 4.2 mg kg^{-1}
Insecticide	Seed dressing of Promet 800 SCO insecticide at 50 mℓ kg^{-1}
Nutriprill	Commercial seed coating (excluding fungicide and insecticide)
Nutriprill plus	Commercial seed coating (including trace elements, fungicide and insecticide)

3.7 Results

7.3.1 Climatic conditions

The trial year (1994) was drier than the previous two years. Daily maximum, minimum air temperature and soil temperature, wind run and rainfall were measured for six months in 1994 (Table 7.2). The mean maximum and minimum air temperatures and 10 cm soil temperature were typical of the trial site. The daily mean wind run was low from January to April, 1994 compared to the previous two years (Table 7.2). The total monthly rainfall was very low during February (19 mm) and March (23 mm) for 1994 which affected the 10 cm soil moisture (26% GSWC) at the time of sowing.

7.3.2 Response of seedling survival to seed treatments

The interaction between seed treatments and legume species was significant over all the seedling measurement times ($P<0.05$; Figure 7.1). Seed germination and seedling density were higher for the seed coat treatments than bare seed up to 30 DAS, and there was no significant difference between the bare seed and seed coat treatments beyond 45 DAS ($P<0.05$; Figure 7.1; Table 7.3). The fungicide seed dressing treatment gave a lower seedling density for both the species compared to all other treatments ($P<0.05$; Figure 7.1; Table 7.3).

The Nutriprill treatment gave the highest seedling densities up to 30 DAS but there was no significant difference beyond 45 DAS ($P<0.05$; Table 7.3). There was no significant difference between the Nutriprill and Nutriprill plus treatments except at 15 DAS. The insecticide treatment gave significantly higher seedling numbers than the fungicide treatment ($P<0.05$; Table 7.3). The overall seedling survival (plant m^{-2}) for each species, for each seed treatments and seedling survival of the two legume species from bare seed is given in Appendix 7.1.

Table 7.2 Climatic data (monthly average) recorded at Poukawa AgResearch at 0900 hours for 1994

Climatic factors	Months					
	January	February	March	April	May	June
Maximum air temperature (°C)	28.4	26.2	23.6	22.2	17.9	13.6
Minimum air temperature (°C)	10.3	6.3	4.8	1.3	2.3	3.8
10 cm soil temperature (°C)	23.3	22.7	18.6	15.1	11.2	8.3
Wind run (km/day)	38.6	38.6	38.6	38.7	129.5	232.2
Rainfall (mm/month)	59	19	23	43	51	56

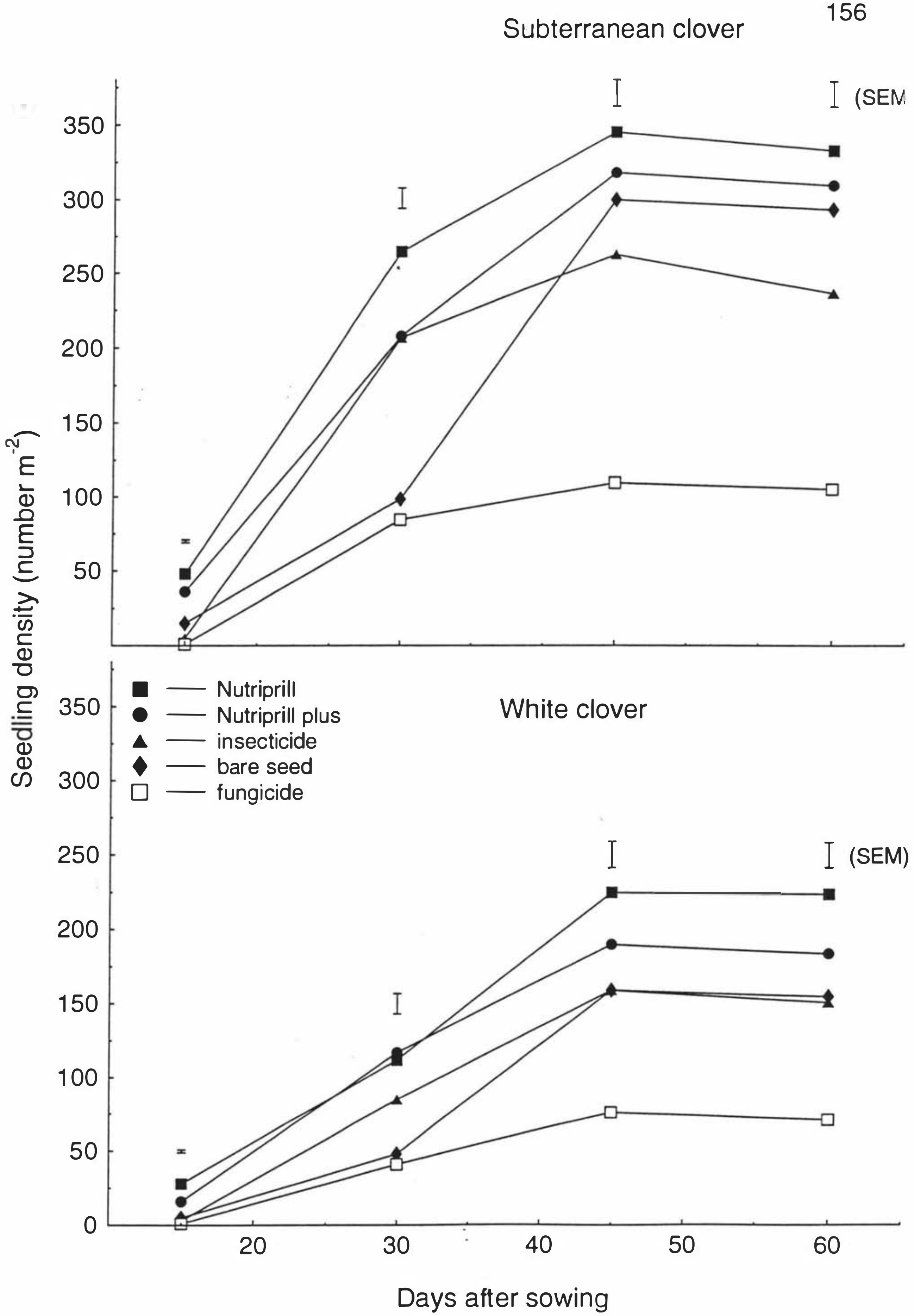


Figure 7.1 The average response of seedling number per unit area of two legume species under five seed treatments over time.

Table 7.3 Comparison and contrast of mean seedling density (number m^{-2}) of two legume species oversown with different seed treatments.

Treatments	Days after sowing			
	15	30	45	60
Bare seed vs	10	73	229	224
Nutriprill	38	188	284	278
Significance	***	***	ns	ns
Bare seed vs	10	73	229	224
Treated seed	17	139	210	202
Significance	***	***	ns	ns
Fungicide vs	1	62	93	88
All others	39	142	224	236
Significance	***	***	***	***
Insecticide vs	4	145	210	194
All others	19	121	215	209
Significance	ns	*	ns	ns
Nutriprill and nutriprill plus vs	32	175	269	262
All others	4	70	133	126
Significance	***	***	***	***
Nutriprill vs	38	188	284	278
Nutriprill plus	26	162	254	247
Significance	*	ns	ns	ns
Fungicide vs	1	62	93	88
Insecticide	4	145	210	194
Significance	**	***	***	***

***; P<0.0001, **; P<0.001, *; P<0.05, ns: no significant difference

7.4 Discussion

Seed coating increased seedling number by 30% at 15 DAS but by 45 DAS there was no significant difference between coated and bare seed. The sowing conditions were dry (GSWC 26%) at the time of sowing which possibly contributed to the early germination of the coated seed by more moisture absorption compared to bare seed. Some seed coatings are known to increase the uptake of moisture by seed for germination (Dowling *et al.*, 1971) and to improve the penetration of seed through residual pasture to the soil surface due to its heavier weight (Hay, 1973). The early germination of coated seed could improve later seedling survival when the period of adequate moisture for seedling growth was brief. Scott (1975) found the major advantage of seed coating was often the increased weight rather than from the chemical properties of coating. The germination of the bare seed treatment was low soon after sowing due to low soil moisture but increased one month after sowing when rainfall fell resulting in the seedling density increasing to the same as for coated seed. This suggested that soil moisture was the major factor that affected the germination, and coated seed imbibed better than the bare seed. If the soil moisture was adequate at the time of sowing both bare and coated seed may have exhibited similar seedling densities following sowing.

The percentage of seedling survival was higher under this trial than to trials in Chapter 3. The number of viable seed in this trial was based on the AA test whereas Chapter 3 viable seed was based on the standard seed germination test. The number of viable seed was 90 and 83% for subterranean clover and 81 and 14% for white clover accounted by standard seed germination test and AA test (Table 3.7). The results of Chapter 3 suggested that low GSWC and high soil temperature conditions determined maximum seedling numbers during the germination phase and low minimum temperature and wind run determine later seedling survival. The values for these conditions were good during this trial (Table 7.2) which ultimately enhanced the seedling survival rate.

A high standard of inoculants and inoculated pelleted seed is required for oversowing in tussock grasslands because the specific rhizobia for oversown legume are not present and soil moisture conditions are severe (Lowther, 1977). In this trial the resident rhizobia was already present for the test species. Seed coating with rhizobia is necessary to aid legume nodulation where legume inoculation is a problem (Brockwell, 1962). Seed coating may increase seedling numbers under certain conditions soon after sowing (Scott, 1989) but there are other possibilities for increasing seedling number like increasing the seed rate above that usually recommended rate (Chapman *et al.*, 1993; Awan *et al.*, 1994) or using the heavier seed within the seed lot of some species (Hunt, 1954; Black, 1956; Chapter 4).

Seed dressed with Apron 35 fungicide produced very low seedling numbers. Presumably this fungicide had some residual toxic effect when directly applied to the bare seed. Cotyledons which emerged from the dressed seed were stunted and leaves were curly and small in size. Cameron *et al.* (1993a) dressed seed with the fungicide Metalaxyl and found a significant increase in the seedling number of red clover over untreated seed at 4 and 8 weeks after sowing, but fungicide treatments significantly reduced the number of rhizobia (Cameron *et al.*, 1993b). Promet showed no residual effect on seed germination.

Slugs are a major economic pest in temperate climates (Runham and Hunter 1970). Losses of legume seedlings after oversowing in New Zealand hill country have been attributed to slugs as well as management and climate (Suckling, 1949; Madden, 1952 and Charlton, 1977). Charlton (1978) showed that seedlings were easily killed by slugs up to the true leaf stage of development. There was no slug damage observed Poukawa AgResearch Farm but anti-slug pellets were used in every trial including this one.

7.5 Summary

Overall, it was concluded that seed coating improved the early germination rate but not final seedling density. Possibly the earlier germination of coated seed could have improved seedling survival in some of the other trials at Poukawa but as the soil was more often too wet rather than too dry it is doubtful that the general findings from the trials at Poukawa (Chapter 3) would have been affected by the use of coated rather than bare seed.

The commercial seed coating increased seed germination rate but not final seedling density compared with bare seed. There was a 30% increase in visible germination due to coating at 15 DAS.

Apron fungicide should not be used as a seed dressing for legumes.

Chapter 8

Chapter 8

OVERSOWN SEEDLING SURVIVAL AND ESTABLISHMENT MODEL

8.1 Introduction

Modelling is widely used in agricultural research. It may allow artificial expansion of the experimental time frame, and provision of possible answers to questions such as what happens when changes in the system occur (Jones, 1983). Models have been used to predict the behaviour of individual species, group of species or whole plant communities.

There are several models for seed germination from sowing that mainly use rainfall frequency and intensity, and surface temperature (Smith and Johns, 1975; McKeon and Kalma, 1977). It was observed in Chapter 3 (Section 3.3.8.4) that gravimetric soil water content (GSWC), soil temperature, minimum air temperature and wind run were more highly related to seedling establishment for seven legume species and seven sowing conditions than other environmental factors. These factors may have potential for developing a simple model that summarises the large data set from the field trials at Poukawa (Chapter 3) and might enable the best time for oversowing to be predicted. The approach in this chapter was to use modelling as an interpretative tool rather than to develop a definitive model.

Models for predicting seedling establishment have concentrated on the relationship between establishment and soil moisture. Dowling and Smith (1976) used the weekly soil moisture budget based on the WATBAL model of Keig and MacAlpine (1974) and transformed the simulated soil moisture data into percent establishment of seedlings using an empirically derived function. With this model they identified the optimum time of aerial sowing for perennial pasture on the Northern Tablelands of New South Wales. Cornish (1985) used a similar soil moisture budget procedure and combined it with a simple explicit germination and seedling survival model to construct growing conditions for annual Medicago spp. in Western Australia and New South Wales. Barker *et al.* (1988) used rainfall data for before and after oversowing of Grasslands Tahora white clover and found that germination was unrelated to rainfall in the week before or 3 weeks after sowing, but was linearly related to rainfall in the 1 or 2 weeks after sowing.

Leslie (1984) used more complex soil moisture and grass establishment models to investigate the effect of time of sowing on the establishment of warm season grasses. He found that while the model predictions agreed quite well with observations over 4 years, establishment probabilities estimated from long run climate data differed greatly from the corresponding probabilities estimated from his experimental results. His conclusion was that modelling provided the only practical approach to predicting optimal management decisions, including the choice of sowing time.

Bellotti and Moore (1993) commented on the models of Dowling and Smith (1976), Cornish (1985) and Leslie (1984) and concluded that these covered a variety of different environments, but all focused on establishment of individual plants and all treated soil moisture availability as the primary factor. Despite their potential utility all have remained as research models and have not been developed to the stage where they could be used to aid on-farm decision making.

Keeping in view the comments of Bellotti and Moore (1993) an attempt was made in this chapter to develop a simple, practical model from the field data covering a wide range of legume species and key environmental factors (GSWC, soil temperature, minimum air temperature and wind run). The principal aim was to develop a relatively simple model that could easily predict the number of germinated seedlings (given 100 viable seed sown), seedling survival and establishment at 90 DAS for oversown legume species in a drought-prone environment.

8.2 Methodology

8.2.1 Model concepts

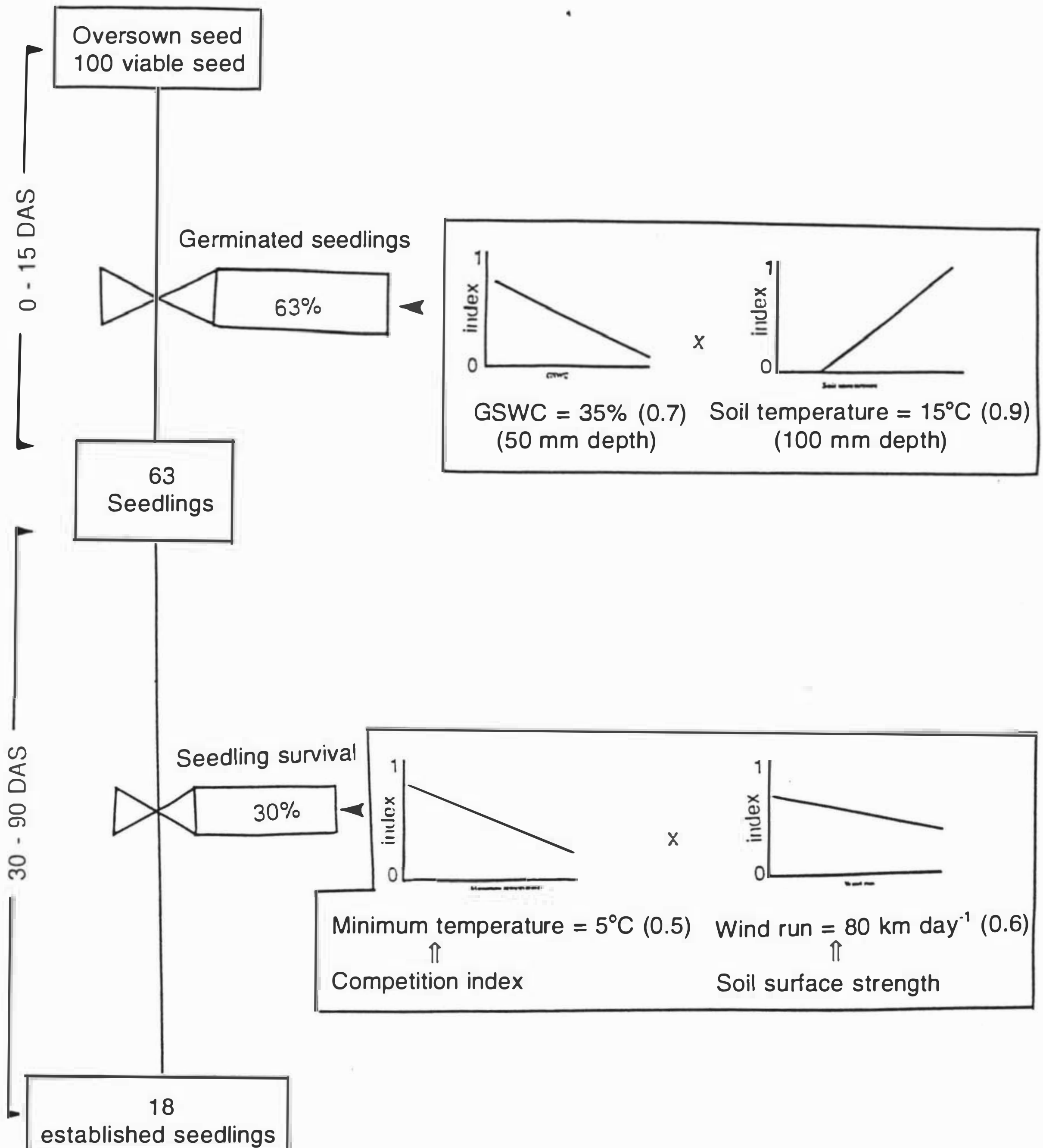
The flow diagram in Figure 8.1 gives the basic structure of the model and the relationship between the environmental factors of GSWC, soil temperature, minimum air temperature and wind run. The equations relating each environmental factor to the number of visible seedlings per viable seed developed in Chapter 3 were used. The GSWC to 50 mm depth and soil temperature at 100 mm depth were the best predictions for seed germination 0 - 15 DAS, and minimum air temperature and daily wind run were the best predictions for seedling survival 30 - 90 DAS. The period from 15 - 30 DAS was not used as it was a transitional period. The model input was 100 viable seed and the output was visible (net) seedling number from oversown seed that germinated 15 days after sowing, and number of seedlings established 90 days after sowing when subjected to the four climatic variables.

8.2.2 Meterological data

Five years of meterological data (1990 to 1994) from the trial site at Poukawa and 10 years of meterological data (1982 to 1991) from Lawn Road, Hastings (12 km from Poukawa) were used to demonstrate the model. Lawn Road was the closest location to Poukawa at which daily mean soil temperature (100 mm depth), minimum air temperature, and wind run were measured. Lawn Road data were not used previously in development of the model.

GSWC (50 mm depth) was not measured at Lawn Road and therefore was calculated from actual evapotranspiration (AET) and effective rainfall (ER) (Equation 8.1), with AET and ER on day_n being driven by GSWC of the previous day (day_{n-1}) (Dr. Dave Barker pers. comm.) (Equations 8.2 and 8.3). An assumption was that volumetric soil water content (VSWC) equalled gravimetric

Figure 8.1 Flow diagram showing basic structure of the model for predicting germinated seedling, seedling survival and establishmentindices from four environmental factors (values shown are for one possible case).



soil water content (GSWC) (bulk density = 1.0).

$GSWC_n = [(GSWC_{n-1} \times 50) + ER_n - AET_n] / 50$ (Equation 8.1)

$AET_n = 0.08 \times GSWC_{n-1} \times PET_n$ (Equation 8.2)

$ER_n = [0.4904 + (-0.0096 \times GSWC_{n-1})] \times rainfall_n$ (Equation 8.3)

Where PET (potential evaporation) was calculated from class A pan evaporation x 0.75. The equation parameters were fitted to measured GSWC (50 mm) from the Poukawa field site during 1992 and were validated against Poukawa, 1993 field data.

Means for 15 day periods between 1 January to 31 December were calculated for each year (5 at Poukawa, 10 at Lawn Road) for the four environmental variables.

8.2.3 Model parameterisation

The germinated seedling and seedling survival indices were based on a scale from 0 to 1 (Ritchie, 1981). The following equations for these indices were derived by multiplying the equations of the two individual environmental factors from Chapter 3 (Fitzpatrick and Nix, 1975).

A) For all legume species

i) germinated seedling index (0 - 15 DAS) =

$-175.81 + 33.35\ ST + 3.82\ GSWC - 0.73\ ST \times GSWC$, (Equation 8.4)

where ST was 100 mm soil temperature and GSWC was 0-50 mm gravimetric soil water content, derived from Equations 8.1, 8.2 and 8.3

ii) seedling survival index (30 - 90 DAS) =

$105.79 - 11.86\ WR - 0.33\ MT + 0.037\ MT \times WR$, (Equation 8.5)

where MT was minimum air temperature and WR was total daily wind run.

B) For subterranean clover

i) germinated seedling index (0 - 15 DAS) =

$$-1977.80 + 293.97\ ST + 45.09\ GSWC - 6.70\ ST \times GSWC \quad \text{(Equation 8.6)}$$

ii) seedling survival index (30 - 90 DAS) =

$$133.99 - 0.56\ WR - 16.63\ MT + 0.07\ MT \times WR \quad \text{(Equation 8.7)}$$

C) For birdsfoot trefoil

i) germinated seedling index (0 - 15 DAS) =

$$27.01 + 11.62\ ST + 0.0007\ GSWC - 0.27\ ST \times GSWC \quad \text{(Equation 8.8)}$$

ii) seedling survival index (30 - 90 DAS) =

$$50.47 + 0.22\ WR + 7.44\ MT + 0.03\ MT \times WR \quad \text{(Equation 8.9)}$$

The product of the two equations was used to calculate the seedling establishment index at 90 DAS. The establishment index for a particular sowing date was, therefore, the result of climatic conditions affecting seed germination 0-15 DAS, and climatic conditions affecting seedling survival 30-90 DAS. The seedling establishment trials in Chapter 3 were at Poukawa in 1992 and 1993 and therefore, the meterological data for these two years was used to construct the model. The predicted establishment for these two years was compared with the actual establishment. An independent data set for seedling establishment was not available.

8.3 Results

8.3.1 General

The germinated seedling index was the product of GSWC and soil temperature from 0 to 15 DAS (Figure 8.2). It was observed that GSWC from 25 to 37% and soil temperature (100 mm) from 13 to 20°C gave more than 80% seed germination. The seedling survival index was the product of minimum temperature and daily wind run from 30 to 90 DAS (Figure 8.3). The minimum air temperature within the range 0 to 2°C and wind run from 50 to 90 km per day gave more than 80% seedling survival.

8.3.2 Performance of the model

8.3.2.1 Poukawa

Of the five years of meterological data available at Poukawa, two years (1992 and 1993) were used to generate the equations used in the model. The actual values of the seedling establishment index for the Poukawa trials (1992, 1993) were compared with the values predicted by the model for these two years (Table 8.1). The actual and predicted mean seedling index were identical in 1992. In 1993 the mean seedling index for the Poukawa trials was 0.10 and predicted by the model was 0.11 (Table 8.1)

Figure 8.4 shows that there was a germinated seedling index of ≥0.5 for oversowing between October and February. The seedling survival index was 0.5 on only two dates (15 February and 1 July). Overall, the highest seedling establishment index was 0.25 when seed was oversown in the second half of February (Figure 8.4). That is, the best time for oversowing was mid to late February. The coefficient of variation (CV) for this time of sowing was 58%. The seedling establishment index was less than 0.05 for seed sown between August

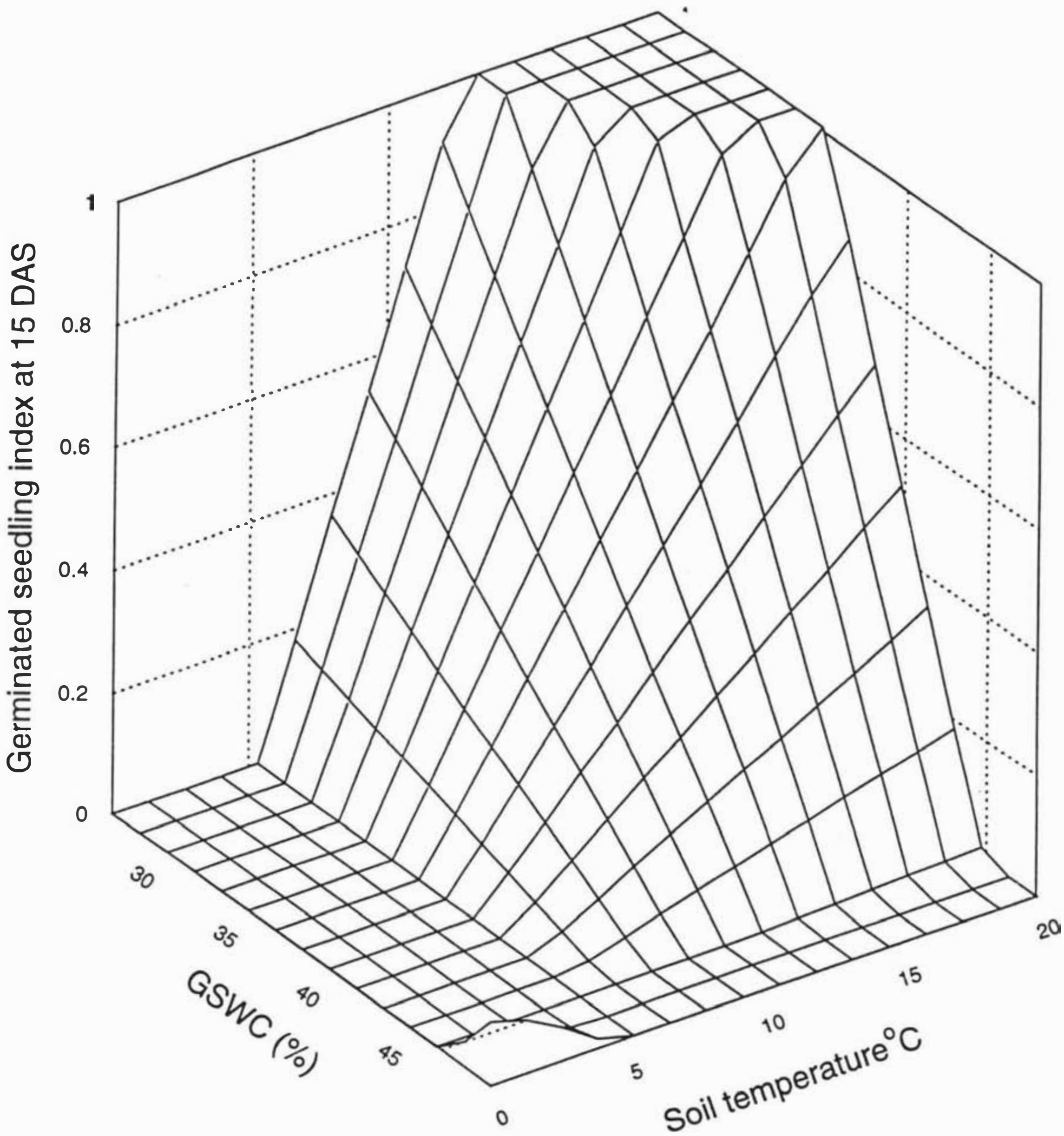


Figure 8.2 Interaction of GSWC and soil temperature on the germinated seedling index.

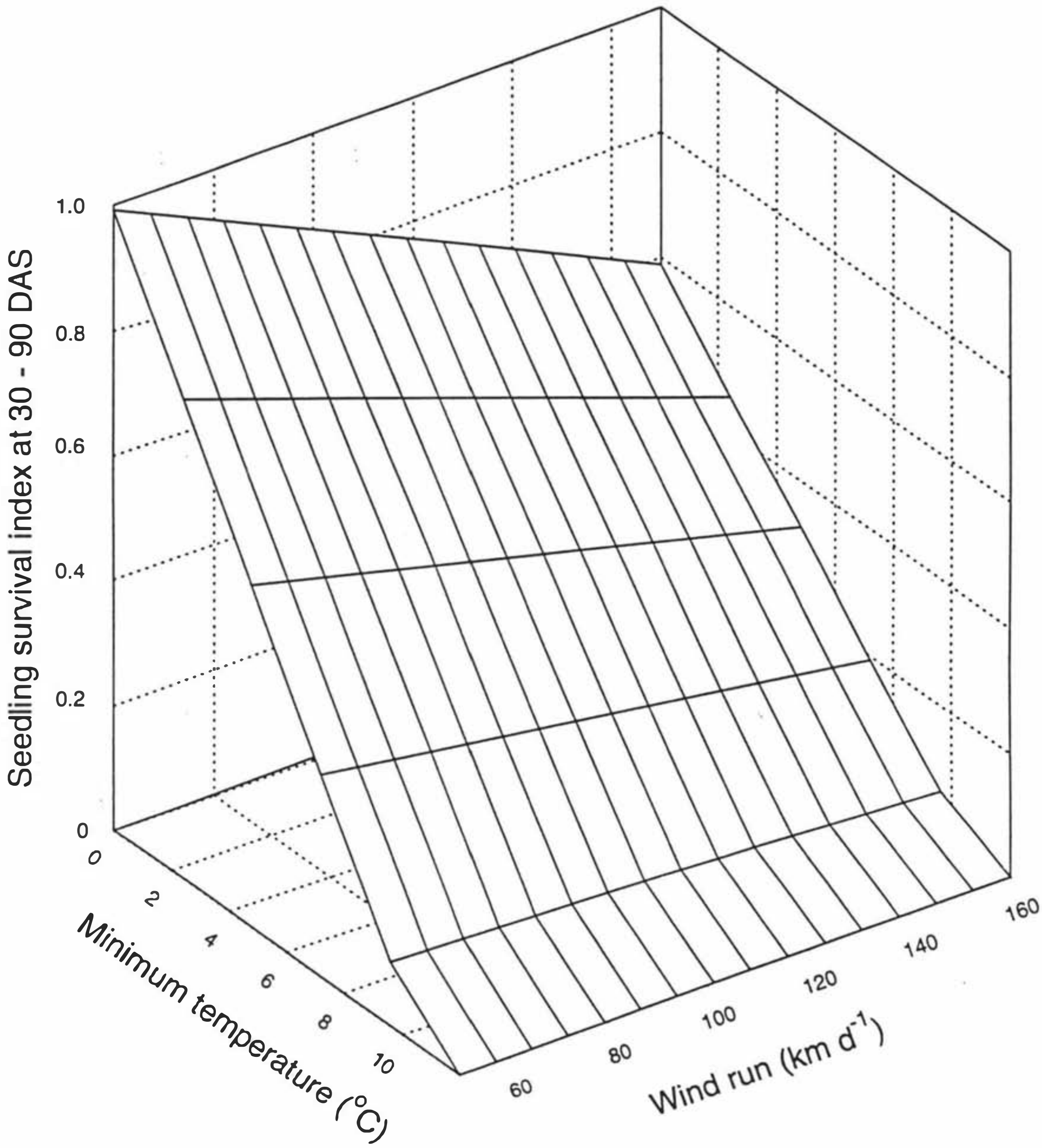


Figure 8.3 Interaction of minimum temperature and wind run on the seedling survival index.

Table 8.1 Comparison of actual and predicted values of seedling establishment (mean for 7 legume species) for different sowing conditions (seasons) during 1992 and 1993 at Poukawa.

	Seedling establishment index at 90DAS	
Sowing season (sowing date)	Actual from Chapter 3	Predicted for Poukawa 1992*
Autumn A 1992 (27 March, 1992)	0.10 (±0.004)	0.13
Autumn B 1992 (10 April, 1992)	0.06 (±0.004)	0.04
Winter A 1992 (12 June, 1992)	0.08 (±0.004)	0.06
Winter B 1992 (26 June, 1992)	0.04 (±0.004)	0.05
Mean	0.07	0.07
	Actual from Chapter 3	Predicted for Poukawa 1993*
Autumn 1993 (05 April, 1993)	0.09 (±0.004)	0.14
Winter 1993 (22 June, 1993)	0.11 (±0.004)	0.09
Mean	0.10	0.11
Spring 1993 (10 September, 1993)	0.06 (±0.004)	0.10

* No SEM (only data for one year)

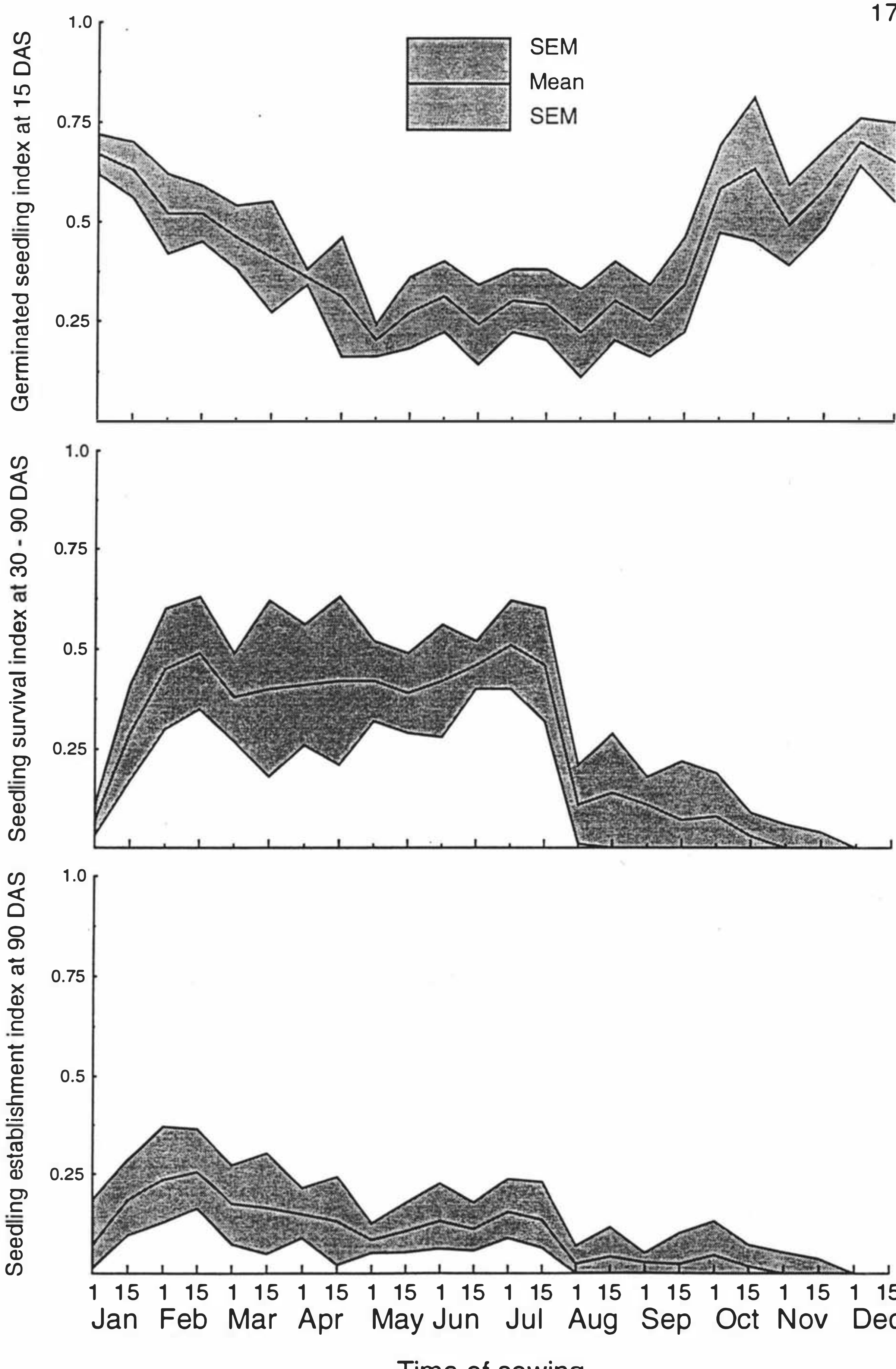


Figure 8.4 Model predictions for germinated seedling index, seedling survival index and seedling establishment index for the mean of seven legume species oversown at Poukawa (1990 - 1994).

and December.

8.3.2.2 Lawn road, Hastings

Figure 8.5 shows that the germinated seedling index was ≥0.35 between mid-November and mid-February. The seedling survival index was ≥0.4 between mid February and end of April and was 0 for November and December (Figure 8.5). The seedling establishment index was a maximum of 0.12 when seed was oversown in mid-February (Figure 8.5) and variability in the weather could result in establishment failure at almost any time of the year. The maximum and mean index values were 0.25 and 0.13, respectively, during mid February.

8.3.2.3 Individual species behaviour

The responses of birdsfoot trefoil (cold tolerant) and subterranean clover (cold intolerant) to oversowing were examined with the model using the meterological data from Lawn Road, Hastings. The germinated seedling index for subterranean clover was greater than 0.8 between mid-October and late February (Figure 8.6). There were no germinated seedlings from mid-May to mid-July. The seedling survival index was more than 0.5 from mid-February to mid-May (Figure 8.6). The seedling establishment index was more than 0.2 when seed was oversown from late February to early March (Figure 8.6). Variability was greater (CV 65%) at either end of this period, and the most reliable period for oversowing was late January to late February for subterranean clover.

The response of birdsfoot trefoil was quite different from that of subterranean clover. Its germinated seedling index and seedling survival index were more than 0.12 throughout the year. The maximum germinated seedling index was 0.30 from early January to early May (Figure 8.7). The seedling survival index of

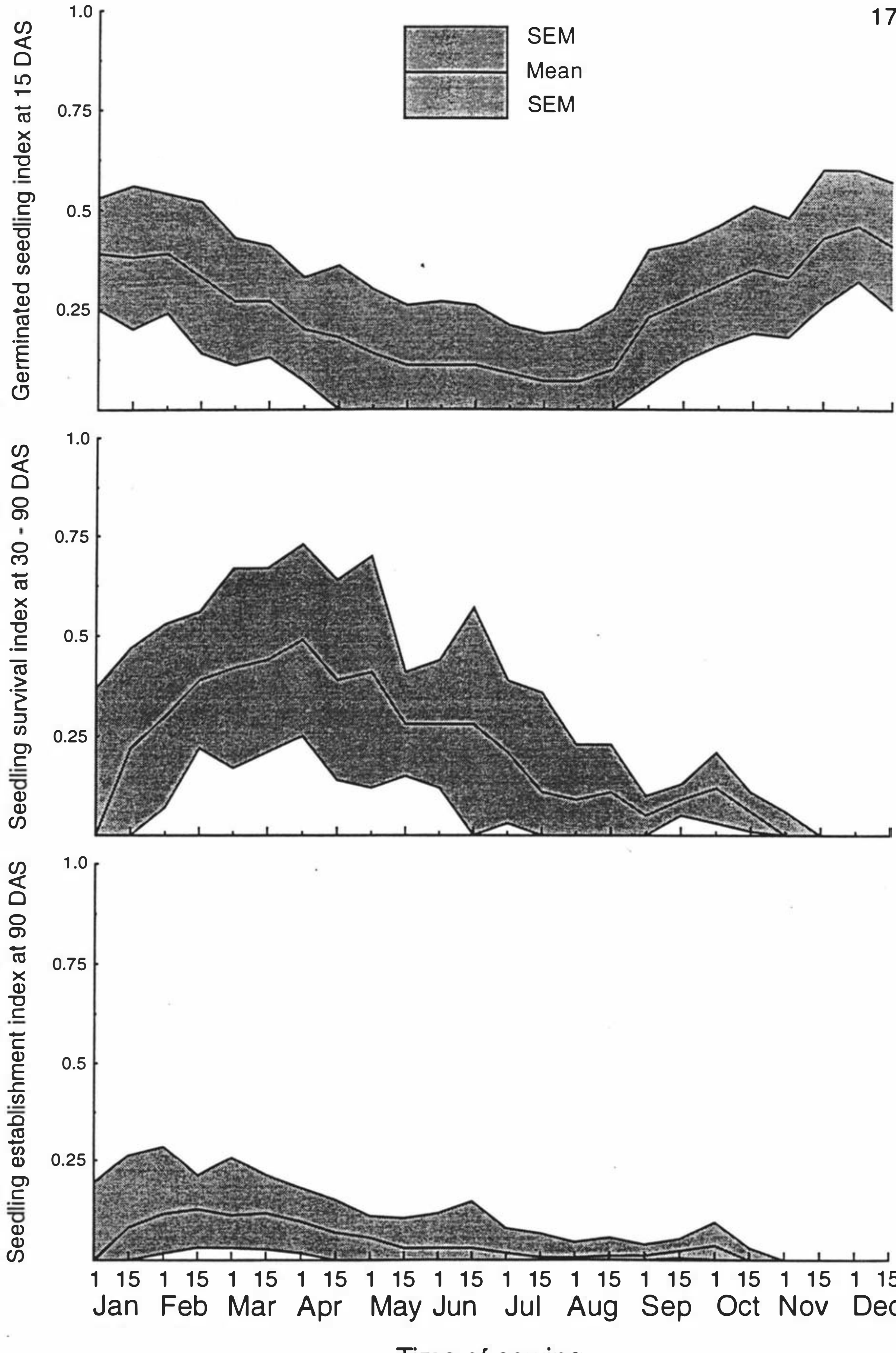


Figure 8.5 Model predictions for germinated seedling index, seedling survival index and seedling establishment index for the mean of seven legume species oversown at Lawn road, Hastings (1982 - 1991).

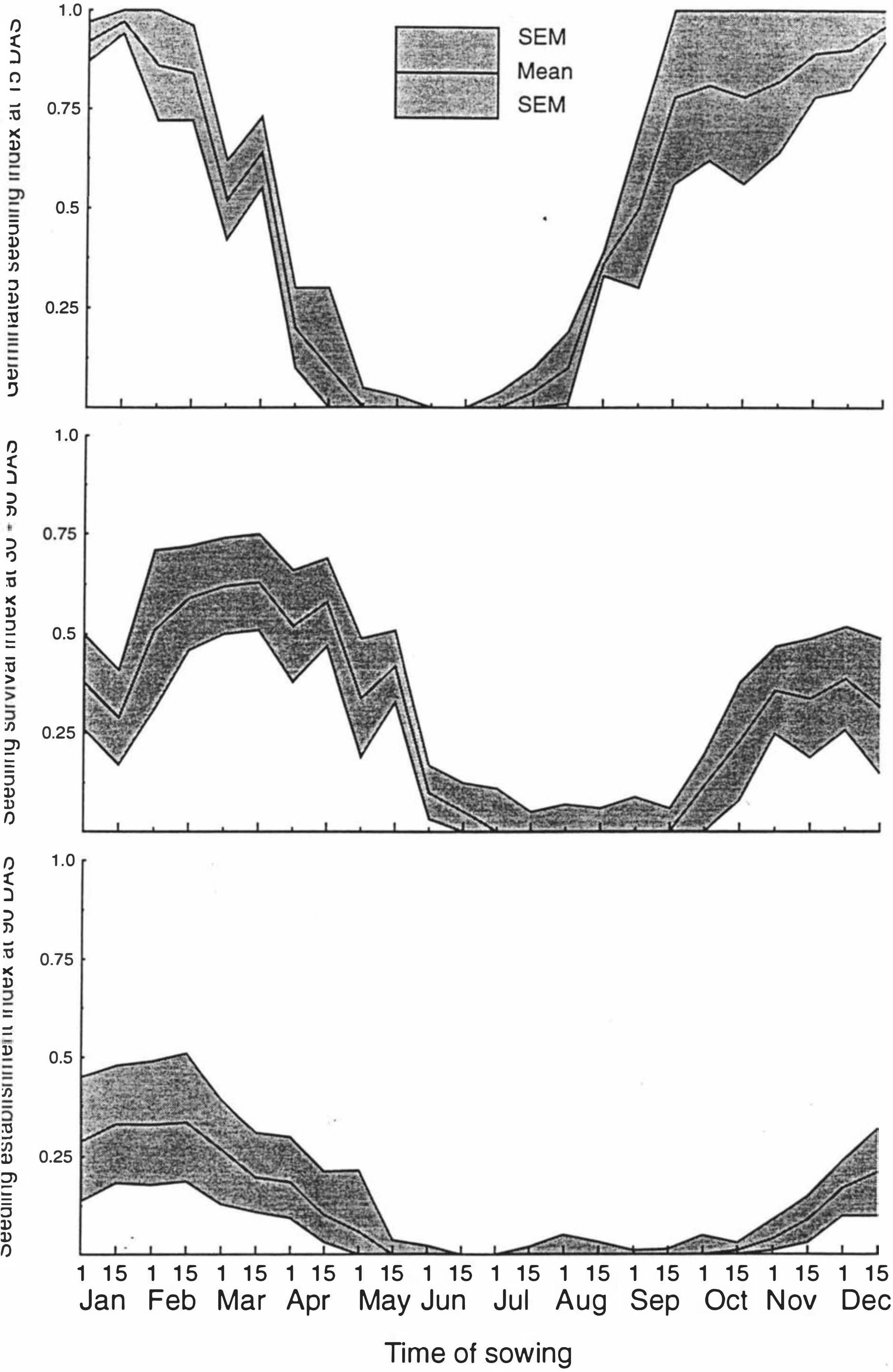


Figure 8.6 Model predictions for germinated seedling index, seedling survival index and seedling establishment index for subterranean clover oversown at Lawn road, Hastings (1982 - 1991).

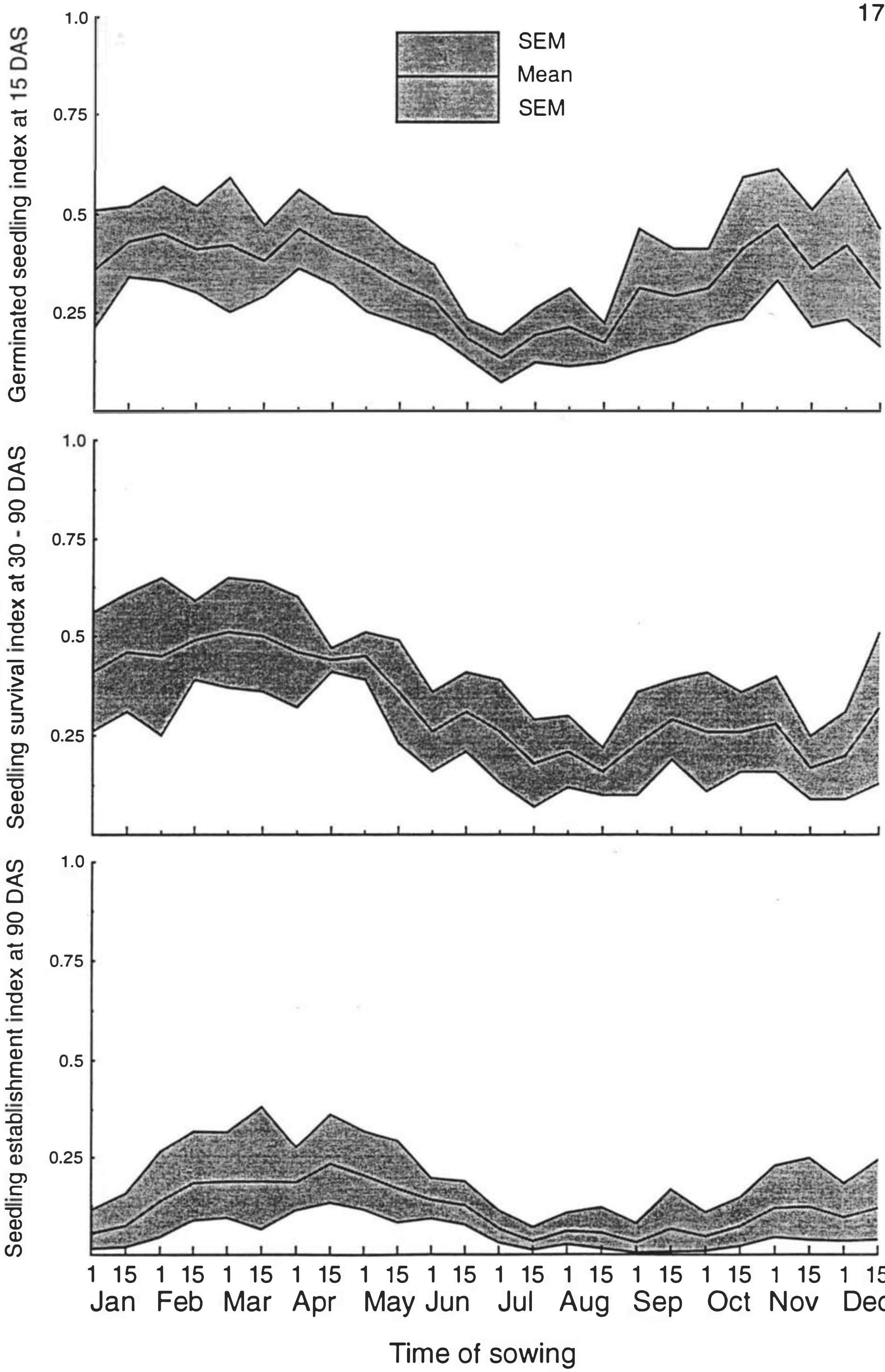


Figure 8.7 Model predictions for germinated seedling index, seedling survival index and seedling establishment index for birdsfoot trefoil oversown at Lawn road, Hastings (1982 - 1991).

birdsfoot trefoil was 0.4 during January to mid-May (Figure 8.7). Overall, the seedling establishment index was greater than 0.1 from February to June if seed oversown during this period. The CV was 69% for this period.

8.4 Discussion

The model developed in this chapter was a complex interaction of four environmental factors (GSWC, soil temperature, minimum air temperature and wind run) and contrasted with single environmental factor models (Dowling and Smith, 1976; Comish, 1985; Barker *et al.*, 1988). Most of the predicted values for seedling establishment from the model were similar to the actual values for different sowing seasons. Bellotti and Moore (1993) concluded that most of the establishment models developed had a greater focus on the pattern of establishment model rather than the values for germination and establishment probabilities. Under both environmental conditions (Poukawa and Lawn Road) the overall seedling establishment was predicted to be less than 25% which is similar to legume establishment from oversowing in field trials (Chapter 3; Suckling, 1976; Charlton and Brock, 1980; Campbell, 1992).

The model showed that the best oversowing time at Poukawa for the germinated seedling index (>0.5) was during summer (January, February) and mid spring (October) and for the seedling survival index was late autumn and winter (mid March to July) (Figure 8.4). The seedling establishment index was best for seed oversown in late February. Although summer and spring oversowing of legume seed was predicted to produce high seedling germination, there was less likelihood of seedling survival because the best time for high seedling survival started during late autumn (from mid March). This suggested that to get high numbers of established seedlings, mid February to early March was the only suitable time for oversowing under Poukawa conditions for most of the legume species. The same trend was observed for Lawn Road. The only difference was that the Poukawa data resulted in a higher (>0.5) germinated seedling index compared to the Lawn Road data (< 0.4; Figure 8.5). The maximum seedling establishment index was 0.25 (mid February) at Poukawa and 0.12 at Lawn Road. The best time to get high seedling establishment was February under the Lawn Road conditions. Late February and early March (autumn) sowing in North

Island hill country gave better establishment than in spring (Madden *et al.*, 1949; Suckling, 1976; Charlton, 1977; Chapman and Fletcher, 1985 and Lambert *et al.*, 1985). The Ballantrae site was moist hill country, and although the model was developed to dry hill country would require validation for different suites of climatic data.

The model predicted the behaviour of cold intolerant and tolerant legume species well. The model predicted high subterranean clover establishment in early autumn but none in winter, similar to results observed by Campbell (1963); Watkin and Vickery (1965) and Chapman and Fletcher (1985). Birdsfoot trefoil can be established from oversowing from February to June, as predicted by model. Lowther (1980) also observed the superior establishment of birdsfoot trefoil over subterranean clover in the cold climate of South Island hill country. A consideration not covered by the model is the need for annual species to set seed in their first year to ensure their future survival. Although the germinated seedling index was high for subterranean clover when oversown in late spring and summer there would not be an adequate time after these sowing times for good seed production (Smetham, 1977).

There are factors such as seed-soil contact (Campbell , 1992), seedling vigour (Hampton and Hill, 1990), radicle life time in air (Campbell and Swain, 1973a), radicle penetration into the soil surface (Campbell, 1968), insect and slug predation (Charlton, 1978) and rhizobia infection (Lowther, 1977) which were not included in this model. However, in the field trials treatments such as herbicide application, post-sowing treading, and use of anti-slug pellets were used to ensure successful oversowing and are discussed in Chapter 3. These factors also influence the degree of seed germination and seedling survival.

The average seedling survivorship pattern in Figure 3.12 showed that establishment beyond 90 days was stable. Once the seedling has established, other factors such as management (Lambert *et al.*, 1985), competition (Bellotti and Blair, 1989), self thinning, and fertility (During, 1984) affect subsequent performance.

Factors like sowing rate (Chapter 4) and seed coating (Chapter 7) have been shown to affect the establishment index. For example high sowing rates resulted in a greater number of seedlings at 90 DAS but the proportion of seeds that produced a seedling was lower, and there was no evidence that this effect was dependent on time of sowing nor that the pattern of seedling survival differed from that determined by the environmental conditions.

The model allowed extension of the Poukawa field trials. The best time for oversowing was easily predicted under Poukawa conditions whereas the field data cannot give an accurate sowing time. The model also provided an understanding of how environmental conditions affect establishment from oversowing, emphasising that not just conditions during the first 15 DAS, but conditions over the subsequent 3 months need to be considered. The coefficient of variation was high (>60%) demonstrating that even ideal conditions at sowing could still result in establishment disorder.

8.5 Limitations of the model

One limitation of the model was that the environmental conditions under which field data were collected were not as extreme as can occur. The range for the factors was limited to the Poukawa field data and was 27 to 50% for GSWC, 0 to 20°C for soil temperature, 0 to 6°C for minimum air temperature and 30 to 165 km per day for wind run. The average maximum wind run at Poukawa was 192 km per day yet at Lawn Road it was 412 km per day which affected the seedling survival index. As the model used linear equations, extrapolation may lead to a rapid, and unlimited, increase or decrease in the predicted seedling survival and establishment if the range for an environmental factor at a site exceeds that at Poukawa. Therefore, use of the model other than at Poukawa (dry hill country) is limited until the range for GSWC, soil temperature, minimum air temperature and wind run is extended.

8.6 Conclusion

The model was simple but practicable and could easily predict establishment index values for any time of the year. The predicted values matched field data.

At Poukawa, the autumn and early winter, and at Lawn Road early autumn were the best time to oversow legumes to get high seedling survival. Due to the higher wind run the establishment success will probably be lower at Lawn Road.

The model suggested that subterranean clover should not be sown in winter, whereas birdsfoot trefoil can establish if sown in winter.

The best environmental conditions for seed germination were 25 to 37% for GSWC, 13 to 20°C for soil temperature and for seedling survival were 0 - 2°C for minimum air temperature and 50 - 90 km per day wind run.

This was a preliminary attempt to develop this model. The approach appeared to be an effective way of developing an empirical model for predicting the level of establishment from oversowing legumes at different times of the year.

Chapter 9

Chapter 9

GENERAL DISCUSSION AND CONCLUSION

9.1 Introduction

The underlying aim of this study was to determine the environmental, seed and plant factors that affect the successful establishment of legume species oversown into drought-prone hill country swards. Despite previous research in this area there was still a need to understand reasons for the high failure of legume establishment following oversowing. The environmental, seed and plant factors responsible for low legume seedling establishment are summarised in Figure 9.1. The environmental factors (left side, Figure 9.1) have a relatively greater influence on legume establishment than seed and plant factors (right side, Figure 9.1).

The effects of some environment, seed and plant factors on twelve legume species were examined in the field (Chapters 3, 4 and 7) and in the glasshouse (Chapters 5 and 6). On the basis of the strong relationship between four key environmental factors and seed germination and seedling survival, a simple model for predicting the best conditions for oversowing was developed (Chapter 8). In this chapter the results are considered in the form of an integrated discussion. More detailed discussion occurs within each chapters.

9. 1. 1 Oversown seed

In the field a large proportion of seed (about 80%) failed to germinate and appear as seedlings following oversowing, despite attempting to provide good seed-soil contact through herbicide defoliation and sheep treading after oversowing. This loss was not related to a phytotoxic effect of glyphosate residue or dead plant material since no major effect on seed germination and seedling survival was found (Chapter 6). The average percentage of germinated seed from oversowing

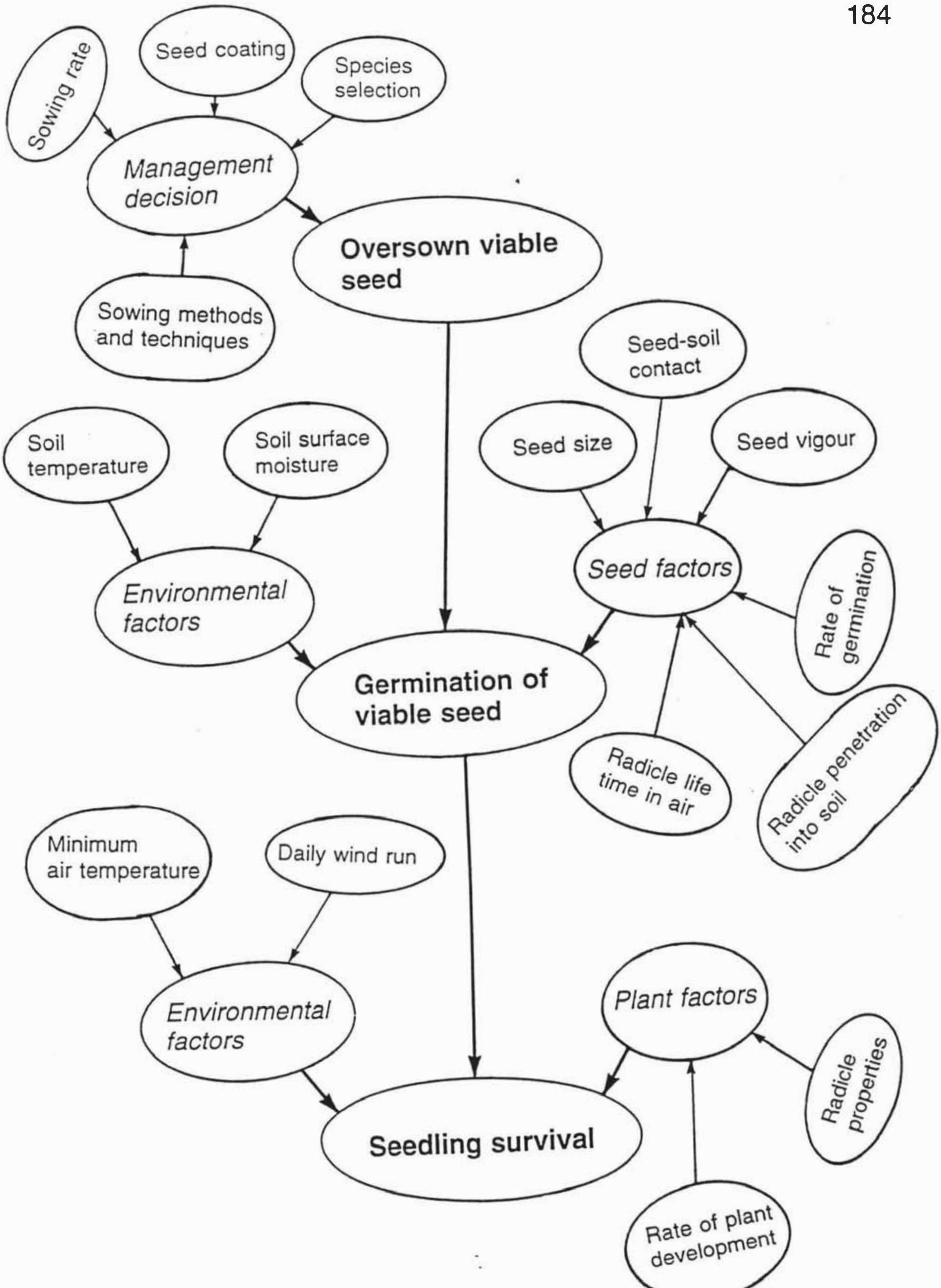


Figure 9.1 Some environment, seed and plant factors contributing for oversown legume seed germination and seedling survival.

was approximately half of the viable seed shown by laboratory germination tests (Section 5.3.5; Table 5.7). The number of viable seed from germination tests does not equate to the field appearance of oversown legume seed. Chapman *et al.* (1985) and Barker and Zhang (1988) found the greater proportion of G. Huia white clover seed failed to appear when oversown into hill country, but the cause of this was unknown.

With simulated treading, only 51% of oversown seed of three legume species was buried by soil (Section 5.3.4). The remaining (49%) seed remained on the surface, facing a relatively harsh environment. The simulated treading suggested that seed burial was also poor in the field. Seed on the soil surface may or may not produce a radicle but even when it does, the radicle must penetrate the soil before it is desiccated (Section 5.4.3; McWilliam *et al.*, 1970; Campbell and Swain, 1973a). Germination of legume seed following oversowing in drought-prone hill country is clearly a critical phase in the successful establishment of seedlings.

9.1.2 Germination of viable seed

The environment and seed factors influencing the germination of oversown seed are given in Figure 9.1.

Soil surface moisture and soil temperature were the most important environmental factors soon after oversowing of seed (Sections 3.3.8; 3.4.3). Soil moisture to 100 mm during the early stages of seed imbibition and germination had a negative influence on the percentage of visible seedlings (Figure 3.11). The availability of soil water in the micro-environment of the seed on the soil surface was the dominant factor that controls germination (Harper and Benton, 1966; McWilliam and Dowling, 1971; Dowling and Robinson, 1976). Adequate surface soil moisture with a suitable soil temperature was essential for germination of viable seed on average for all legume species. Soil temperature showed a positive influence on seed germination soon after oversowing (Figure 3.11) in agreement with Lambert (1977); Radcliffe and Lefever (1981); Charlton *et al.* (1986); Hampton *et al.* (1987). This was not the case for specific species, however, as birdsfoot trefoil seed germinated well under cold temperature (Table 3.6), and caucasian clover had poor germination due to low relative growth rate but not soil temperature (Table 3.8; Section 3.3.4.2).

The GSWC range under field conditions was 27 to 50% (Section 3.4.2.4) and the field capacity of this silt loam soil was 35%. Figure 3.11 showed that high soil moisture conditions actually reduced germination success, a previously unreported result for oversowing. Soil moisture at 30 mm depth was a poor indicator of seed germination compared with the surface soil moisture (Table 5.1 Section 5.3.2). A glasshouse trial showed that 45% GSWC was the optimum for the germination of slow germinating species (Figure 5.4). A $CoCl_2$ saturated paper strip technique was developed to measure the soil surface moisture. This technique was an easy method to measure the soil surface moisture and it, or a refinement of it, will be useful in future studies on the environmental conditions affecting oversown seed.

Seed coating improved the early germination rate (by 30%) but not final seedling density for subterranean and white clovers (Chapter 7). Seed coating may (Scott, 1989) or may not (Dowling, 1978) increase seedling numbers under certain conditions. Some seed coats increase the uptake of moisture compared to bare seed (Dowling *et al.*, 1971). The main benefit of seed coating appeared to be an increased rate of germination which was vital since the window for germination is narrow, but seedling survival was still be dependent on subsequent conditions.

Seed vigour of oversown legume species was not a useful predictor for seedling establishment (Section 3.4.2.2). Seed germination in the field was also found by Perry (1977) to be poorly related to seed vigour due to the influence of environmental conditions. Seed vigour did not appear to be a critical or limiting factor in establishment of legumes by oversowing but if there is choice preference should be given to higher seed vigour.

Heavy seed was useful when oversowing legume species as it improved the seed to soil contact and usually resulted in more vigorous seedlings (Chapter 4; Kneebone and Cremer, 1955; Black, 1957). Seed size within legume species affected seedling density during the early stages of establishment (Sections 4.3.2.2 and 4.4.2; Asher and Ozanne, 1966; McWilliam *et al.*, 1970; Evans, 1973). Although there was a general relationship between seed size and seedling growth rate, some species like caucasian clover do not exhibit this relationship. Caucasian clover had a relatively large seed size but an extremely slow rate of germination and seedling growth. Such a species was not suitable for oversowing under poor environmental conditions or where competition from existing vegetation is high but has been successful in some environments; for example relatively open tussock regions of the South Island high country (Patrick and Lowther, 1994).

Legume species varied in both their maximum number of germinated seedlings, and in the time taken for half of the seed to germinate (Figure 5.6; Table 5.4). The slow germinating species were more sensitive to soil surface moisture (Figures 5.2 and 5.3; Dowling *et al.*, 1971). The faster germinating species (subterranean clover) had superior seedling survival compared to slow germinating species.

Radicle death probably occurred due to insufficient soil moisture, and although radicle elongation occurred, entry into the relatively harder soil surface was prevented (Section 5.4.3; Campbell *et al.*, 1987). Tadmor and Cohen (1968) found that the ability of the radicle to enter the soil and rate of its extension during early seedling development was important under conditions where the soil surface moisture dried rapidly. Seeds that had the radicle die would have contributed to the high proportion of seed that failed to produce a visible seedling.

The radicle length for all the legume species was greatest on the soil surface at the low moisture level in the glasshouse trial (Section 5.3.6; Figure 5.7). Campbell *et al.* (1987) noted legume radicles tended to grow along the soil surface until they encounter a suitable entry site. The radicle tips of the slower germinating species were observed to desiccate and if the tip of the radicle was killed, the remainder of the radicle though healthy had no chance of entry into the soil (Sections 5.3.6; Campbell and Swain, 1973b).

The epigeal germination of pasture legume species tend to handicap their chances of higher seedling survival from oversowing. The combined forces of radicle and hypocotyl extension push the germinating seed away from the soil surface from the point of radicle penetration (McWilliam and Dowling, 1970). The radicle tip of the slower germinating species desiccated in low soil surface moisture conditions (Dowling *et al.*, 1971).

A sowing rate greater than that usually recommended increased the legume contribution to the total herbage mass through an increased plant density (Sections 4.3.2.1 and 4.4.1; Chapman *et al.*, 1993). The increased plant density enabled the sown legume species to be more competitive against regrowth from the existing sward. Although proportionally fewer seeds produced a seedling at high sowing rates, the total number of established seedlings increased progressively and these seedlings improved the competitiveness of the sown legume species.

9.1.3 Seedling survival

Good germination does not mean good seedling survival. The factors affecting the survival of oversown legume seedlings are given in Figure 9.1.

High minimum air temperature and daily wind run were the major climatic factors which caused low seedling survival. The response of seedling survival to minimum air temperature and daily wind run was not found to have been reported previously. Although a low minimum air temperature slowed seedling growth, this did not result in seedling death, and was preferable to severe competition from recovering resident vegetation that might occur at a higher minimum air temperature. Too low an air temperature (< -0.1°C) might, however, be harmful for seedling survival. Competition from re-establishing resident pasture is a major cause of seedling mortality (Dowling *et al.*, 1971). Campbell *et al.* (1987) found that one of the major problems in the surface establishment of pasture was poor control of competition from the existing pasture during the first two years.

High wind run was a major cause of low soil surface moisture (Chapter 5), resulting from both, evaporation from the soil and transpiration from the young seedlings. In these trials, high wind run decreased seedling survival (Figure 3.11) in agreement with Charlton and Brock (1980) who observed that low rainfall and high wind run caused high seedling mortality of white clover. A germinating legume is extremely vulnerable to desiccating wind, either directly, through reduced imbibition due to dehydration of the soil surface (McWilliam and Dowling, 1970), or indirectly, through the exposed radicle drying and by increasing soil impedance to radicle penetration (Campbell and Swain, 1973a).

Seedling survival was better for species with a fast rate of development (Figure 3.8; McKell, 1972; Grime and Hunt, 1975). Subterranean clover development rate was highest following autumn sowing whereas persian clover development rate was highest following winter sowing (Figures 3.9 and 3.10). Plant development rate for caucasian clover was low for winter and autumn sowings, as was also observed in South Island by Paljor (1973).

9.2 Seedling establishment

Figure 3.12 showed that establishment of legume species 90 days after sowing was stable. After the establishment of seedlings other factors like management, competition, self thinning, fertility and plant characteristics are important.

The contribution of sown legumes to herbage mass was dependent on environmental factors and plant characteristics (Section 3.4.3). Winter sowing gave higher herbage mass than the autumn sowing probably due to less competition from regrowth of the existing vegetation. Overall, herbage mass contribution by oversown legume species was less than 15%. The low contribution of legume species to total herbage mass from many of the sowings emphasised the importance of establishment. Nevertheless, herbage mass of introduced legumes can increase with time under effective management (Chapter 4; Lambert *et al.*, 1985). Moderate to high grazing pressure and soil fertility status will favour persistence of introduced cultivars once satisfactory establishment (after at least one year) has been achieved (Lambert *et al.*, 1985). Annual legume species like subterranean clover which must reseed for continued survival, may disappear if rotationally grazed at frequent intervals during flowering in spring (Suckling, 1954).

9.3 Seedling survival and establishment model

The seedling survival and establishment model proposed in Chapter 8 was a simple but practical model that included the complex interaction of GSWC, soil temperature, minimum air temperature and daily wind run. The model could predict the best time for oversowing at Poukawa (or similar climates) and provided an understanding of how environmental conditions affected establishment from oversowing. The emphasis was on seedling establishment over 90 days rather than just the germination phase. The model used GSWC and soil temperature for seed germination, and for seedling survival, minimum air temperature and daily wind run. No research has previously been reported on the relationship of minimum air temperature and daily wind run to seedling survival.

The best conditions for seed germination were 25 to 37% for GSWC, 13 to 29°C for soil temperature, and for seedling survival were 0 to 2°C of minimum air temperature and not more than 90 km daily wind run. However, due to the vagaries of weather these never coincided to give optimum (> 80%) establishment success. Although the data in Chapter 3 and the model suggested the best time for oversowing at Poukawa, there was still year to year variation in the optimum time. Furthermore some legume species varied from the average response and further development and validation of the model for specific species is warranted. Nevertheless this was the most comprehensive attempt to model the establishment of pasture legume species by oversowing in New Zealand.

9.4 Conclusion

The results indicated the importance of four environmental factors, GSWC and soil temperature (for seed germination) and minimum air temperature and daily wind run (for seedling survival). The experiments in the glasshouse also confirmed the importance of low surface soil moisture and wind run. A cobalt chloride ($CoCl_2$) saturated paper strip technique was developed as a cheap indicator for surface soil moisture. High soil moisture at depth was a poor indicator of seed germination and seedling survival, compared with the surface moisture. Three different legume species showed a dependence on soil surface moisture for germination and early seedling growth in a glasshouse trial, where the causes of low soil surface moisture were wind run and withholding water.

The greatest loss of potential plants after field oversowing was non-appearance of visible seedlings for up to approximately 80% of viable seed sown however this could be reduced to 40% under ideal conditions in the glasshouse. The seed germination tests in the laboratory did not adequately predict the proportion of viable seed in the seed oversown. The percentage of ungerminated seed was higher under oversown conditions compared to the standard seed germination test. The glyphosate sprayed 20 days before oversowing each trial did not have any major phytotoxic effect. Dead material of the resident pasture killed by herbicide did not show any residual toxicity. Use of fungicide, insecticide and slug bait had some benefits on seedling appearance, but not in the same scale as failure for seedlings to appear due to environmental conditions.

A higher seed rate (but not seed size) was an effective means of improving both overall establishment, and the legume contribution to total herbage mass. Although large seed size within the same species increased initial seedling survival there was no effect on seedling establishment. However, some species may not be suitable for oversowing regardless of their seed size. A series of trials found that not all species were suitable for oversowing in North Island dry hill country, and a list of suitable legume species under different sowing conditions is given in Table 3.19.

Seed coating increased seed germination rate but not the final seedling density compared with bare seed. There was a 30% increase in visible seed germination due to coating soon after sowing. In some environmental conditions seed coating might give an advantage to seedling establishment but its cost needs to be compared with an increased sowing rate.

A model developed for Poukawa predicted the best time for oversowing to get high seedling establishment was late February to early March, and for Lawn Road, Hasting was early February. The model showed that subterranean clover should not be sown in winter whereas birdsfoot trefoil can establish successfully if oversown in winter. The model was an effective means of integrating the effect of environmental conditions on the establishment from oversowing of a range of pasture legume species.

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Appendices

Appendix 3.1 The standardized coefficients of slopes for sowing conditions over seven legume species from 0 - 90 DAS.

Autumn A 92

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	-1.0066	-0.1714	0.1307	-0.0246	81	***
Arrow leaf clover	-0.7667	-1.2185	0.2176	0.3653	74	***
Persian clover	-0.1842	0.4983	0.3651	-0.1758	63	***
Alsike clover	-0.7651	-0.9234	0.1253	-0.0412	48	***
Birdsfoot trefoil	-1.0733	-1.0086	0.3440	-0.0175	72	***
Caucasian clover	-0.1838	-0.8465	0.0461	0.1278	50	***
Strawberry clover	-1.2025	-1.0907	-0.0007	-0.0282	67	***

*:P<0.05; **:P<0.01; ***:P<0.001; ns: No significant difference

Appendix 3.1 cont'd

Autumn B 92

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	-0.8289	-0.1891	0.0299	0.0521	44	ns
Arrow leaf clover	-	-	-	-	-	-
Persian clover	1.5460	1.4904	-1.0344	0.2352	66	***
Alsike clover	-1.3189	-1.6132	0.7109	-0.0482	32	ns
Birdsfoot trefoil	-0.8786	-0.4267	0.3349	-0.1048	33	ns
Caucasian clover	-1.0130	-1.9714	0.6264	0.5595	74	***
Strawberry clover	-1.3910	-1.6285	0.4451	-0.3644	23	ns

Appendix 3.1 cont'd

Winter A 92

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	0.5262	0.8817	-0.4083	0.2931	75	***
Arrow leaf clover	0.7312	0.6584	-0.3742	0.7781	87	***
Persian clover	0.6884	0.7662	-0.5519	0.8366	92	***
Alsike clover	0.6592	0.7416	-0.4396	0.6899	82	***
Birdsfoot trefoil	0.7637	0.7222	-0.3378	0.6670	92	***
Caucasian clover	0.6075	0.7222	-0.3378	0.6670	92	***
Strawberry clover	0.6497	0.7066	-0.3418	0.5732	76	***

Appendix 3.1 cont'd

Winter B 92

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	0.1531	0.3561	-0.2125	0.0671	14	ns
Arrow leaf clover	0.0503	0.5856	-0.2131	0.3003	40	*
Persian clover	0.0411	0.6519	-0.2484	0.2832	48	**
Alsike clover	0.1202	0.7322	-0.3430	0.3600	64	***
Birdsfoot trefoil	0.1115	0.7409	-0.3187	0.3415	63	***
Caucasian clover	0.0018	0.7426	-0.2725	0.3901	61	***
Strawberry clover	0.4281	0.6529	-0.2617	0.3105	45	***

Appendix 3.1 cont'd

Autumn 93

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	1.5053	2.3196	-0.4674	0.3541	80	***
Arrow leaf clover	2.7303	3.2679	-0.9639	0.3958	57	***
Persian clover	0.7641	0.3810	-0.2939	0.4660	44	*
Alsike clover	5.0939	7.1841	-1.8798	0.3754	80	***
Birdsfoot trefoil	-	-	-	-	-	-
Caucasian clover	4.0724	5.5021	-1.5094	0.1162	78	***
Strawberry clover	3.4607	4.5140	-1.0924	0.0999	65	***

Appendix 3.1 cont'd

Winter 93

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	-0.0289	-0.0464	-0.0312	-0.6153	40	*
Arrow leaf clover	0.1115	-0.3550	-0.2138	-0.8306	76	***
Persian clover	-0.1799	0.1888	-0.0874	-0.8799	76	***
Alsike clover	-0.2269	-0.0297	-0.2238	-0.6965	84	***
Birdsfoot trefoil	-0.3076	0.2299	-0.1658	-0.6792	86	***
Caucasian clover	-0.3155	-0.0911	-0.2700	-0.5122	74	***
Strawberry clover	-0.2737	0.2218	-0.2068	-0.7045	88	***

Appendix 3.1 cont'd

Spring 93

Legume species	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
Sub-clover	0.1181	0.9777	-0.5148	-0.5905	22	ns
Arrow leaf clover	0.4531	1.7155	-1.0480	-0.9481	38	*
Persian clover	0.7214	1.4894	-0.7952	-0.0816	32	ns
Alsike clover	0.1746	1.1134	-0.6832	-0.6638	23	ns
Birdsfoot trefoil	0.2873	1.9732	-1.0695	-1.2153	81	***
Caucasian clover	0.7491	1.8382	-1.1974	-0.8915	33	ns
Strawberry clover	0.4502	1.6158	-1.0126	-0.8670	32	ns

Appendix 3.2 The standardized coefficients of slopes for different legume species over seven sowing conditions from 0 - 90 DAS.

Subterranean clover

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	-0.4248	0.4637	0.2236	0.0412	86	***
30	-0.4367	0.9552	-0.7058	1.0611	43	***
45	-0.6776	-0.3371	-0.0469	0.2602	17	***
60	1.9149	0.7689	-0.0198	-1.5152	64	***
75	-0.0845	-1.1183	0.7495	-0.7725	68	***
90	-1.1997	-1.7383	0.4154	-0.0120	71	***

*:P<0.05; **:P<0.01; ***:P,0.001; ns: No significant difference

Appendix 3.2 cont'd

Arrow leaf clover

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	0.7516	1.3208	-0.0746	-0.2011	72	***
30	2.1706	3.7846	-2.9881	1.1072	88	***
45	0.3441	0.2638	0.0518	-1.0632	61	***
60	1.9192	-0.3465	0.6809	-2.8412	90	***
75	0.8989	0.6278	-0.5744	-1.1195	81	***
90	0.2067	1.6301	-1.8366	0.2994	70	***

Appendix 3.2 cont'd

Persian clover

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	-0.4001	0.3538	0.5870	-0.1715	77	***
30	0.4200	1.7016	-1.5402	0.9114	25	ns
45	0.1415	-0.3583	0.1331	-0.5967	17	ns
60	0.7352	-0.1493	-0.3607	-0.8845	68	***
75	1.4691	0.5166	-0.4829	-1.3361	81	***
90	0.5609	1.0753	-1.4343	0.0803	70	***

Appendix 3.2 cont'd

Alsike clover

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	0.0142	0.5712	0.5540	-0.0406	87	***
30	0.5119	0.8957	-0.8789	-0.0959	26	***
45	-0.1555	-0.7672	-0.0856	-0.9378	74	***
60	-0.6114	-0.9801	-0.0692	-0.3232	80	***
75	-0.1334	0.2084	-0.8288	-0.0618	60	***
90	0.7372	1.1242	-0.8115	-0.0351	20	***

Appendix 3.2 cont'd

Birdsfoot trefoil

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	-1.5844	-0.8574	-0.3837	0.3502	69	***
30	-0.6696	-1.7315	1.8689	-1.9319	79	***
45	-0.7523	-0.4393	-0.3490	-0.3741	81	***
60	0.2999	-0.3952	-0.0111	-0.9931	59	**
75	2.2762	1.7461	-0.7728	-1.3873	69	***
90	0.3933	1.5985	-1.2864	0.4905	29	ns

Appendix 3.2 cont'd

Caucasian clover

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	0.4752	1.0375	0.2189	0.0765	81	***
30	0.5247	1.5662	-1.2409	0.8872	18	ns
45	-0.1017	-0.5918	-0.1710	-0.5659	42	**
60	0.5470	-0.5877	-0.0259	-1.0752	78	***
75	-0.4419	-0.8383	-0.2994	0.1578	55	***
90	-0.5229	-1.5769	0.6353	-0.1029	57	***

Appendix 3.2 cont'd

Strawberry clover

Days after sowing	GSWC	Soil temperature	Minimum temperature	Wind run	R^2	Significance
15	-0.1536	0.6787	0.1884	-0.0457	80	***
30	0.0495	1.0479	-1.0107	0.3295	39	**
45	-0.5017	-0.9579	0.0248	-0.8478	67	***
60	-0.0921	-0.8975	0.0773	-0.8780	90	***
75	0.3991	0.2530	-0.6783	-0.5120	57	***
90	0.2178	0.8772	-1.0830	0.3165	21	ns

Appendix 4.1 Response of seedling density (number m $^{-2}$) over time for different seed sizes and legume species.

		Days after sowing				
Species	Seed size	15	30	60	90	120
		Seedling density (number m $^{-2}$)				
Subterranean clover cv. Karridale	Large	202	176	153	143	115
	Small	134	133	130	126	98
Strawberry clover cv. G. Onward	Large	53	115	96	86	46
	Small	47	90	76	70	24
Crimson clover	Large	150	152	119	105	67
	Small	103	112	102	96	55
SEM		13.4	10.1	10.2	11.0	9.8
Significance		ns	ns	ns	ns	ns

Appendix 7.1 Responses of seedling density (plant m^{-2}) over time under different treatments.

	Days after sowing			
Overall species	15	30	45	60
Subterranean clover	21	172	266	255
White clover	11	81	162	157
SEM	3.1	6.2	7.9	7.5
Significance	***	***	***	***
Overall seed treatments				
Nutriprill normal	38	188	284	278
Nutriprill plus	26	162	254	247
Insecticide	4	145	210	194
Bare seed	10	73	229	224
Fungicide	1	62	93	88
SEM	3.9	9.7	12.4	11.9
Significance	***	***	***	***
Bare seed only				
Subterranean clover	15	98	299	293
White clover	5	48	159	155
SEM	4.7	13.7	17.6	16.8
Significance	**	**	***	***