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**Aspects of Biology of the
Weed of Arable Crops broom corn millet
(*Panicum miliaceum* L.)**

A thesis submitted in partial fulfilment
of the requirements for the degree of
Masters of Science
in Biological Sciences

by

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Abstract

Grass weeds such as *Panicum miliaceum* L. (broom corn millet) are a persistent problem for agriculture, causing significant crop losses. A weedy biotype of broom corn millet is already a troublesome weed on North American arable farms, and has recently emerged as a threat to New Zealand corn and maize crops. This thesis describes aspects of the biology of broom corn millet under New Zealand conditions. Experiments were designed to understand under what conditions broom corn millet is mostly likely to affect crop growth.

Experiments were conducted in a glass house and a laboratory to observe the effect of temperature on germination and early growth of broom corn millet. The pot-based glasshouse experiment compared germination and growth between a range of controlled substrate temperatures. The response of broom corn millet to temperature was typical of that expected for a C₄ plant. At 10°C seeds germinated later and in lower proportions compared to 15°C, 20°C and 25°C. Growth and above ground dry biomass accumulation also increased with increasing substrate temperature, with the highest dry biomass accumulated at 25°C, primarily because of increases in germination rate. In a laboratory experiment conducted at temperatures ranging from 5°C and 34°C, temperatures $\geq 20^\circ\text{C}$ were more favourable for germination of broom corn millet seeds. The optimum temperatures for germination were 27°C to 34°C. The threshold germination temperature for the seed lot used was 7.4°C.

Broom corn millet seeds were tested for their ability to germinate and emerge from a range of planting depths when planted in pots containing 16 soil types from around New Zealand. Seedlings emerged from 120 mm depth in all soil types. In six soil types seeds of broom corn millet were able to emerge from the greatest depth tested of 170 mm, very deep compared to most herbaceous weeds. In general, seedling emergence reduced with increasing depth, whereas suicidal germination increased. Step-wise binomial regression of emergence against various soil physical properties did not reveal any significant relationship between soil physical properties and seedling emergence.

To observe the affect of competition on both the weed (broom corn millet) and the crop (sweet corn), plants of both species were grown together in pots at a range of planting ratios. Plants were also grown in monoculture to observe growth without competition. In the competition trial broom corn millet emerged after sweet corn and affected sweet corn above ground biomass during the first four weeks. However, this effect did not persist as sweet corn biomass increased irrespective of the level of competition from broom corn millet plants. The monoculture experiment indicated that sweet corn grew better without competition whereas growth of broom corn millet was stimulated while growing in competition. The poor competition by broom corn millet plants was assumed to be the result of unseasonal low temperatures during the period immediately after sewing and demonstrated that broom corn millet plants emerging after the crop may not affect crop growth.

The likely persistence of New Zealand broom corn millet seeds in soil is unknown. A laboratory based Controlled Ageing Test (CAT) was therefore evaluated for its ability to predict the persistence of seeds. The test was conducted using seeds of nodding thistle (*Carduus nutans*), for which real time persistence data is available. In two additional experiments, the CAT was used to estimate the potential persistence of New Zealand sourced broom corn millet seeds. The CAT derived half life time (P_{50}) of nodding thistle seeds did not compare well with the field derived P_{50} for nodding thistle seeds, with the CAT results suggesting less persistence compared to actual persistence. Examination of the CAT results for broom corn millet showed a decline in seed viability from 30 to 50 days, followed by a sharp decline at 75 days. The midpoint of the initial decline (40 days) was taken as the P_{50} for broom corn millet. This value is similar to existing information for broom corn millet in North America, and indicates that broom corn millet will form a moderately persistent seed bank in New Zealand.

In conclusion, results suggest that higher temperatures favour the growth of broom corn millet. Planting of crops earlier in the season may reduce competition by this weed. However, increasing temperatures as a result of global climate change will enhance conditions for broom corn millet and may increase future crop losses caused by this weed. The ability of broom corn millet's relatively

large seeds to germinate and emerge from depth will limit the efficiency of conventional weed control practices, such as ploughing and stale seed beds. The ability to form a moderately persistent seed bank suggests that once introduced, broom corn millet will be challenging to eradicate because of its prolific seed production. Significant changes in weed control practices will therefore be required to manage broom corn millet in the future.

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**Chapter- 1 Introduction and literature
review**

1.1 Weeds

1.1.1 Introduction

A weed is a plant growing where man wishes no plants, or other plants to grow which has some economic, ecological or aesthetic implications for man and/or his activities (Bridges, 1994). In simple terms, a weed is a plant that grows vigorously in a place where it is unwanted (Hollingsworth, 2000).

A plant becomes a weed when it has negative impacts on human or animal health, crops or aesthetic values (Bridges, 1994). Four main sectors of the economy are impacted by weeds, ultimately affecting every person in society in some manner. i) agriculture production: field crops, fruit trees, nut crops, pastures and other crops; ii) forestry: forest regeneration and nursery production; iii) non crop land: aquatic sites, industrial sites, landscapes, sides of the way etc. and iv) health: human and animal health. Efforts to maximise crop development and productivity also benefits undesirable plants, ultimately resulting in their adaptation to agricultural environments and their classification as ‘weeds’ (Bridges, 1994). For example, to eliminate or control weeds high efficacy herbicides have been developed over a long period, but weeds have also rapidly evolved herbicide resistance (Rajcan & Swanton, 2001). Any plant has the potential to be called a ‘weed’ if humans decide so, thus making the term ‘weed’ general rather than definable in rigorous scientific terms (Holzner & Numata, 1982).

This literature review examines the problem of weeds in the arable industry, with an emphasis on a comparatively new weed of maize and sweet corn farming in New Zealand that also has the potential to spread and affect other crops. *Panicum miliaceum* L. (broom corn millet) is an emerging weed of arable farming in New Zealand and the primary research subject of this thesis. Weeds such as broom corn millet compete with the desired crop plant for available resources, thus section 1.2 discusses the effects of weed competition on crop growth. The botanical and agronomic characteristics of broom corn millet are described in section 1.3. broom corn millet is a prolific seed producer and this factor has an important part to play in the weediness of this plant. Section 1.4 therefore elaborates on the characteristics of seeds in relation to their survival in the soil. Finally, section 1.5

concentrates on the ability of some seeds to emerge from deeper depths and the impact of this characteristic on weed management practices.

1.1.2 The Problem of weeds in New Zealand

Weeds are often better colonisers, fast reproducers and better survivors than non weedy species. Generally, weeds are successful because of their ability to outcompete non weedy and/or desired species (Sutherland, 2004). In his comparative study of life history traits of exotic and native species, Sutherland (2004) found that weeds are more likely to be annuals or biennials than perennial. In case of New Zealand, 85% of the plants here are not found anywhere else in the world and this uniqueness is threatened by new introductions (Hollingsworth, 2000), as introduction of new competitors as well predators can result in loss of natives (Savidge, 1987). A survey regarding perception of crop losses from pests was carried out in 1982 in New Zealand and England. This survey revealed that in almost all cases weeds were considered the most serious pests compared to any other factors causing crop loss (Mumford, 1982). This coincides with the fact that weeds are considered the major pests in most cropping system (Buhler, 2002).

Weeds are a threat to the unique flora and fauna of New Zealand, agricultural production, and the tourism industry. Annually, it costs \$450 million to New Zealand through the various impacts of weeds; \$350 million of this is spent on limiting their spread (Landcare Research, 2009). A large proportion of weeds are known to have been deliberately introduced as either ornamental or agricultural plants. The numbers themselves illustrate the size of the problem; there are approximately 2400 native vascular plant species currently growing in New Zealand, compared with an estimated 20,000 introduced species. Of the introduced species, 2000 are classified as naturalised (self reproducing) and 200 of them have been declared problem weeds by the Department of Conservation (Hollingsworth, 2000). Every year approximately 12 exotic plant species become naturalised in New Zealand, a pattern that has been observed for about the last 150 years, since records began (Lee et al. 1999).

1.1.3 Impact of weeds

As discussed above, weeds are a threat to native flora and fauna and can cause significant loss in agricultural outputs. Oerke (2005) estimated that the global loss in wheat and cotton yield were 50% and 80% respectively in the years 2001 to 2003, in which weeds caused the highest loss of 34%. These significant losses were just in yield, presumably this data does not include the cost of controlling weeds, either chemically or manually, which would utilise significant amounts of the resources available to farmers. The recent awareness of the environmental and health impacts of pesticides has resulted in more cautious applications of them. This is because the application of herbicides for weed control can result in an increased risk of herbicide contamination of ground and surface waters, negatively impacting human health, increasing the cost of water purification, and decreasing fishery and recreational values (Tilman et al. 2002; Tilman, 1999). Use of pesticides can also result in a decrease in species richness along with negative impacts on soil microorganisms (Fantroussi et al. 1999; Relyea, 2005). Classical biological control, i.e. the introduction and release of exotic insects, mites, or pathogens to give permanent weed control and eradication is the predominant method in weed biocontrol (Cruttwell McFadyen, 1998). As suggested, organisms from other regions of the world and different ecosystems are introduced into the place where biological control of the weed is to be conducted. Such introductions are usually irreversible and once introduced these organisms are almost impossible to eradicate. These organisms can also impact on desired and rare plants related to the target weed (Harris, 1988). All of the above mentioned problems, caused by weeds, ultimately have an economic impact, which makes them undesirable.

1.2 Weeds- Resource competitors for crops

The major concern with weeds is the reduction they cause in crop yield and quality, resulting from their utilisation of light, nutrients and moisture. In particular, weeds compete with desired plants for light, affecting their growth by reducing their biomass (Watt et al. 2003). There is also growing evidence for light quality effects, in terms of the far-red/red ratio perceived by plants which plays an important role in influencing interactions among neighbouring plants (Ballare et

al. 1990; Ballare & Casal, 2000). Considerable numbers of environmental factors can be altered when crops grow in competition with weeds, thus adversely affecting the growth processes of crops. Detection of neighbouring plants during the early growing stages may extensively affect the morphology of the adult crop plant (Rajcan & Swanton, 2001). Under natural conditions it can be difficult to determine which of these factors is causing the damage to the crop. In most scenarios multiple factors, interrelated in a complex manner, affect the crop (Shadbolt & Holm, 1956)

Competition by weeds during the early part of the growing season depresses crop growth more significantly than competition at any other time (Buchanan & Burns, 1970). In general crop yields reduce proportionately as weed populations increase (Knake & Slife, 1962). Therefore, it is essential to predict the effects of weeds on crop plants before planning management programmes to control weeds (Kropff & Spitters, 1991). Kropff & Spitters (1991) have further suggested in their review that the relative time of emergence of the weeds compared to the crop, and weed density, are the two most critical factors to consider. Both these factors are indicators of the competitive relationship between the crop and weeds and precise predictive models of yield loss can be made based on these two factors. However, in the field weeds can emerge in successive flushes, and in some cases throughout the growing season, thus making the use of predictive models more difficult. In these situations, the relationship between the relative leaf area of the weed versus the crop and yield loss was found to be more appropriate (Kropff, 1988).

Considerable amounts of research, including various aspects of weed biology, have been performed to control and reduce the degree of impact caused by weeds. These range from developing herbicides and crop rotation practices (Liebman & Dyck, 1993) to the recent trend of preparing stale seed beds in organic farming (Lamour & Lotz, 2007). However, as discussed in the section 1.1 the adaptability exhibited by weeds has kept humans at bay in the majority of attempts either to study or control them.

1.3 Broom corn millet (*Panicum miliaceum* L.)- A weed of arable farming

Panicum miliaceum L., commonly known as either proso or broom corn millet, is an annual grass that grows only by seed (Bough & Cavers, 2009; James et al. 2010). Broom corn millet is grown for human consumption and bird seed, as well as being a weed, thus making it a crop-weed complex (Cavers & Bough, 1985). Originated in the tropics and in temperate latitudes as a warm season grass, broom corn millet was domesticated as a crop in many parts of the world at least 2000 years ago. However, the location of its first domestication as a crop is still debated (Lu et al. 2005). A crop well suited to dry climates, it is now cultivated in Central Russia, Eastern Europe, northern India, China, Africa and the Great Plains area of North America (Karam et al. 2004). The crop and weedy biotypes of broom corn millet are different from each other in their characteristics. Weedy biotypes of broom corn millet are already a problem of arable farming in the USA, Canada and recently in New Zealand (Wilson, 1993; James et al. 2010). It is suspected that origin of the wild type of broom corn millet is through the escape from the cultivated variety as a result of repeated reverse mutations (Carpenter & Hopen, 1985). However, a black or dark brown seeded biotype of broom corn millet has always been considered a weed and is often referred to as *Panicum spontaneum* or *Panicum miliaceum* var. *ruderales* in European literature (Bough & Cavers, 2009). The introduction of the wild type in New Zealand was recorded around the time when it was first considered as a weed on North American farms (Bough & Cavers, 2009; James et al. 2010). In addition, the weedy biotype found in New Zealand is identical in appearance to the weedy biotype found in North America.

Commonly known as broom corn millet in New Zealand, this recent grass weed is under-going a rapid range expansion (James et al. 2010). It was first recorded in 1961 in Auckland and Palmerston North and is now causing significant trouble to arable farmers in the maize growing regions of Hawkes Bay, Gisborne and Marlborough. It is assumed that broom corn millet had multiple entry points into New Zealand through pathways such as bird seed or as a contaminant in imported seed lots (James et al. 2010).

Broom corn millet has the C₄ photosynthetic pathway (Gardestrom & Edwards, 1984), meaning that the first product of carbon fixation is a four carbon organic acid (Ehleringer et al. 1991). The efficiency of the C₄ photosynthetic pathway is mainly associated with a reduction in photorespiration, particularly in warm and arid climates. Plants with the C₄ photosynthetic pathway may also have higher photosynthetic capacities than C₃ plants. The resulting increases in nitrogen, water and in some cases light use efficiencies provide C₄ plants with a significant advantage over C₃ plants under warmer, drier conditions (Ehleringer et al. 1991; Leegood, 2002).

Broom corn millet is one of the most problematic weed of sweet corn and maize in countries like the USA and Canada (So et al. 2009; Bough & Cavers, 2009), and is a threat to both maize and sweet corn farming in New Zealand (James et al. 2010). Its rapid growth, in conjunction with other weedy characteristics such as prolonged germination and seedling emergence, tolerance towards commonly used herbicides and prolific seed production, can make it a troublesome weed under suitable environmental conditions. Difficult to get rid of, its seeds are also contaminants of harvested sweet corn and maize, with the unwanted seeds acting as founding populations (So et al. 2009). Apart from maize, the species is also a weed of other crops like beans, barley and soybean (Wilson, 1993; O'Toole & Cavers, 1983). Aggressive expansion of range by broom corn millet is also evident from the literature. For example, in the USA, after it was first recorded as a weed in the early 1970's, it had infested 4000,000 hectares of prime land by 1980 (Bough & Cavers, 2009). This rapid spread can be readily explained because moderate infestations of broom corn millet can produce 2000 to 3000 seeds per square meter, and heavy infestations can produce 40,000 to 45,000 seeds per square meter (Bough & Cavers, 2009). Small infestations have the potential to become serious infestations within 2 years because the abundant seeds are easily spread by harvesters and other farm machinery.

1.3.1 Morphology

Seedlings of this annual warm season grass grow rapidly into upright plants which may tiller at low densities. The root system is weak and the plant can be easily uprooted from the soil (Baker, 2003). The leaf blades of *Panicum miliaceum* are

more or less hairy on both surfaces and along the edges. They are 1 to 2 cm wide and up to 30 cm long. The leaf sheaths are densely hairy and have overlapping margins (Figure 1.1). The ligule consists of a line of dense hairs. The much branched panicle, 15 to 30 cm long, is compact in some populations and loose in others. The spikelets are borne individually at the end of branches. Spikelets consist of a fertile floret and a sterile floret enclosed between two pieces of chaff. The seeds of broom corn millet are somewhat egg shaped, with a hard, shiny seed coat that firmly encloses the seed when it is shed (Figure 1.2). The seed is approximately 3 mm × 2 mm in size (Bough & Cavers, 2009). The weight of broom corn millet seeds varies between biotypes, from 3.7 to 6.3 mg seed⁻¹ (Moore & Cavers, 1985).

Apart from cultivated varieties, there are six biotypes of broom corn millet that are considered weeds. These biotypes are usually distinguished by their seed colour, which varies from brownish black, olive brown, orange-red, golden and light cream. However, apart from seed colour the weed biotypes also differ in the shape of their inflorescence, whether the head is shattering or non-shattering, the amount of tillering, the time of flowering, plant height, and the ability to form a viable seed bank (Bough et al. 1986). The black seeded biotype of broom corn millet is considered an aggressive biotype since its seed heads shatter when they are ripe and it also forms a seed bank where seeds can be viable in the soil for at least four years. Only the black seeded biotype of broom corn millet can survive winters, thus making it more persistent (Bough & Cavers, 2009). It is also proposed that in broom corn millet, a darker and heavier seed coat is correlated with seed longevity by reductions in imbibition damage (Khan et al. 1996).

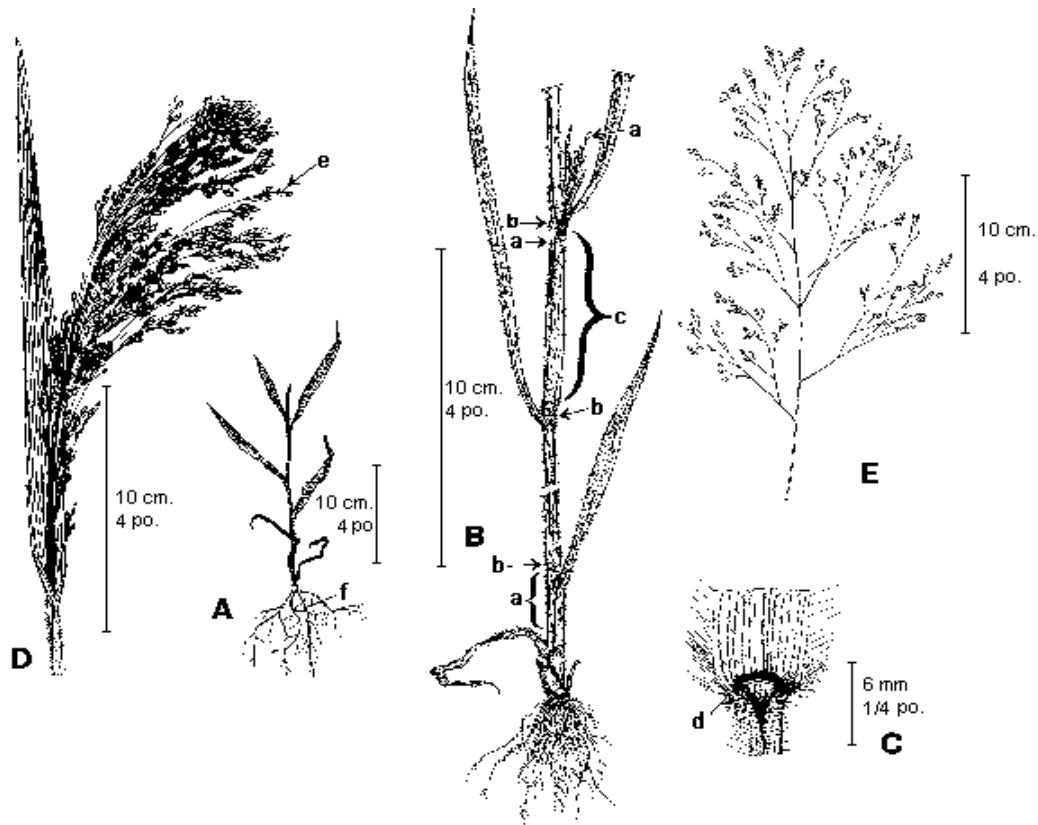


Figure 1.1 Broom corn millet- A. Young plant in the 6-leaf stage with primary roots. B. Lower portion of flowering plant showing one inflorescence emerging from leaf sheath. C. Leaf-base. D. Dense, arching inflorescence. E. Loose, open inflorescence (Figure: Bough & Cavers, 2009).



Figure 1.2 Seeds of the black seeded biotype of *Panicum miliaceum* L. (Photo: Trevor James).

1.3.2 Growth and known aspects of biology in overseas infestations

Broom corn millet has a large seed compared to many other weeds of arable crops (James et al. 2010). The hull of the seed can be found attached to the primary roots even after the plant is mature. Germination starts at the onset of the warm season and is continuous throughout the growing season. Plants may also emerge after harvesting of cereal crops and can still produce seeds before the end of the growing season (Bough & Cavers, 2009). The growth habit of broom corn millet is rapid and vigorous without any competition (Carpenter & Hopen, 1985). In a comparative growth study between cultivated and weedy biotypes of broom corn millet, weedy biotypes grew taller and produced more leaf area, dry weight and seeds. However, cultivated biotypes produced seed heads earlier than weedy biotypes (Eberlein et al. 1990).

Flowering generally begins 30 days after germination depending upon the biotype (Reddy et al. 2007), and occurs continuously. Therefore, maturation and shattering of the seed heads also occurs continuously. The seeds of the black seeded biotype of broom corn millet shatter readily when mature. In addition to the differences in seed colour and other characters between varieties, differences have also been observed in the seed bank pattern of broom corn millet. Seeds of crop varieties form transient seed banks while the seeds of the black seeded biotype are reported to form moderately persistent seed banks. Seed banks of broom corn millet may have seeds that are not capable of immediate germination (Cavers et al. 1992); however, they are known to have very high (> 99%) viability (Eberlein et al. 1990). The major proportion of seeds germinates at or near the soil surface, from an average depth of about 2.5 cm. However, seedlings can emerge from a depth of 7.5 to 13.5 cm, depending upon soil type and tith (Bough & Cavers, 2009).

In summary, characteristics such as prolific seed production in combination with rapid emergence, ability to set seed at any time during the season, and a tendency to form a seed bank make broom corn millet an aggressive weed (O'Toole & Cavers, 1983).

1.4 Reproduction by seeds

Weed problems can be enormous in agriculture where annual weeds with high and prolonged seed production are present (Lamour & Lotz, 2007), as the phenomenon of high seed production is used by weeds to maintain sustainable populations (Reuss et al. 2001). Once shed onto the soil surface, weed seeds either remain on the surface or are incorporated into the soil by natural or artificial means (Thompson, 1992), and ultimately form seed banks. The seeds at maturity are generally dormant or enter into dormancy soon after maturity, resulting in an extended life in the soil (Reuss et al. 2001). Therefore, weeds are often characterised by large seed banks with prolonged seed longevity and adaptive seasonal patterns of germination (Kigel et al. 1992). However, not all seeds produce mature plants as viable seeds are always subject to mortality through biotic or abiotic factors. This section describes the role of seed banks in the success of weeds, and how inherent characteristics of seeds coupled with environmental factors influence the longevity of seeds in soil.

1.4.1 Soil seed banks and their role in management strategies

Almost all major weeds of economic importance form large soil seed banks (Warr et al. 1993). Seeds of weed species can survive in soil seed banks for more than 5 years and examples of persistence up to 20 years or longer have been documented (Kivilaan & Bandurski, 1981; Lewis, 1973; James et al. 1998; James and Rahman, 1999, 2001, 2003). In highly variable and disturbed environments the development of seed banks allows plant populations to persist (Adams et al. 2005). The same phenomenon makes weeds persistent and hard to eradicate. However, there is considerable interspecies variation in the longevity of seeds in soil seed banks, and not all weed species form long-lived soil seed banks (James et al. 1998; James and Rahman, 1999, 2001, 2003).

Soil seed banks also have an important role in perpetuating the genetic diversity (Tanksley & McCouch, 1997) and persistence of weeds in an area. The soil seed bank is therefore an important factor for land managers to consider when undertaking weed monitoring and eradication. Thus, knowledge of seed longevity is vital for planning and executing weed management programmes (James et al.

1998). Germination patterns of weed seeds is also important as seedlings are more vulnerable to control measures than mature plants and are therefore an easier target for weed management programmes (Forcella, 1992; James & Rahman, 2000). Therefore, knowledge of a weed's germination ecology (i.e. environmental factors affecting the germination of seeds, their dispersal range and timing and pattern of germination) is also important for the planning of a weed eradication programme. Continuous monitoring required of sites where weed eradication has been attempted (Cunningham et al. 2003; Long, 2007) makes these aspects of seed biology essential to know. This is because eradication cannot be declared successful as long as a single seed remains alive in the soil (Long et al. 2008). In addition, the current trend towards integrated and organic farming systems has reduced the use of herbicides, resulting in an increase in the size of weed soil seed banks. For example, the transition from conventional to organic farming led to an increase in the size of the weed seed bank of between 11% to 21% per year over 7 years in the Netherlands (Riemens et al. 2007), and 17% per year over 6 years in Germany (Albrecht, 2005). Therefore, it is critical to understand the function of seed banks and seed longevity for economically important weeds.

1.4.2 Characteristics of seeds helping their persistence in soil seed banks

1.4.2.1 Seed viability and seed vigour

A seed is called viable when it has the potential to germinate and viability is the percentage or proportion of viable seeds in a seed bank (Sawma & Mohler, 2002). Testing of seed viability is a critical step that exhibits the liveness of seeds from seed banks and seed longevity experiments (Sawma & Mohler, 2002; Long, 2007).

The definition of seed vigour is given by The ISTA Vigour Test Committee and is reproduced here: "Seed vigour is the sum total of those properties of the seed which determine the potential level of activity and performance of the seed or seed lot during germination and seedling emergence. The aspects of performance which may show variation include rate and uniformity of seed germination and seedling growth, seedling emergence and growth in the field, emergence ability of

seedlings under unfavourable environmental conditions and vigour may influence growth as well as yield.”

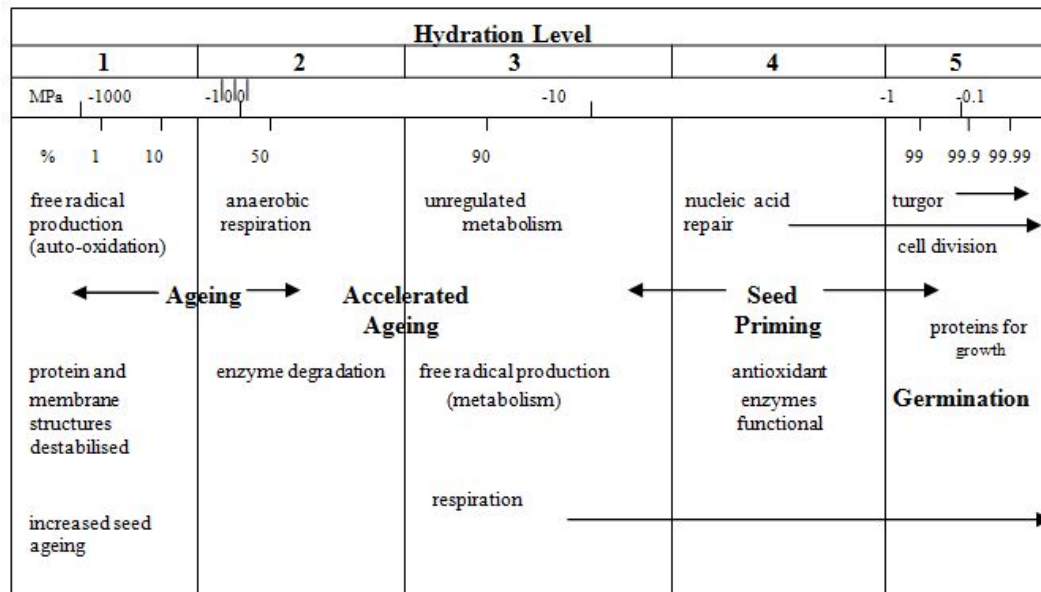


Figure 1.3 The relationship between different levels of hydration and ageing and repair processes in seeds [Adapted from Walters et al. (2005)].

Low moisture content is the key factor in maintaining the viability and vigour in seeds which are known as desiccation tolerant seeds. Vertucci (1989) has quoted that most seeds can be stored at a moisture content of about 8% on a dry weight basis. Viability loss is increased in warm and moist conditions (Mudroch & Ellis, 1992; Figure 1.3). In addition, loss of viability cannot be recovered while there is possibility of regaining the vigour by priming process (wetting and drying cycles) that enable repair reaction to occur inside the seeds in high moisture condition (Bray, 1995; Burgess & Powell, 1984).

1.4.2.2 Seed dormancy

Seeds which do not germinate when placed under conditions normally considered ideal for germination are said to be dormant (Chancellor, 1982). A recent definition was published by Baskin & Baskin (2004) “A dormant seed is one that does not have the capacity to germinate in a specified period of time under any combination of normal physical environmental factors that otherwise is favourable for its germination”. The plant growth regulator abscisic acid is produced by the embryo and acts by inducing dormancy during seed development,

while gibberellins promote germination in non-dormant seeds (Baskin & Baskin, 2004). It has become evident from molecular biology studies that seed dormancy is a typical quantitative genetic trait, involving many genes which are substantially influenced by the environment during seed development, and exhibiting continuous phenotypic variation (Baskin & Baskin, 2004).

Seed dormancy and persistence or longevity have long been considered related to each other in literature. For example, Baskin & Baskin (2006) suggest that dormancy and longevity allow seeds to persist for longer periods in the soil. However, Thompson et al. (2003) argue that there is negative correlation between these two phenomena. Their research from existing seed longevity data of 339 species suggested that the traits are different and that seed persistence is not related to dormancy. As discussed above, in the majority of annual weeds high seed production results in the formation of seed banks that sustain future populations. Simultaneously, in response to resource competition and inbreeding (see section 2.4) various dormancy mechanisms have evolved. This suggests that the hypothesis proposed by Thompson et al. (2003) should be studied extensively, including a broad range of plant taxa and site specific factors.

1.4.2.3 Seed morphology

The morphology of seeds i.e. seed size and shape, seed coat etc., along with environmental factors, has an important role in the persistence of seeds. A recent study by Probert et al. (2009) with 195 species covering broad taxonomic and geographic areas has indicated that seed longevity is related to seed structure and climate of origin. Impermeable seed coats are reported to have an influence on seed longevity by protecting them from environmental influences. Layers of palisade tissue in seed coats do not allow the water gap to open (a specialized anatomical structure in the seed, through which water moves to embryo) until a positive environmental signal is received. This protects them from environmental fluctuations, especially in moisture which can have an impact on their longevity (Baskin & Baskin, 2006). Examples are known of seeds which persisted for 1300 years, protected from microorganisms and moisture by their hard seed coat (Baskin & Baskin, 1998; Smith & Berjark, 1995). Another example of the influence of the seed coat (testa) on seed dormancy, germination and longevity is

given by Debeaujon et al. (2000). They found that mutated seeds varied in testa pigmentation and structures, and that mutated seeds were less competent in natural ageing compared to wild types. Dark seeds of broom corn millet are also known to survive in the soil longer than light coloured seeds. This character of dark seeds is associated with heavier seed coats, which protects the seed from imbibition damage by imbibing water slowly (Khan et al. 1996). In addition, the phenolic components of seed coats of some species, such as *ortho*-dihydroxyphenols, are also proposed to have a decisive role in seed longevity by protecting seeds from herbivory and attack by microorganisms (Hendry et al. 1994).

A significant number of studies have also explored the relationship between seed size and shape and seed persistence in soil (Thompson et al. 1993; Bekker et al. 1998). Bekker et al. (1998) suggested that larger seeds, and seeds with comparatively larger surface to volume ratios, do not incorporate into soil easily compared to smaller seeds. This observation was supported by the research of Thompson et al. (1993) who suggested “seeds do not persist for long periods on the soil surface, and burial is clearly an essential prelude to persistence.” However, the theory of size and shape effecting seed longevity does not seem to be applicable everywhere as no consistent tendency was found in similar analysis done for Australian species. This suggested that persistent seeds are not smaller or more compact in this case (Leishma & Westoby, 1998). However, Moles et al. (2003) also found that for Australian and New Zealand species larger seeds were associated with increase in persistence. This suggests that the relationship between seed size and shape and persistence may be species and site specific.

Evidence from various studies suggests that seed persistence in the soil is not solely determined by seed size and shape, as factors like germination requirements, dormancy mechanisms, seed reserves, and resistance to pathogens also play an important role in persistence (Thompson et al. 1993).

1.4.3 Influence of environmental factors on seed longevity in soil

1.4.3.1 Moisture and temperature

It is long established that variation in the soil environment can affect the degree to which buried seeds persist (Schafer & Chilcote, 1970). Seeds experience a variable environment in the soil at different times. Temperature, water potential and oxygen can vary widely between seasons, days, and even within a day (Allen & Mayer, 1998). Equilibrium is made by seeds with this constant alteration in external conditions that influence the ageing process. In contrast to the common belief, water vapour is the main source of water throughout much of the soil column, instead of liquid water (Wuest, 2007). Liquid water is more abundant towards the soil surface. Seeds near to the surface are therefore more prone to be lost to germination and hence less persistent. Research by Bekker et al. (1998) suggests that even groundwater can have positive or negative impact on the fate of seeds in soil seed bank. Fluctuations in soil moisture and temperature may prime seeds and cause faster germination, ultimately leading to reduced persistence (Schafer & Chilcote, 1970; Gonzales-Zertuche et al. 2001).

1.4.3.2 Soil type

Soil and soil properties also have an important role in the persistence of seeds in direct and indirect ways (Forcella, 1992). Soil seed bank studies usually include chemical properties of seeds such as pH, organic matter, moisture content (MC), nutrients, cation exchange capacity (CEC) and physical properties like percentage of sand, silt and clay which may influence seeds present in soil seed banks (Long, 2007; Buhler et al., 1998), either alone or in combination.

Researchers in New Zealand (James and Rahman, 1999, 2000, 2001, 2003; James et al. 1998) considered both burial depth and soil type when studying the persistence of five broadleaf weed species in a real time burial experiment. Results from this study showed that seeds of all species were primarily affected by depth of burial, with seeds near the surface lost sooner due to germination or other causes such as predation. In the same study, species responded differently in different soil types, suggesting a role of soil properties in their persistence (Figure

1.4). There is no data available in these studies about microclimatic factors such as soil temperature, which could be leading to misinterpretation the results for different soil types. However, the close proximity between sites (within 5 km of each other) supports the assumption that they did not differ significantly in climatic conditions

A similar study was carried out in the USA by Burnside et al. (1996) for 41 economically important weed species. The two sites that were selected for this study differed markedly from each other in soil physical and chemical properties and extremely from each other in temperatures during winter and in annual rainfall. It was considered in this study that soil with a higher percentage of organic matter and clay would retain more rainfall. Result revealed that weed seed germination was higher after exhuming seeds from the soil where rainfall was comparatively lower and temperatures were moderate. This indicates that climate along with soil properties have an important role in seed persistence.

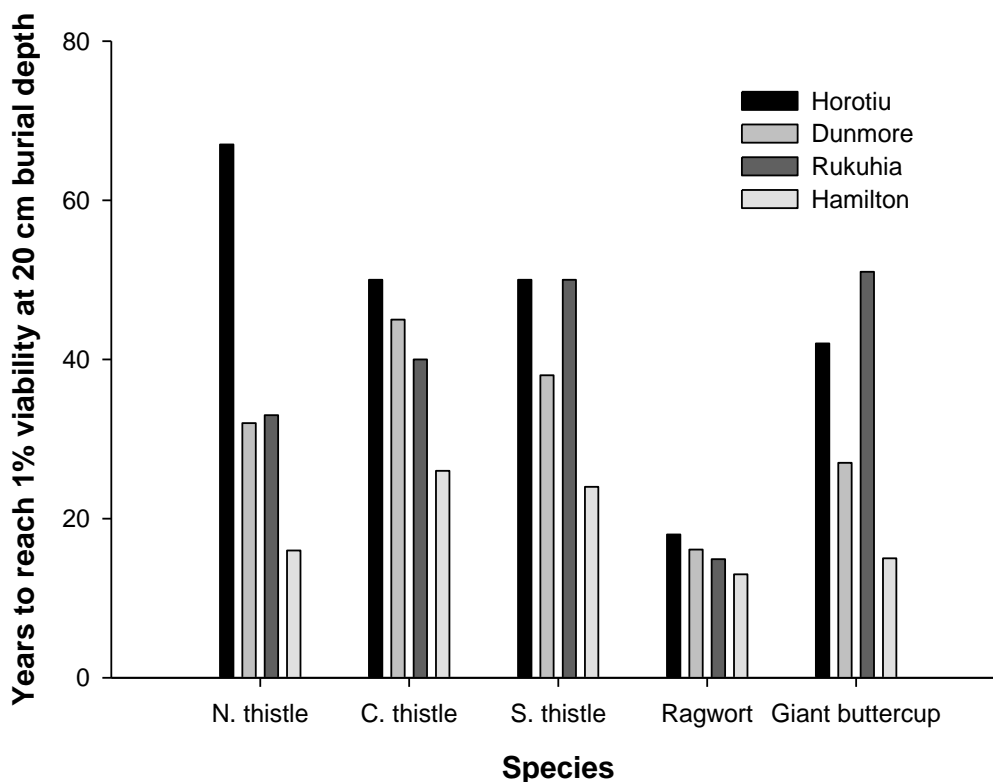


Figure 1.4 Estimated years to reach 1% viability for five New Zealand weed species in four soil types at 20 cm burial depth (James and Rahman, 1999, 2000, 2001, 2003; James et al., 1998).

To summarise on this subject, it is evident that soil type may influence the persistence of seeds in conjunction with climatic factors (Burnside et al., 1996). Therefore, even though the outcomes from James and Rahman (1999, 2000, 2001 and 2003), James et al. (1998) and other similar experiments provide valuable information, inclusion of soil climatic observations would strengthen future studies.

1.4.3.3 Biotic factors

The weed seed bank normally declines in seed number exponentially and this is readily explained in terms of biological processes, such as ageing, faunal parasitism and faunal predation (Rahman et al. 1998). In this context, insects as well as microorganisms have been documented as influencing persistence of seeds through displacement, granivory and decay (Bekker et al. 1998; Kremer, 1993; Forcella, 2003), and thus have been proposed as potential biocontrol agents in weed eradication programmes (Kremmer, 2000).

Combinations of insects and pathogenic microorganisms have been observed being predator of a few weeds. For an example, seed feeding insects facilitate infection by pathogen, resulting in death and decay of seeds. In addition, the vigour and fecundity of the emerging plants are also affected when the seeds are infected with pathogens (Forcella, 2003; Kremer, 1993; Kremer, 2000). The performance and longevity of seeds and seedlings can also be different in the presence of seeds of other species, different biotypes of the same species, or even seeds of the same biotype but of different age, because competition for resources may occur between them post germination (Grundy et al. 2003; Lenz & Facelli, 2005). Hence, the expected lifespan of a seed in a homogenous seed bank could be quite different from that in a heterogeneous seed bank. Reports have also been made of phenolic compounds released by other seeds, fruits and plant tissues which can have negative impacts on the germination of seeds, development of the soil seed bank, growing plants and other plant processes (Williams & Hoagland, 1982).

1.4.4 Previous attempts to estimate seed longevity and classify seed banks

Numerous attempts have been made worldwide to predict the persistence of weed seeds in soil seed banks. The most famous experiment of such nature was initiated in 1879 by Dr. Beal which finished 100 years later in 1980 (Kivilaan & Bandurski, 1981). The work is being carried out on both agriculturally important species as well as problematic weed species (Lewis, 1973; Jianhua & McDonald, 1997; James et al., 1998; James & Rahman, 1999, 2000, 2001, 2003; Long, 2008). Different authors have considered different factors which could be responsible for the persistence of seeds in soil seed banks e.g. soil type and burial depth (Lewis, 1973; James et al., 1998; James & Rahman, 1999, 2000, 2001, 2003), seed size and shape (Thompson et al. 1993). A recent trend has been towards developing laboratory based methods to predict seed persistence (Davies & Probert, 2004; Long, 2007). However, all these attempts do not provide adequate information about what really governs the persistence of seeds in soil seed banks. A recent hypothesis supported by Long (2007) suggests that there is strong genetic determinant to longevity of seeds (Bakker & Bekker, 2003). Real time *in situ* studies to describe seed persistence in seed banks are considered more satisfactory and accurate (Panetta, 2004) but they are time consuming and difficult to carry out in today's world with rapidly changing land use. It is practically difficult to run such experiments with changing personnel through time. In addition, a key limitation to such trials is that the outcomes may be limited to the particular location and seed lot tested (Long, 2007). Therefore, efforts should be made towards developing laboratory based techniques to estimate the persistence of seeds in various conditions and in conjunction with available seed classification systems for interpreting persistence in field scenarios.

Since 1969, ten soil seed bank classification systems have been published that classify plant species based on the longevity of their seeds in soil. Applicability of these system range from being versatile and thus can be used everywhere to specific for certain ecosystems or agriculture systems (Csontos & Tamas, 2003). These systems have between 3 and 12 seed bank categories. Seed persistence is the main factor used to distinguish the categories whereas other factors include

germination, dormancy as well as size and shape of seeds (Bekker et al. 1998). Seed bank classification systems with few categories are generally more widely accepted compared to systems involving high numbers of categories. This is due to the detailed knowledge of each plant species that is required for more complex classification systems, but which is usually lacking (Csontos & Tamas, 2003). In this context, only the commonly cited classification system of Thompson et al. (1998) is discussed here.

Based on the available persistence records the soil seed bank classification system of Thompson et al. (1998) classifies each species into one of three categories: Type 1- species with transient seed banks, where seeds persist for less than one year; type 2- short- lived seed banks, where seeds persist for more than one year but less than five years and; type 3- long- lived seed banks, where seeds persist more than five years. The allocation of the species in to respective category is determined by the ratio of total number of records for type-2 and type-3 seed banks and total numbers of records for that species. The ratio falls within the range of 0 to 1 and indicates the persistence potential of seeds.

$$SPI = \frac{\Sigma(\text{type 2} + \text{type 3})}{\Sigma(\text{type 1} + \text{type 2} + \text{type 3})}$$

Long et al. (2008) gives the simple form of this equation without changing its meaning:

$$SPI = \frac{\text{No.of records for persistence} > 1 \text{ year}}{\text{Total No.of records for that species}}$$

Based on the available records of the seed persistence for each species, problems encountered with the proposed system include that there is no upper limit for type- 3, and persistence scale (1-3) is not linear. In addition, sampling time and inadequate knowledge of vegetation history can easily lead to misinterpretation of results (Thompson et al. 1998). Therefore, to calculate longevity index in a simple manner the authors suggest dividing data in just two types; transient (type 1) and persistent (type 2) and then dividing by the original number of records. A value between 0 (no records of persistence) to 1 (all records indicate persistence) can be achieved with this approach. As discussed before, this system is by far the most

cited and perhaps the most convenient for deciding seed persistence provided enough records of seed persistence for each species are available.

1.5 Ability to emerge from greater depths- A management problem for controlling weeds

Most annual weeds rely on prolific seed production and a seed bank in the soil for maintaining a sustainable population (Reuss et al. 2001). Soil seed banks serve as source of new infestation every year in the case of annual weeds of cropping systems (Buhler et al. 1998). For effective weed management practices, it is therefore essential to have a knowledge of weed emergence patterns in relation to environmental factors such as soil conditions and weather, as the number of seedlings emerged in combination with the timing of emergence have significant effects on crop growth (Kropff, 1988). Emergence of weeds in a field is a two stage process of seed germination, and seedling growth prior to emergence (Vleeshouwers, 1996). Pre-emergence seedling growth is an important factor determining the emergence pattern of weeds (Benvenuti et al. 2001).

Soil characteristics along with soil environment can have considerable influence on seed germination and seedling emergence from depth. Seeds of different species may possess different dormancy characteristics such as requirement of specific temperature range or moisture conditions which can influence their germination in a particular site (Benvenuti et al. 2001). Soil particle size also ultimately impacts on germination and seedling emergence of buried seeds (Cussans et al. 1996). In addition to soil particle size, soil compaction may also directly limit the germination of seeds (Pareja & Staniforth, 1985) and can induce dormancy in them (Terpstra, 1995). Other important soil environment factors include light, soil temperature and soil water content (Ballare et al. 1992; Benvenuti & Macchia, 1993). While the mechanism is not yet completely understood, it is also known that seeds are inhibited by their respective depth in the soil (Stoller & Wax, 1973). The proposed factors influencing the complex mechanism of seed germination and seedling emergence from depth are lack of light, decreasing thermal fluctuation, secondary dormancy induced due to reduced gas exchange, presence of CO₂ derived from soil biology activities and energy reserves (Benvenuti, 1995; Benvenuti & Macchia, 1995; Lafold & Baker, 1986).

In fact, it has already been demonstrated that high soil moisture, soil compaction and microbial activity may reduce soil O₂ concentration or inhibit gaseous movement (Drew, 1992), resulting in production of toxic volatile alkaloids through fermentation, ultimately influencing the germination of buried seeds (Benvenuti & Macchia, 1995).

In the models of the population biology of weeds, it is generally assumed that the seedlings of most weed species arise from seeds in the surface layer of the soil. Most weed species of economic importance have been noted to germinate from depths less than 20 mm and not more than 60 mm (Cussans et al. 1996). However, weed species such as johnsongrass (*Sorghum halepense*) and others are also known to emerge from greater depths, such as 100 mm (Benvenuti et al. 2001). Such information is used to select soil acting herbicides to control these weeds. When seeds and seedlings are located at depth they may benefit from 'depth protection', as the herbicide may remain in the surface layer of soil (Blair et al. 1991) and may dissipate before seedlings emerge.

It is widely accepted that large seeded species can emerge from greater depths compared to smaller seeded species (Froud-Williams et al. 1984; Grundy et al. 2003). Taking into account that the growth of seedlings before emergence is completely heterotrophic in the absence of light, energy reserves available in seeds are vital for growth. Therefore, seeds germinating at excessive depth may have little chance to reach the soil surface in the form of seedlings due to the energy limited heterotrophic phase. However, the fluctuating availability of O₂ in the soil can also influence the process of seedling emergence (Raymod et al. 1985). Weed species may differ from each other in their ability to emerge from various soil depths (Benvenuti et al. 2001), and within populations depending upon site specific conditions (Milberg et al. 1996). In this context, seed weight can have a major role in allowing germination of seeds from significant soil depths, as seed weight is proportionally related to size and reserves available within the seeds (Benvenuti et al. 2001).

1.6 Summary of literature review

Weeds are a serious problem to both agriculture and natural ecosystems due to their invasive nature as well as ability to evolve in response to control measures. They cause a loss in yield by competing with crops for resources. *Panicum miliaceum*, broom corn millet, is a good example. Having evolved as a weed from its crop biotypes, it is now a major problem in many countries where sweet corn and other crops are grown. Prolific seed production, a typical characteristic of many annual weeds, acts as a key factor in its weediness. Apart from providing sufficient propagules for future populations, these seeds also form seed banks and stay viable within them. The inherent characters of these seeds protect them from external environmental fluctuations that otherwise may have deteriorative effect on them. Therefore, attempts are being made to understand the mechanism of survival of seeds in the soil and predict their persistence in a particular set of conditions in both field as well as laboratory based studies.

Panicum miliaceum (broom corn millet) is an emerging weed in maize and sweet corn farming in New Zealand. Therefore, the aim of this thesis is to study aspects of the biology of the biotype present in New Zealand, as outlined below:

- Chapter 2 describes the effect of temperature on germination and the early growth stages of broom corn millet. The knowledge obtained from this study may be used for modelling exercises predicting the time for crop planting to reduce competition from broom corn millet.
- Chapter 3 focuses on the ability of broom corn millet seeds to emerge from various planting depths in a range of New Zealand soils. It is hypothesized that the large seeds of broom corn millet with plenty reserves allow it to germinate and grow from higher depths than other weeds.
- Plants often respond to presence or competition from neighbouring plants by accumulating more biomass in shoots than roots. Therefore, a wide range of crop to weed ratios are explored in chapter 4 to identify the

threshold density at which broom corn millet begins to affect the above-ground biomass of sweet corn.

- In chapter 5 a laboratory based 'Controlled Ageing Test' is evaluated by using it on a broadleaf weed species for which real time persistence data is available. The technique is then used on broom corn millet seeds for which we have limited information regarding its persistence in soils.

**Chapter- 2 Influence of temperature on
germination and growth of broom corn
millet**

2.1 Introduction

It is known that the germination and growth of plants are temperature dependent, and that the influence of temperature on germination and seedling development varies among species (Wiese & Binnings, 1987). Temperature is usually the dominant environmental factor determining the rate of germination when the soils are wet. It also frequently remains the dominating influence during early shoot and root growth. Generally, plants start growing once the lower temperature threshold for development or germination has been reached. It has also been well established that soil temperature affects many aspect of growth, including water and nutrient uptake by roots, development and expansion of leaves, dry matter production, flowering and harvestable yield. Considerable research has shown that the base, optimum and maximum temperatures differ for the phases of growth as well as between species (Wiese & Binning, 1987; Russell, 1988). This chapter examines the effect of soil temperature on germination and early growth of the C₄ weed *Panicum miliaceum* (broom corn millet). Below, the significance of temperature for growth processes in plants is reviewed, including the particular importance of temperature for C₄ plant growth.

The rate at which roots take up water and nutrients from the soil is affected by temperature. For example, the uptake of phosphate generally increases with elevated temperatures (Russell, 1988; Gavito et al. 2001). Gavito et al. (2001) demonstrated that in wheat, increasing soil temperature induced more N uptake and positively affected plant size during vegetative growth. Apart from nutrient uptake, soil temperature also increased N-use efficiency, under ambient CO₂ conditions. An increase in early root production in their experiment also suggests that temperature had a significant effect on germination. As an outcome of this research it was found that variation in soil temperature during vegetative growth can alter root development, biomass allocation and nutrient uptake. Importantly, the effect of soil temperature was clear even when air temperature was unchanged. Soil temperature is more stable and predictable than air temperature, making it an important variable to be included in models of plant growth. In temperate regions where plant growth is inhibited during the early growing season by low soil temperatures, predictions that do not consider the effect of soil temperature are

often misleading (Gavito et al., 2001). However, the effect of temperature on nutrient uptake is species specific, and the optimum temperature for uptake may vary amongst the elements (Turner & Lahav, 1985; Menzel et al. 1987). For example, in passion fruit (*Passiflora edulis*) the highest rate of uptake of nutrients, and the highest concentration of elements such as P, Ca, Fe, Mn, Cu, B and Al, occurred at 20°/25°C (Menzel et al. 1987).

The rate and proportion of seed germination for a species generally increases with elevated temperatures from a base temperature, reaches a maximum at an optimum temperature, and decreases when temperature is beyond the optimum (Garcia-Huidobro et al. 1982). Temperature also affects the growth process in similar manner. However, considering the role of temperature in growth, distinguishing the duration of the process from the rate of process is useful. Generally, the rate of process increases as temperature increases from the base temperature to the optimum, and the duration reduces as the rate of processes increase (Bhattacharya & De Datta. 1971; Russell, 1988). Crops, in particular cereals, provide good examples of these effects of temperature on growth. If temperatures are below the optimum, increasing temperature increases growth rate of cereals, and shortens the duration from germination to harvest maturity. The shoot meristem of cereals, which is the site of temperature perception, lies below the soil surface for a considerable amount of time (Russell, 1988). Thus, it is likely in the case of cereals that limited or stunted growth during the early growth phases is related to unfavourable soil temperature.

Tolerance of supra-optimal temperatures by seeds is also an important aspect of weed biology. Solar heating of soil using transparent polythene mulch has been developed as an effective method for disinfecting soil with plant pathogens (Katan, 1981). Earlier research also suggested that a similar approach of solarisation of the soil effectively controlled various annual weeds (Jacobsohn et al. 1980). Natural heating of seeds to temperatures above optimum for germination can result in a reduced germination rate. The effect may also interact with seed size, as species with larger seeds can emerge from deeper depths, and this may serve as an escape mechanism when natural or artificial soil heating

occurs. However, the emerging seedlings are still vulnerable to higher soil temperatures (Rubin & Benjamin, 1984).

Determining the base and optimum temperatures facilitating germination and growth for species of economic importance, and how temperature affects physiological processes in such species, have been the main focus of research conducted to date (Meyers et al. 1984; Garcia-Huidobro et al. 1982). For example, Porter & Gawith (1999) have quoted in their review that temperatures beyond a tolerable threshold can have significant consequences on the growth process of plant, and both high and low temperature can slow the biomass accumulation of plants. There is much yet to be known about how individual species respond to fluctuating or changing temperatures. With global climate change, it is increasingly important to know how species will respond to increasing temperatures, how these responses will vary from region to region, and how they will interact with other factors associated with climate change, such as elevated atmospheric CO₂ partial pressure [p(CO₂)] and changes in soil water availability.

This century is predicted to see a rise in global mean temperature ranging from 1.5 to 4.5°C, with local variations (Houghton et al. 1992; NIWA, 2008). It is likely that global change will modify p(CO₂) and temperature in a range where significant responses by plants can be expected. Such changes will alter processes like plant carbon assimilation, growth, biomass allocation and nutrient uptake (Veteli et al. 2002). Studying plant responses to global changes of the past can provide valuable information for predicting future responses to a rapidly changing environment due to anthropogenic effects (Edwards et al. 2007; Jackson, 2007). Recent studies on C₃/C₄ vegetation shifts during glacial change on the Chinese Loess Plateau suggested that C₄ plants were dominant over C₃ plants when average growing season temperature was more than 13-15°C and p(CO₂) was lower (Zhang et al. 2003).

Under current CO₂ conditions, the C₄ photosynthetic pathway allows plants to grow in conditions that are comparatively unfavourable for the growth of C₃ plants (Schulze et al. 1996). Therefore, modern C₄ plants are generally found in hot and dry environments where they have potential to dominate over C₃ plants under increasing temperature in regions where CO₂ level are also tolerable for

them (Zhang et al. 2003). Including broom corn millet, a large proportion of weeds are C₄ plants (Patterson, 1995), making them important species to be studied in a changing climate. Recent studies have shown that the growth of many C₄ plants responds positively to elevated p(CO₂) even under well watered conditions, contrary to previous observations (Ghannoum et al. 2000). It has also been suggested that the expected growth stimulation of plants in doubled p(CO₂) from current ambient p(CO₂) will also benefit C₄ plants. In addition, it is estimated that the average growth stimulation will be 22-33% for C₄ plants and 40-44% for C₃ plants, and that growth stimulation of C₄ plants will increase with decreasing soil water availability. One important prediction that can be derived from this review is the advantage that may be gained by C₄ weeds compared to C₄ crops in an altered climate, which could have significant impact on arable farming (Ghannoum et al. 2000).

Seeds of each plant species have a temperature range including minimum and maximum temperature at which germination is possible. This temperature range is considered a characteristic of the species (Bewley & Black, 1994). However, this temperature range can vary with geographic origin in some species (Probert, 2000). In addition, efforts to control weeds are mainly concerned with the threshold temperature for germination, seedling or early growth stages (Wiese & Binning, 1987). Therefore, the experiments described in this chapter are aimed to determine the effect of temperature on germination and early growth of the broom corn millet ecotype found in New Zealand. The origin of this biotype and its temperature threshold for germination and early growth, are unknown. The results can be used in models for farmers that predict the best time of the season to plant crops to reduce the impact of competition by broom corn millet. Since broom corn millet is a C₄ grass, its possible spread in range and growth in relation to climate change scenarios will be discussed.

2.2 Methods and materials

Two separate experiments were carried out, the first at AgResearch, Ruakura Research Centre and the other at the Massey University, Palmerston North.

2.2.1 Effect of soil (substrate) temperature on germination and growth

The experiment was commenced on the 23rd of December, 2009 and continued until 12th of February, 2010. Four temperatures $10 \pm 0.5^{\circ}\text{C}$, $15 \pm 0.5^{\circ}\text{C}$, $20 \pm 0.5^{\circ}\text{C}$ and $25 \pm 0.5^{\circ}\text{C}$ (here after discussed as 10°C , 15°C , 20°C and 25°C) were chosen in this experiment to observe the effect of soil (substrate) temperature on germination and early growth of broom corn millet. The four desired temperatures were achieved using custom made water baths equipped with both heating and cooling mechanisms, installed in a glasshouse.



Figure 2.1 The experimental setup. Broom corn millet plants were grown with substrate temperature controlled by floating pots in temperature controlled water baths.

Plants were grown in $160 \times 160 \times 190$ mm pots filled with potting mix (Daltons, Table 2.1) to provide plants with enough nutrients during growth. The potting mix was compressed to provide soil like conditions that also aids in holding moisture. Nine seeds were planted per pot in three rows of three. Seeds were planted at a depth of approximately 5 cm, a depth from which broom corn millet is known to

germinate and emerge successfully. The average distance between two seeds was 55-60 mm. Four replicate pots were allocated to each temperature regime.

Pots planted with broom corn millet seeds were kept afloat in the water baths with the help of stands (Figure 2.1). Pots were fixed in those stands in a manner that bottom of the pot was always immersed in water with the desired temperature. The pots were kept apart from each other to reduce competition for light after seedling emergence and growth. Gaps on the stands as well as exposed areas of the water surface were covered using polystyrene sheets to maintain the desired soil temperature and to prevent it fluctuating with air temperature (Figure 2.1). Since this experiment was carried out in midsummer, the pots were watered frequently to provide enough water to the plants and to assist in maintaining even soil temperature within the pots (by keeping the soil moist). The temperature of the moistened potting mix was checked at regular intervals using a 'Checktemp 1' temperature probe.

Table 2.1 Ingredients and their respective percentage in Dalton's potting mix.

Ingredient	Percentage
C.A.N fines	50
Coco fibre	15
Fibre	15
Pumice 7mm	20
Total	100

Supplemental lighting (400W Na vapour lamps) was used during early morning to provide a photoperiod of 16 hours for the 50 day duration of the experiment, with the light sources fixed above the water baths.

2.2.1.1 Data collection

Each seedling that emerged in any temperature regime was noted along with day of seedling emergence. For each emerging seedling the day of third and fifth leaf emergence was noted and heights of seedlings were measured. Observation was also made of the day when seedlings produced a tiller. All shoots from two seedlings were harvested randomly from each temperature regime when a tiller

was produced. However, seedlings were harvested only when enough seedlings had emerged for further observation of growth in the respective temperature regime. Harvested seedlings were subsequently dried in a heated glasshouse for 96 hours and dry weight recorded. At the end of the 50 days of this experiment, heights of individual plants was recorded and shoots of all plants were harvested by cutting from their roots, and dry weight recorded as described above.

2.2.1.2 Data analysis

One-way ANOVA analyses were performed for retrieved data, with temperature as the treatment factor, and total dry biomass produced per pot and average biomass produced per pot as response variables (Minitab[®], 2006; version, 15.1.0.0).

2.2.1 Laboratory determination of optimum germination temperature

To determine the threshold, optimum and maximum temperature for germination for broom corn millet seeds, a Temperature Gradient Plate (Grant Instruments Limited, Cambridge, United Kingdom) was used. The temperature range was set as minimum 5°C to maximum 34°C and checked regularly with a calibrated thermometer. A single layer of water soaked K-22 Kimpak (Anchor Paper Company, St. Paul, Minnesota) was placed on the plate and two layers of steel blue germination blotters also soaked in water were placed on top. Placed in the laboratory, the plate received natural light.

Same seed lot as in Experiment 1 was used in this experiment. Four replicates containing 45 seeds each were placed at even increments up the plate with each increment representing a 1°C change in temperature. Seeds were left on plate for 64-66 days and after that the plate was turned off and the remaining seeds were allowed to germinate at room temperature to assess viability. Radicle emergence was scored as germination and germinated seeds were left on the plate for assessing normal development of seedlings. A seedling was considered as normal when it exhibited a healthy primary root and a coleoptile free of splits at the base (ISTA, 2003).

2.2.2.1 Data analysis

Percent germination and time to reach 50% germination were not distributed normally. A non-parametric Kruskal-Wallis test was therefore used to test for the effect of temperature on these two variables (SAS, version 9.2, SAS Institute Inc., Cary, USA). Regression analysis of the rate of germination (expressed as $1/T_{50}$, where T_{50} is time to reach 50% in hours) against germination temperature was conducted as described by Coolbear et al. (1984) using Prog Reg in SAS.

2.3 Results

2.3.1 Effect of soil temperature on biomass

Among all the temperature regimes, the most delayed seedling emergence was observed for the lowest temperature of 10°C, with the first seedling emerging on day 9 after planting. In comparison, increased temperatures resulted in earlier germination and seedling emergence (Table 2.2). Seedling emergence was sporadic with seeds germinating throughout the 50 days of the experiment. However, the majority of seedlings emerged within 20 days of planting (Table 2.3). Germination percentage also generally increased with elevated temperature (Figure 2.2). At the lowest temperature (10°C) total percentage germination in a single pot never exceeded 33% (3 seedlings/pot), whereas, at the highest temperature (25°C) up to 100% of seedlings emerged in a single pot (data not shown). Total above ground biomass produced per pot also increased with each elevation in temperature (Figure 2.4), although this trend was not statistically significant ($F = 1.44$, $p = 0.28$), due to high levels of variation in germination rate and individual plant size between pots. Most of the increase in biomass production with substrate temperature was the consequence of increased germination and seedlings per pot, rather than increases in individual plant biomass ($F = 2.24$, $p = 0.14$).

Table 2.2 Day of first seedling emergence for broom corn millet at each temperature regime along with average numbers of tillers and final height.

Temperature (°C)	Day of first seedling emergence	Average numbers of tillers observed/plant	Average height (cm)
10	9	6	64.6
15	8	5	75.2
20	6	4	87.0
25	4	4	76.2

Table 2.3 Cumulative numbers of broom corn millet germinants at 10 day intervals for four substrate temperatures, from 36 seeds planted.

Temperature (°C)	Germination after day-10	Germination after day-20	Germination after day-30	Germination after day-40	Germination after day-50
10	1	5	7	8	8
15	5	7	7	7	7
20	16	17	17	17	18
25	16	25	27	27	27

The growth of seedling at the lowest temperature (10°C) was more horizontal than vertical with comparatively more tillers produced compared to the other temperatures (Table 2.2). Generally, plants grew more in height with increasing temperatures and the highest individual plant height was observed at 20°C. Average plant height was also highest at 20°C (Table 2.2). However, more plants attained heights above 100 cm at 25°C. Except for 10°C, at all the temperatures plants produced seed heads and the plants that produced seed heads reached a height near to or more than 1 m. While no quantitative data were recorded for root growth, it was observed that in the 20°C and 25°C treatments the broom corn millet plants developed much more extensive root systems, making them more difficult to uproot from the substrate (Figure 2.3).

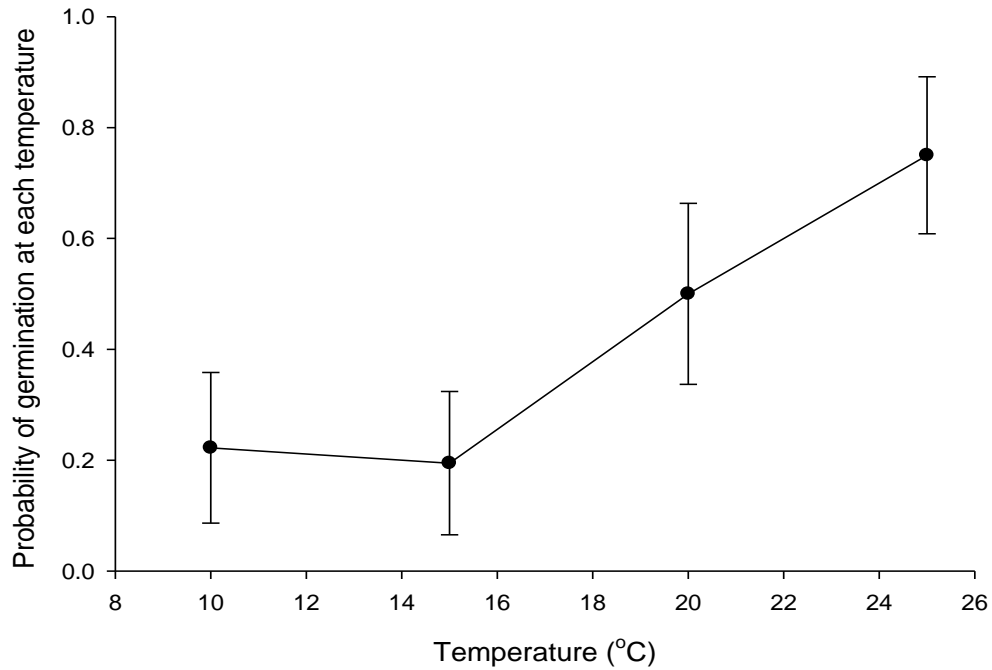


Figure 2.2 Probability of germination for broom corn millet plants grown at four substrate temperatures (n = 36 seeds) (error bars indicate the 95% confidence interval of the germination percentage based on a normal approximation to the binomial distribution).



Figure 2.3 Extensive root systems were developed by broom corn millet plants grown at temperatures $\geq 20^{\circ}\text{C}$.

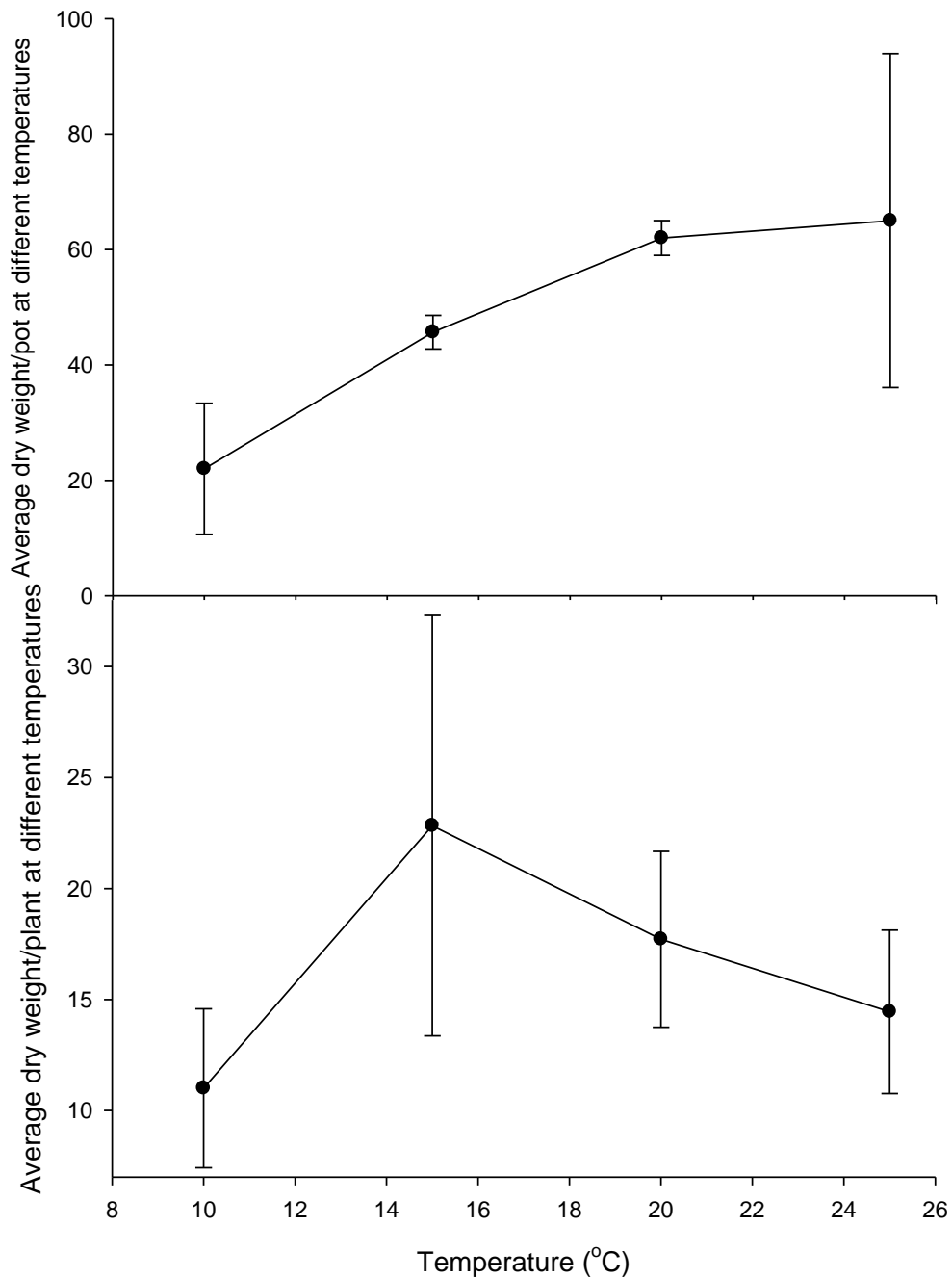


Figure 2.4 Average above ground dry weight produced by broom corn millet plants at each temperature regime, error bars = 1 SEM.

2.3.2 Influence of temperature on germination

The seed lot used in the temperature gradient plate experiment had high viability and germination rates. 86% germinated successfully, and a squash test revealed that of the 14% that did not germinate, 11% were viable.

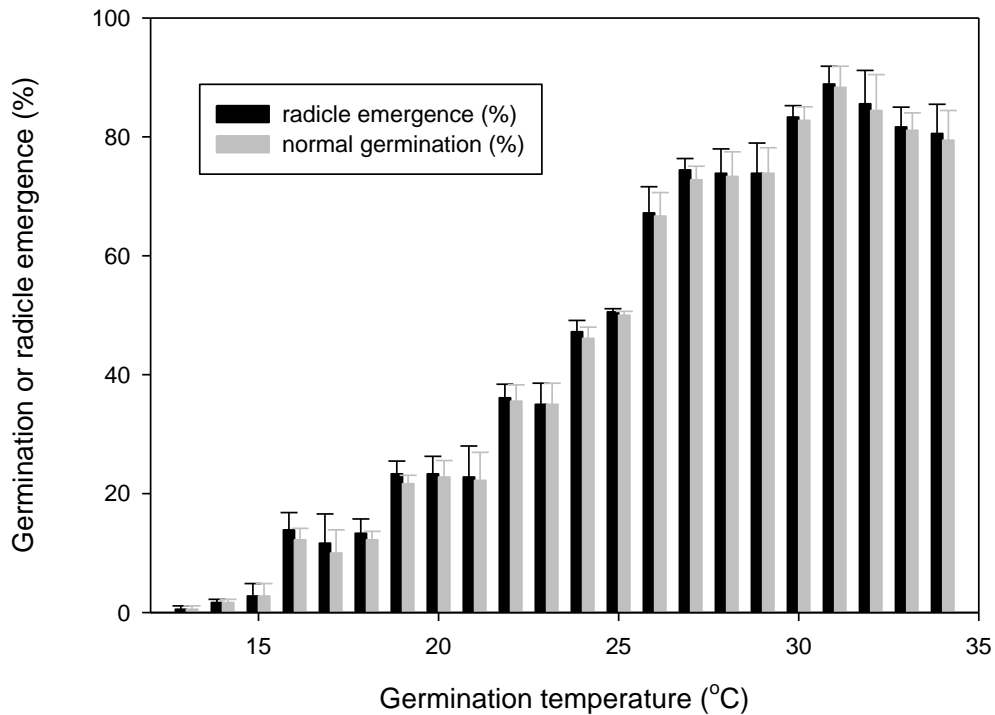


Figure 2.5 Radicle emergence (%) or normal germination (%) of *Panicum miliaceum* seed after 64-66 days at different germination temperatures (error bars = 1 SEM).

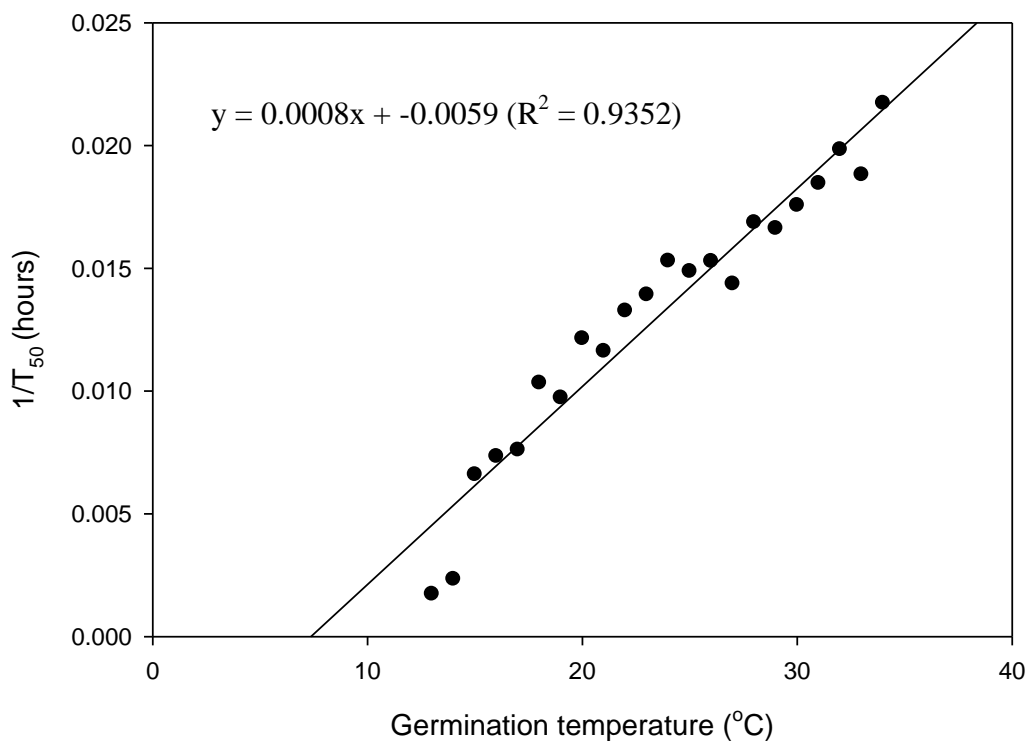


Figure 2.6 Rate of germination, expressed as $1/T_{50}$, the time to 50% germination as a function of temperature on the temperature gradient plate.

On the gradient plate, seeds germinated at temperatures from 13°C to 34°C, the highest temperature tested (Fig. 2.5). Of the seeds that germinated, 88% were considered normal. The highest percentage of germination was observed at 31°C. However, there was no significant difference in germination percentage between 27°C and 34°C ($P < 0.0001$ for normal germination at all respective temperatures) as revealed by regression analysis. Germination percentage decreased with decreasing temperature. Although seeds were kept on the temperature gradient plate for 64-66 days in this experiment, germination at any given temperature has ceased after 5-7 days. The rate of germination increased with temperature (Figure 2.6), with the lowest time taken to reach 50% germination occurring at 34°C. Regression analysis of the inverse of time to 50% germination against germination temperature suggested 7.4°C to be the threshold temperature for broom corn millet germination, even though no seeds germinated between 5 and 13°C (McGill, 2009, unpublished data).

2.4 Discussion

It was clearly evident from both experiments that increasing temperature was favourable for broom corn millet in both germination and vegetative growth. Accumulation of biomass during vegetative growth increased with increasing temperature. Similarly, germination rate was also positively related to temperature in the same experiment. These observations from the experiment simulating the soil environment were also supported by laboratory determination of the optimum germination temperature. In this experiment seeds of broom corn millet showed significantly increased germination percentage and faster germination at temperatures above 20°C. At the lowest temperature (10°C) tested in the experiment simulating soil conditions, seedlings attained less height and produced more tillers, resulting in prominent horizontal growth.

Provided the same growth conditions one out of four replicates at 15°C in glasshouse experiment had no germination. The reason behind this is not clear since it was difficult to retrieve the seeds after the experiment due to their morphology and resemblance to components of the potting mix. Although estimates of germination percentage at this temperature were reduced, the positive

effect of increasing temperature was clearly evident in the biomass accumulation at this temperature.

The highest two temperatures included in this study demonstrated the typical characteristics of a C₄ species with optimum growth occurring at 20°C or higher (Ghannoum et al. 2000). Only final biomass at respective temperature regime was taken into account to observe the combined effect of temperature and germination on final biomass, as it will relate to competition by broom corn millet in the field. Biomass accumulated by broom corn millet at 20°C and 25°C was two fold greater than biomass of plants produced at 15°C. This can be readily explained by the greater numbers of seedlings emerged at temperatures higher than 15°C. However, growth rate of plants at higher temperatures was also higher compared to temperatures lower than 20°C. There was no significant difference in accumulated biomass by seedlings growing at 20°C and 25°C. However, if the seedlings had not been harvested at the tillering stage to measure the accumulated biomass at 25°C, presumably final biomass would have been significantly higher.

Considering that all the plants were growing in similar ambient air temperatures, a negative correlation between individual plant biomass and temperature suggested that the substrate temperature only affected shoot biomass through its effect on seed germination. However, effects of substrate temperatures on shoot morphology were evident as seedlings grew faster and in a more upright direction at higher substrate temperatures. This indicates that broom corn millet plants exhibit their competitive traits at higher temperatures. In addition, competition could also have occurred between the seedlings, at higher temperatures due to comparatively higher seedling emergence. This could also be assumed as a responsible factor for upright growth of plants to compete for light.

It was also evident from the second experiment that higher temperatures, especially above 25°C, were favourable for germination of broom corn millet. Compared to the first experiment, seeds of broom corn millet showed an even higher optimum germination temperature. In the soil experiment 50% germination was attained at 20°C, where as in the gradient plate experiment 50% germination was achieved at 25°C. Taking into account that the same seed lot was used for

both the experiments, it can be concluded that seeds of broom corn millet do have temperature dependant dormancy. However, the differences observed for percentage germination at the same temperature in different experiments indicate that apart from temperature and moisture other factors may also influence germination in the field. Overall, it can be concluded, as expected, that low temperature does not give a growth advantage to the C₄ plant broom corn millet.

The threshold temperature for broom corn millet germination observed in this experiment was very close to that previously reported by Wiese & Binning (1987). Both of the results suggest that broom corn millet has a lower threshold temperature compared to other weeds of maize, such as redroot pigweed (*Amaranthus retroflexus*) (Guo & Al-Khatib, 2003; Wiese & Binning, 1987). This may give it a growth advantage over such species. On the contrary, fat hen (*Chenopodium album*); another weed of maize, is known to germinate at lower temperatures than broom corn millet (Wiese & Binning, 1987; Young et al. 1980). Therefore, this weed could compete with broom corn millet for resources. In comparison, the base temperature for the growth and germination of maize differs between germplasm lines, ranging from 6°C to 10°C (Stewart et al. 1998). However, as observed in this experiment, temperatures lower than 20°C are not favourable for broom corn millet. This indicates that lower temperatures early in the growing season may provide large seeded maize a growth advantage compared to broom corn millet.

Evidence suggests that various anthropogenic and natural activities have resulted in increased global average concentration of CO₂ in the atmosphere from 280 parts/million in 1750 to 380 parts/million in recent times, with half of this increase occurring in the last 30 years. The average temperature for New Zealand has already increased 0.9°C in the last 100 years (NIWA, 2008). Plants with C₃ photosynthetic pathway are favoured by higher CO₂ because of decreased oxygenase and increased carboxylation activity by RUBISCO (the primary enzyme involved in carbon fixation) (Percy et al. 1981; Tissue et al. 1995). On the other hand, C₄ plants are less affected by reduced CO₂ level in the environment (Dippery et al. 1995). However, a significant reduction has been observed in total biomass and leaf area of C₄ species at low temperatures (Ward et

al. 2008) suggesting a negative effect of low temperature on growth. This is because the light saturated rate of net photosynthesis decreases in C₄ plants at temperatures below 20°C (Long, 1983). It has also been demonstrated that C₄ plants growing at low temperature (<17°C) show limited photosynthetic activity due to reduced rubisco activity (Pittermann & Sage, 2002). The research conducted so far suggests that vegetative growth of C₃ plants should be favoured compared to C₄ plants with global increases in temperature and CO₂ level (Ziska, 2000). However, from the same aspect it is also assumed that C₄ plants will have a growth advantage in next few decades in the regions that have climates facilitating their growth (Ghannoum et al. 2000).

Most 'troublesome' weedy species are C₄ plants, whereas most major crops are C₃ plants (Patterson, 1995). Ziska (2000) argues that a decrease in weed competition from C₄ weeds to C₃ crops with increasing CO₂ cannot be considered a universal phenomenon. It has also to be taken into account that crop/weed interactions may vary with region, temperature, precipitation and other factors. The research so far suggests that C₃ crops will be favoured compared to C₄ weeds with increasing CO₂ while C₃ weeds will have a growth advantage over C₃ crops in the same conditions (Ziska, 2000). Therefore, considering the global climate change, it is essential to study the response of the economically important weeds to climate change. In that respect, broom corn millet (C₄ weed) will have advantage over C₄ crops in elevated CO₂ and temperature (Ghannoum et al. 2000), and regions currently not suitable for its growth may become suitable (Sage & Monson 1999; Kenny, 2001). It is likely that although both plants will benefit from increasing CO₂ and temperature in terms of growth, conditions such as an increase in warm days and long dry periods will provide broom corn millet with significant advantages over maize. It will have both growth and survival benefits as it is drought tolerant and can stand arid temperatures better (Baker, 2003).

The history of broom corn millet in New Zealand is short, with the first record in 1961 (James et al. 2010). It has been well known that new populations of organisms are usually founded by small number of individuals with limited genetic diversity (Schwaegerle & Schaal, 1979). The evolutionary significance of such a genetic bottleneck is known as the founder principal (Mayr, 1942). It has been observed that unusual allozyme frequencies can occur due to linkage

disequilibria in population of *Drosophila* founded by very small numbers of homozygous lines (Powell & Richmond, 1974; Jones & Yamazaki, 1974). Such disturbances in genotypes of organisms can lead to novel outcomes in population phenotypes (Schwaegerle & Schaal, 1979). Similarly, in the case of broom corn millet in New Zealand; it is likely that populations were started with very few individuals (James et al. 2010). Therefore, considering the founder effect, there may be novel traits developed by the populations found in New Zealand. It has already been reported in one of the experiments that contrary to overseas observations (Baker, 2003) extensive root system was developed by broom corn millet growing at temperatures $\geq 20^{\circ}\text{C}$. However, to be confirmed, this observation will require detailed study of the effect of temperature on root development.

2.5 Recommendations

If conditions and facilities were favourable, more replicates of each temperature regimes would have been tested. In addition, adding few more parameters such as leaf area index, seedling height at regular intervals and root biomass would aid significantly in assessing the effect of temperature on growth rate and phenology of the plants.

It is clear from these experiments that, being C_4 plant, broom corn millet's growth is aided with increasing temperature. In addition, current CO_2 levels and temperatures during warm seasons facilitate growth and establishment of broom corn millet. Hence, further research should be concentrated towards investigating the response of broom corn millet to temperatures higher than 25°C , which were not included in this experiment due to a lack of time as well as technical problems and restriction with the apparatus available.

A rise in temperature due to elevated CO_2 will have an effect on agricultural systems. Laboratory and field experiments simulating future climate scenarios should be conducted to predict effects on agriculture systems in New Zealand. Such investigations should include both C_3 and C_4 crops/weeds as much needs to be known about the effect of climate change on *in situ* agriculture systems where plants grow in complex relationships.

**Chapter- 3 Effect of burial depth on
emergence of broom corn millet in
various soil types**

3.1 Introduction

The recent trend towards controlled and minimum herbicide application as a way of reducing herbicide use and on going efforts to improve the crop yields has raised interest in weed biology and its place in management strategies (Bhowmik, 1997; Benvenuti et al. 2001). Knowledge of weed biology is important to develop efficient, economic and sustainable practices for weed control (Grundy et al. 2003) and for better agronomic practices (Forcella et al. 1993). It is known that, for weed control purposes, the most important characteristics are describing the emergence pattern of weeds, number of seedlings emerging and their time of emergence (Vleeshouwers, 1996). Thus, it is also essential to know the vertical position of weed seeds in soil and their potential to germinate and emerge as a seedling from given depth (Grundy et al. 1996).

It is evident that burial depth of seeds has influence on their ability to germinate and emerge, and this effect is site as well as species specific (Benvenuti et al. 2001, Grundy et al. 2003). Seeds at shallow depth experience much more moderate environment (e.g. moisture and temperature) compared to deeply buried seeds. This helps the germination and seedling survival of seeds at shallow depth in contrast to deeply buried seeds, where the germination is low and pre-emergence mortality rate is usually high due to lack of oxygen, light and/or temperature fluctuation (Seiwa et al. 2002). In the two stage process of seedling emergence, seeds must germinate successfully first and then it has to have adequate reserves to aid the seedling to reach the soil surface before it commences photosynthesis (Grundy et al. 1996). Hence, energy reserves are vital when considering seedling growth from the depth, since the germination and growth to surface rely heavily on the energy source available in seeds.

In adverse environmental conditions such as soil compaction, flooding or excessive burial, the seeds may avoid fatal (suicidal) germination until better conditions are available (Benvenuti et al. 2001). However, a major cause of seed bank depletion is premature germination at depths from which seedlings cannot emerge successfully (Fenner, 1985). However, seeds which do not germinate are always prone to either degradation or being consumed by soil biota, as well as

dying through normal aging (Buhler et al. 1998). Light, temperature, soil water content and degree of soil compaction represent the main factors limiting germination of buried seeds (Benvenuti et al. 2001). Seedlings of most weed species arise either from the seeds in the surface layer of soil, or just below (Cussans et al. 1996), although there are reports of many weed species such as Johnson grass (*Sorghum halepense*), velvetleaf (*Abutilon theophrasti*), mayweed (*Matricaria spp.*), fat-hen (*Chenopodium album*) etc., which are capable of emerging from greater depths (Benvenuti et al. 2001; Grundy et al. 1996). In an experiment conducted by Grundy et al. (2003) and in some other experiments, it was observed that weed species with larger seed size successfully germinated from the burial depth of 8 cm and deeper. One common observation from all these studies shows that percent emergence decreases with increasing soil depth. Burial depth could also possibly be inducing dormancy in seeds (Benvenuti et al. 2001), and ultimately inhibits the germination of seeds at great depth (Stoller & Wax, 1973). Genetic differences between and among the species are also proposed to be interfering with this phenomenon of emerging from different burial depths (Obeid et al. 1967).

Numerous studies have been carried out to observe the effect of vertical distribution of seeds on emergence of seedlings. Various factors such as soil aggregate size, light, temperature, soil water content, soil compaction, availability of gases, seed size etc. have been considered to find the most influential factors affecting germination and emergence (Chantre et al. 2008; Cussans et al. 1996; Seiwa et al. 2002; Benvenuti et al. 2001). Research conducted by Grundy et al. (2003) tested three hypotheses relating emergence capability of weed seeds to seed density, weight and shape. Their experiment with seeds of different species which varied in weight, shape and seed density concluded that relationship between seed size and shape and emergence is complex and possibly species specific. They also suggested that if seeds of the same species are scattered in a small area they can influence negatively their own germination. Further, they also added the possibility of interspecies competition between seeds of different species which may also affect germination. They further suggest including the parameter of pre-emergence seedling mortality while developing models to predict the emergence of seedlings from depths. However, soil nutritional

availability along with effect of soil type on seedling emergence seems to have been left out in such investigations. These parameters could also be as important as the others since weeds compete vigorously for nutrients and water and are proposed to be more efficient in utilizing them (Landcare Research, 2009). In addition, radical emergence as a result of germination forms the primary root which may utilise moisture and other elements from the soil prior to emergence.

Knowledge of the effect of depth on germination and seedling emergence also has practical implications for planning weed management, such as deeply burying seeds to avoid germination or shallowly burying seed to promote germination, such as in the 'stale seed bed' technique. In this chapter, the affect of burial depth on germination and emergence of *Panicum miliaceum* (broom corn millet) seedlings in different soil types from around New Zealand is examined. It is hypothesized that the relatively large seeds of broom corn millet, which have more nutritional reserves than the seeds of most other herbaceous weeds, enables them to germinate and emerge successfully from greater depths. The secondary objective of this experiment was to investigate the effect of different soil types and soil properties on germination seedling emergence.

3.2 Methods and materials

This emergence study was conducted using 16 different soils (Table 3.1). The soils used in this study are all cropping soils and were sampled from different locations in New Zealand. The soils were sourced from regions where broom corn millet is present as well as other cropping regions. The soil samples were selected and provided by FAR (Foundation of Arable Research).

On arrival, each soil sample was first checked for presence of broom corn millet seeds in them. Soil sub-samples were placed in a small pot and kept in the glass house for 15 days to check for the presence of any broom corn millet seeds by germination. Dry bulk density was measured for each soil samples using the standard protocol (McLaren & Cameron, 2002) and soil analysis was conducted in the laboratory to investigate the basic characteristics and elements of the soils (Table 3.2, Appendix 1). To measure the bulk density soils were dried in a glasshouse to remove most of the moisture (down to 5% to 10%). Subsequently,

soils were filled in petri dishes of the same volume and weighed. Three replicates were made for each soil. Petri dishes filled with soil were kept in an oven set at 35°C for 12 hours. Soils were weighed again and the ratio of the mass of dry soil to the total volume of soil was taken as the bulk density.

The soils that were found through seedling emergence to contain broomcorn millet seeds were autoclaved and subsequently kept in the open for five weeks. Autoclaved soils were moistened and mixed frequently to ameliorate the adverse effects of autoclaving (Boyd, 1971).

The Waikato and Gisborne soils were used in a pilot experiment with four replicates to evaluate suitable burial depths for emergence of broomcorn millet seeds. Both the soils were passed through a 4 mm sieve. In this experiment, the planting depth of seeds were 30 mm, 50 mm, 75 mm, 100 mm, 120 mm and 150 mm. Experimental set-ups were modified after the pilot trial was completed to incorporate an additional greater depths. To allow for this with the limited soil available, the two shallowest burial depths (30 mm and 50 mm) were eliminated and one deeper burial depth of 170 mm was used in the main experiment. The 170 mm burial depth was also subsequently tested for the two soils used in pilot experiment. The main experiment using the remaining 14 soils comprised only two replicates due to the limited amount of soil available as a result of incorporating the greater emergence depths that required significantly more soil than originally expected.

Poly vinyl chloride (PVC) pipe of 100 mm diameter was used in this experiment. The lengths of these pipes varied from 5 mm to 100 mm and were combined to give the desired depths (Figure 3.1). The bottom 30 mm tube section was filled with the test soil and 10 broom corn millet seeds were planted on top. Tubes to the desired depth were placed on top and again packed with respective soil. Care was taken to avoid placing the seeds within 10 mm of the outside edge. Soil was pressed into the tubes with a flat based still ram at a constant pressure to achieve normal compaction. The assembled tubes were then laid on the glasshouse bench in a randomised block layout. For the duration of this study temperature of the glasshouse was maintained between 15°C (night) to 25°C (day). All the tubes

were watered regularly and seedling emergences of undesired species were removed manually. Each soil set up was maintained for 40 days.

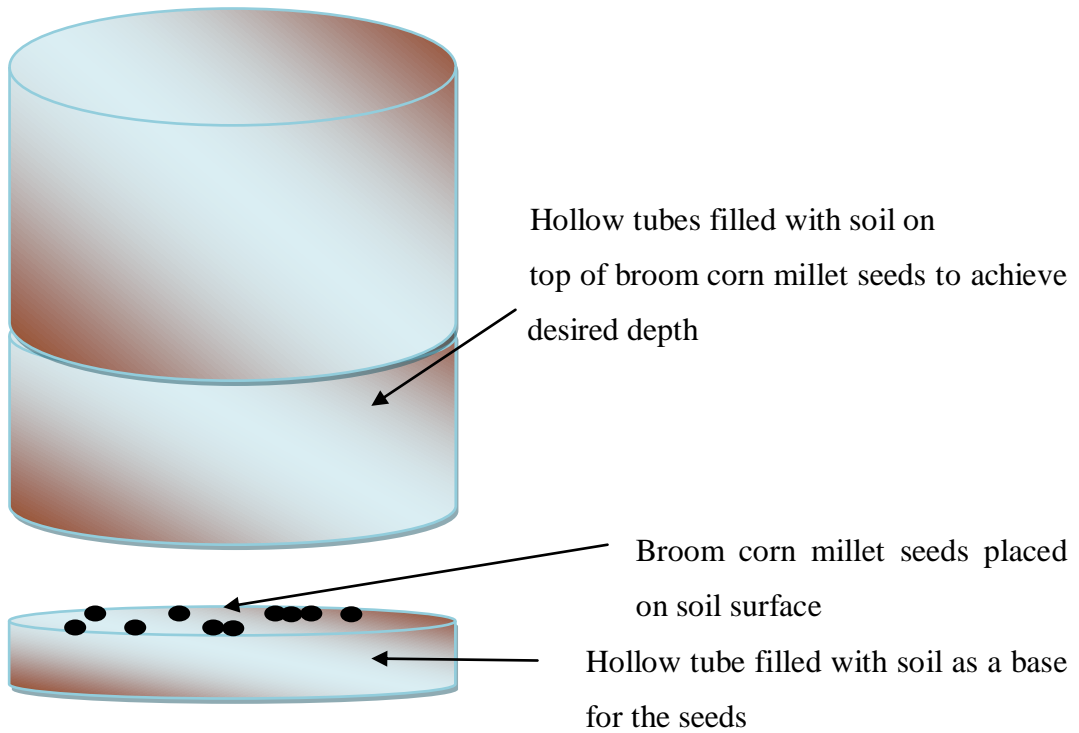


Figure 3.1 A typical experimental set-up to evaluate seedling emergence from different burial depths.

Table 3.1 Locations from where soils were sourced for this experiment and their respective soil type.

	Location	Soil Type
1	McLeod Road, Havelock North, Hawkes Bay	Meeanee silt loam
2	Fleming paddock, Manawatu	Carnarvon black sandy loam
3	Brookfield Rd. between King and Hale Road, Napier, Hawkes Bay	Farndon silt loam over sand
4	Lawn Road, Hastings, Hawkes Bay	Te Mata Mangateretere silt loam on clay
5	Valley Road, Te tua station, Hawkes Bay	Havelock sandy loam
6	Stock Road, Hastings, Hawkes Bay	Pakipaki ash over taupo pumice lapilli
7	Corner Brookfield and Gilbertson Roads, Pakipaki, Hawkes Bay	Moteo silt loam/clay loam over old top soil
8	Maraekakaho Road, Washpool Stn. Hastings, Hawkes Bay	Ngatarawa sandy loam over gravel
9	Fleming paddock, Manawatu, Hawkes Bay	Kairanga fine sandy loam
10	Wenley Road, Hastings, Hawkes bay	Havelock sandy Loam
11	New Plymouth, Taranaki	- ¹
12	Wanganui, Manawatu	Marton clay loam
13	Sedon, Marlborough	- ¹
14	Otorohanga, Waikato	Otorohanga silt loam
15	Gisborne, Poverty Bay	Kaipaki silt loam
16	Peat, Waikato	Kaiti silt loam

¹ Soil type is not known

3.2.1 Data collection

The tubes were inspected daily for broom corn millet and each emergence along with the number of seedlings was recorded. All the set-ups were dismantled after 40 days and depth of emergence was confirmed. Suicidal (fatal) germinations were counted where seed had germinated but the seedling failed to reach the soil

surface. Retrieved non-germinated seeds were tested for viability using the seed crush method (Sawma & Mohler, 2002).

All non-germinated seeds were initially assessed visually under a dissecting microscope and badly deteriorated as well as empty seeds (as viewed through damaged seed coat) were removed and immediately classed as nonviable. Seeds that had damage associated with germination were also removed. The remaining seeds were crushed using the blunt end of a pair of forceps. Seeds were considered viable if the embryo was found to be white in colour and the flesh indicated presence of lipid (oil). Seeds were considered non viable if the endosperm was found to be dry with brown to black appearance.

3.2.2 Data analysis

The data were analysed using GenStat (Version 11.1.01575, 11th Edition for Windows, VSN International, Oxford). The proportion of seed germinated was analysed using the GLMM (Generalised Linear Mixed Model) procedure, with the depth as a fixed effect and site/soil and the interaction with depth as random effects (Schaal, 1991). Other random effects analysed for also included soil type and soil properties such as pH, bulk density, organic carbon, organic matter and soil elements Ca, P, K, Mg, Na and S(SO₄). Binomial stepwise regression was used to determine which of these site factors affected the proportion of seed that emerged from a particular depth.

3.3 Results

Basic characteristics of soils are given in Table 3.2. From the pilot experiment, it was evident that the majority of broom corn millet seeds were able to germinate and emerge from the shallow depths of 30 mm and 50 mm. It was found that 80-90% seedlings emerged successfully from these depths (Table 3.3).

In the main experiment with 16 soil types, seeds emerged from the shallowest depth of 75 mm in all but one soil type tested, while in all soil types seedlings emerged from the depths of 100 and 120 mm. However, seedling emergence from the two greatest burial depths (150 and 170 mm) was comparatively very low as seedlings emerged from only seven and six soil types respectively (Table 3.4).

Further, the proportion of the seedlings emerging from a given depth in each soil type differed considerably (Table 3.4). Generally, in a soil type where greater than 50% of the seedlings emerged from the 75 mm depth, seedlings also emerged from the greater depths. Overall the number of seedling emergences declined with increasing planting depth (Figure 3.2 and 3.4, Table 3.4). A few seedlings also died after emerging from the depths of 150 and 170 mm.

In the pilot experiment no suicidal germinations were observed from the planting depths 30 mm and 50 mm, suggesting that they did not restrict the emergence of broom corn millet. This was one of the reasons for excluding these depths from the main experiment. However, when emergences from greater depths were evaluated considerable numbers of suicidal germinations were observed (Figures 3.3 and 3.4) with increasing depth. Numbers of suicidal germination generally increased with deeper planting depth except the greatest depth investigated where fewer suicidal germination were observed. The numbers of non-viable and missing seeds also increased with increasing planting depth (Table 3.5).

Table 3.2 Basic characteristics of 16 soil types as revealed from various assays.

	Soil type	Organic matter (%)	pH	Bulk density (g/cm⁻³)
1	Meeanee silt loam	4.4	6.9	0.83
2	Carnarvon black sandy loam	9.6	5.1	0.86
3	Farndon silt loam over sand	3.9	5.6	0.93
4	Te Mata Mangateretere silt loam on clay	6.0	5.7	0.77
5	Valley Road, Havelock sandy loam	8.6	7.0	0.82
6	Pakipaki ash over taupo pumice lapilli	7.0	5.2	0.78
7	Moteo silt loam/clay loam over old top soil	5.0	5.9	0.83
8	Ngatarawa sandy loam over gravel	6.2	4.6	0.91
9	Kairanga fine sandy loam	3.7	5.3	0.84
10	Wenley Road, Havelock sandy Loam	⁻¹	⁻¹	0.84
11	New Plymouth, Taranaki	8.1	6.1	0.78
12	Marton clay loam	6.8	5.9	0.78
13	Seddon, Marlborough	5.4	5.9	0.73
14	Otorohanga silt loam	10.8	6.0	0.85
15	Kaipaki silt loam	50.1	5.3	0.48
16	Kaiti silt loam	3.1	7.0	0.80

¹ Information not available

Table 3.3 Number of broom corn millet seedlings which emerged from the two shallowest planting depths in the pilot trial.

Soil type	Seedling emergence (40 seeds) from planting depth			
	30mm	% emergence	50mm	% emergence
Otorohanga silt loam	32	80	35	87.5
Kaipaki silt loam	31	77.5	36	90

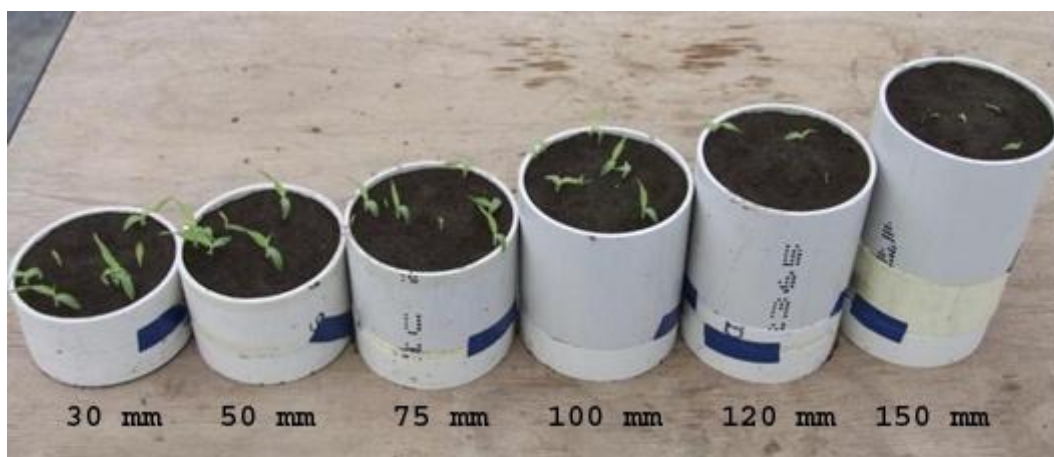


Figure 3.2 Broom corn millet seedlings emerged from different planting depths in Otorohanga silt loam soil during pilot study.

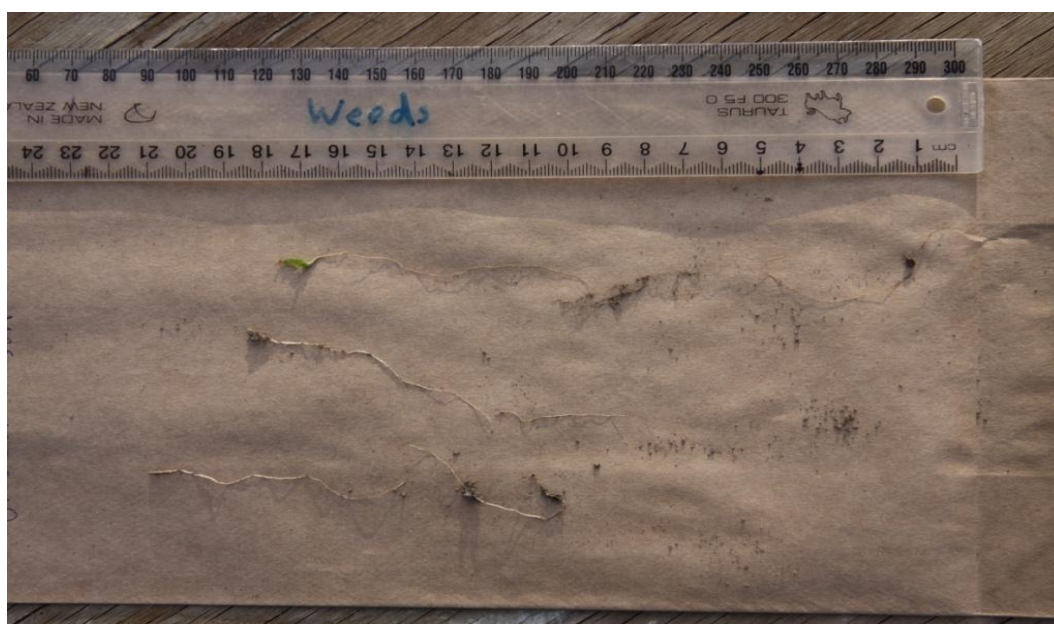


Figure 3.3 Suicidal (bottom seedling) and normal germination (top two seedlings) of broom corn millet seedling from the depth of 170 mm.

Table 3.4 Seedling emergence of broom corn millet (20 seeds) from 16 different soils.

Soil Type	Planting depth				
	75 mm	100 mm	120 mm	150 mm	170 mm
Meeanee silt loam	0	1	3	0	1
Carnarvon black sandy loam	10	9	2	1	0
Farndon silt loam over sand	5	3	2	0	0
Te Mata Mangateretere silt loam on clay	3	4	1	0	0
Valley Rd.-Havelock sandy loam	9	5	1	0	0
Pakipaki ash over taupo pumice lapilli	7	6	8	0	0
Moteo silt loam/clay loam over old top soil	11	8	3	1	0
Ngatarawa sandy loam over gravel	20	10	9	0	1
Kairanga fine sandy loam	3	2	1	0	0
Wenley Road- Havelock Sandy Loam	15	6	7	1	4
New Plymouth	7	8	4	6	0
Marton clay loam	7	8	7	1	0
Sedon (Marlborough)	10	6	5	0	0
Otorohanga silt loam	18	16	4	2	1
Kaipaki silt loam	1	1	4	0	3
Kaiti silt loam	13	17	7	1	1

Table 3.5 Fate of non-germinated broom corn millet seeds (total 320 seeds/depth) at various planting depths in the main experiment.

Seed planting depth	Viable seeds ¹	Non-viable seeds ²	Missing seeds
75 mm	22	15	81
100 mm	46	24	69
120 mm	59	37	85
150 mm	42	41	149
175 mm	42	56	172

¹ Viability evaluated by unimbibed seed crush test (Sawma & Mohler, 2002)

² Embryo brown to black in colour with dry appearance of endosperm

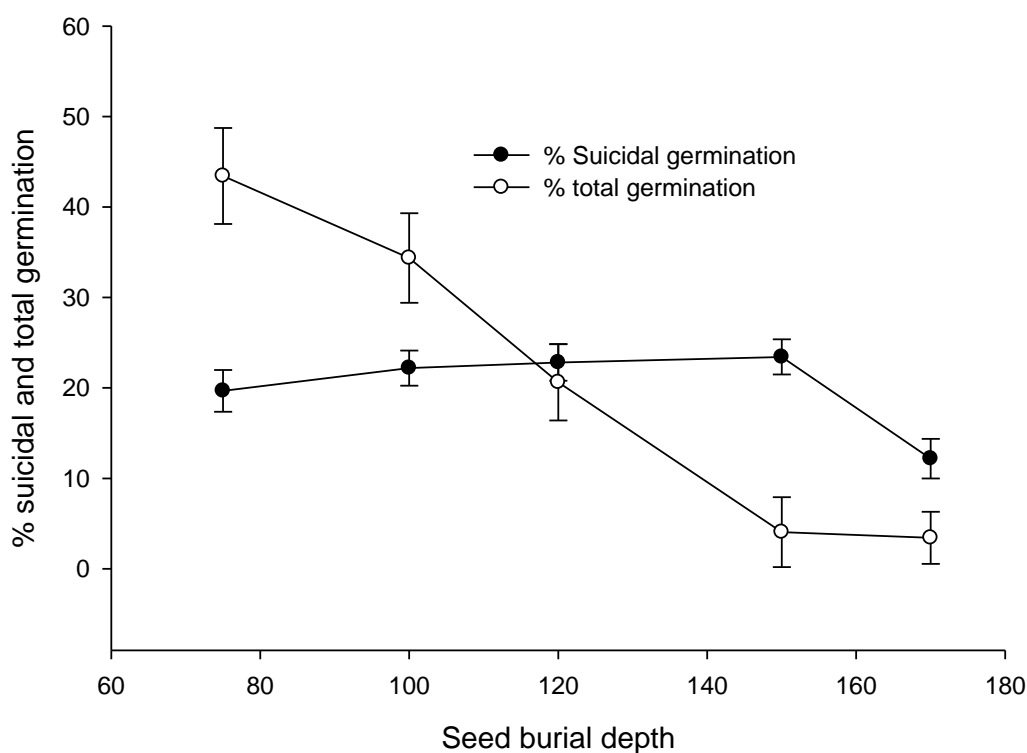


Figure 3.4 Percentage of suicidal and total germination observed at different planting depths, error bars = 1 SEM

Analysis by binomial stepwise regression using generalised linear mixed model, revealed that planting depth was the only factor to consistently impact emergence of broom corn millet. Soil type and other investigated factors such as soil texture, bulk density, pH, organic carbon, organic matter and other soil elements did not consistently affect emergence of broom corn millet.

3.4 Discussion

It was clearly evident from this study that with increasing planting depth both seed germination and seedling emergence was reduced for broom corn millet. Although few in number, seeds of broom corn millet were able to emerge and successfully establish seedlings from the greatest depth of 170 mm included in this experiment. The effect of soil type was evident as broom corn millet emerged from 170 mm depth only in 6/16 soils and from 150 mm in 7/16 soils. In an attempt to find an explanation for this difference the emergence data was regressed against several soil characteristics. However, no significant relationships were detected. Despite this it still appears that there is a soil type effect but the actual soil property or combination of properties that caused it could not be identified.

Irregularity in the time of seedling emergence was also observed in the different soil types. For example, seedling emergence from 75 mm depth was generally observed during the second week after planting. However, emergence of seedlings from this depth was also observed to start during the fifth week after planting in a few soil types. The proportion of seedlings emerging from each depth for each soil type also varied significantly. In some soil types seedlings emerged from greater depths but not from the previous shallower depth. In such cases, it was assumed that seedlings can also emerge from the shallow depths. In a few cases, the seedling response to greater planting depth was more positive than the shallow depth resulting in more seedling emergence from the greater depth compared to the shallow depth. The reason for this anomaly is not clear as for each soil type all the depth studies were carried out simultaneously in identical conditions.

Seedling emergence from various soil depths is known to be related with seed energy reserves (Lafond & Baker, 1986). A positive relation between seed size and emergence has already been reported for both crop and wild species (Bond et al. 1999; Benvenuti et al. 2001). Seed reserves are known to be crucial for seeds to allow activation of metabolism even under stressed O₂ conditions (Al-Ani et al. 1985). Seeds germinating at excessive depth usually have little chance to reach the soil surface as reserves may be exhausted during heterotrophic growth

resulting in suicidal germination. Therefore, in this respect, seed specific weight can significantly influence the seedling's ability to reach the surface. Hence, seed weight represents a valid parameter to study seed emergence, as seed weight generally reflects the proportion energy reserves available in the seed (Benvenuti et al. 2001).

Benvenuti et al. (2001) has demonstrated the role of seed size on emergence of seedlings from vertical distribution of seeds in arable soil conditions. In this experiment with 20 weed species, no seedlings emerged from planting depths greater than 100 mm. Thousand-seed weights of the 20 species ranged from 0.088 g to 11.41 g. It was observed that heavier seeds were able to produce seedlings from deeper depths than the smaller seeds. Benvenuti et al. (2001) also showed a possible correlation in seed weight and depth mediated emergence inhibition. In comparison, the thousand-seed weight of broom corn millet is 4.35 g, which is in the mid range of the seeds studied by Benvenuti et al. (2001). As reported here seeds of broom corn millet were able to emerge from the depths of more than 100 mm in all soils tested. This may have occurred due to comparatively low bulk density (< 1) of New Zealand soil types as shown in Table 3.2 (James et al. 2002).

The result observed here coincides with the previous observation for broom corn millet where seeds were able to emerge from the depth of 150 mm in Canada (Bough & Cavers, 2009). In addition, broom corn millet seeds also emerged from a greater depth (170 mm) than for any other seeds. A study was conducted to observe the effect of the vertical distribution of seeds on seedling emergence for other weed species in New Zealand soils (James et al. 2002). A conclusion from their research was that many weed species were able to emerge from the depth of 50 mm in New Zealand soils but not from 100 mm. The soils used by James et al. (2002) were very similar to the two Waikato soils (Table 3.2) used in the experiment reported here. It was also evident that outcome from overseas studies may not be applicable for New Zealand soils due to the prevailing lighter soil types.

A relationship between seed unit weight and seed longevity has also been proposed (Thompson et al. 1993). It has been hypothesized that variations in seed

unit weight also exist within the populations of species which could interfere in germination process and aid depth mediated dormancy in weed seeds (Milberg et al. 1996). This phenomenon will favour smaller buried weed seeds to stay dormant and maintain germination ability despite agronomic disturbances such as herbicide incorporation, tillage or harvesting, giving unfavourable situations for germination (Hodkinson et al. 1998). As discussed previously, this mechanism is essential for annual weeds to maintain sustainable populations.

A significant proportion of seeds remained ungerminated in this experiment which tended to increase with depth of planting. Not all seeds were successfully retrieved at the end of this experiment (Table 3.5). However, although low in number the retrieved ungerminated seeds were mostly viable as decided by the seed crushing test (Sawma & Mohler, 2002). It has been well documented previously that burial depth induces secondary dormancy in seeds (Benvenuti et al. 2001). The mechanism of dormancy imposition in seeds due to burial depth is believed to be an important survival strategy, allowing formation of a persistent seed bank formation (Burnside et al. 1996). In the case of broom corn millet it is a very important characteristic of seeds as broom corn millet is an annual species and large number of seeds are required in soil seed bank to ensure a persistent seed bank and a sustainable population.

Increased rate of decay has also been observed for deeply buried seeds due to senescence and soil microbial activity with the increased availability of moisture (Kremer, 1993). In this experiment, it was observed that with increasing planting depth the number of non-viable seeds and missing seeds increased (Table 3.5). Therefore, soil microbial activity could be responsible for some of the missing and non-viable seeds at the greater depths. As the span of this experiment was short (40 days), soil microbial activity may not account for all the missing or non-viable seeds, leaving the fate of some missing seeds unexplained.

Grundy et al. (2003) suggests in their study that when a large number of seeds of the same species are contained in the relatively small area, severe sibling disadvantage can result, leading to reduced germination and seedling emergence. In their study some species had significant density effect on their germination and

seedling emergence. Progeny could have identical requirement for resources and inbreeding at maturity may be encouraged (Willson & Traveset, 2000). Thus, it has been proposed that avoidance of germination at the same time may have been evolved to facilitate spatial dispersal of seeds and dormancy as a mechanism to distribute germination over time (Venable, 1989). Seeds of weed species *Parthenium hysterophorus* are known to contain organic inhibitors in sufficient quantities to inhibit germination when seeds are present in high densities (Picman & Picman, 1984). However, there is little evidence and knowledge on how this phenomenon of autotoxicity operates, except from the assumption that manipulating dormancy of seeds by this means could be one such inhibition mechanism (Hillhorst & Karssen, 2000). Weedy biotypes of broom corn millet are known to produce average seeds/plant in the range of 69,000 to 94,000 (Eberlein et al. 1990). Hence, it is likely that large numbers of seeds can be found at the foot of parent plant. Thus, phenomenon such as autotoxicity and dormancy could aid the species by reducing the germination at high seed densities where intraspecific competition existed for a limited resource. However, it is unlikely that such effects could have occurred here. The seed density of the sown seed was equivalent to about 1000/ m² and it has been observed in New Zealand that weed seeds can establish seedlings at much higher densities, such as 4000/ m² (Rahman and James, 1993).

It is hypothesized that in the field, in addition to exposure to mortality factors such as predators, microorganisms and the drying and wetting of seed bed, other factors may persist which prevents germination occurring at greater depths (Grundy et al. 2003). This effect was not evident in the present experiment as seeds of broom corn millet emerged from each burial depth, however, seedling emergence reduced with increasing depth. Numbers of suicidal germinations were also observed to reduce considerably at the 170 mm depth, suggesting that effect of increasing burial was perhaps inducing dormancy in seeds (Stoller & Wax, 1993). It has been established that seeds of broom corn millet have the ability to emerge from all the depths investigated. Therefore, observed suicidal germinations were perhaps the reflection of seed specific reserves. It has to be taken into account that with increasing depth, generally the time of seedling emergence also increased. Although the experiment ran for 40 days, pre-emerged

seedlings in transition to soil surface might have been considered as suicidal germination in the two deepest burial depths. However, the outcome from this experiment supports the previous research conducted on effect of burial depth of weedy biotypes in Canada (Bough & Cavers, 2009). In addition, it was revealed from this research that seedlings of broom corn millet can emerge from depths greater than generally reported (Bough & Cavers, 2009).

The result obtained from this study has important implications for management practices to control or reduced the impact of broom corn millet. It was observed in the experiment that seeds of broom corn millet can emerge from ≤ 120 mm burial depths regardless of soil type. Thus, continuous monitoring and available control measures are required to be carried out in early growing season. Tilling the soil prior to sowing can also push seeds from seed bank towards soil surface and will facilitate conditions for germination and growth. This suggests that significant measures to control the weeds will be required in infested areas due to abundant seed availability in soil. Also, ploughing, a traditional method to reduce weed emergence by burying seeds at depth, buries the seeds only up to 150 mm deep (Rahman et al. 2000). Therefore, it will effectively bury broom corn millet seeds below a depth from which it can germinate and emerge and thus making the ploughing less effective.

Preparing a stale seed bed is mostly recommended for organic farming but it can be adopted to reduce the weed density and deplete the seed bank in conventional farming as well (Lamour & Lotz, 2007). Normally this technique works well for prolific seed producers and seed bank forming annual weeds. The technique is based on the early preparation of shallow 'seedbed' by disturbing upper soil layer and then removing the seedlings which emerge before sowing the crop seeds. Removal of the weed seedlings which emerge is usually performed mechanically (Kurstjens & Kropff, 2001) but they can also be killed with non-residual spray. The process can be aggressive and efficient due to absence of crop. Subsequent seedling emergence can again be removed and this process can be repeated several times following seed bed preparation and sowing if required. This will reduce the density of seeds in soil bank and ultimately result in less weed competition while crops are present (Lamour & Lotz, 2007). However, the

technique becomes less effective when there are delayed and prolonged emergences throughout the season from seeds buried at depth. In addition, it may not be practical to repeat the procedure as recommended to plant the crops at appropriate time. However, if we have delayed emergence, as in the case of deeply buried broom corn millet seeds, then the basis of stale seedbed technique collapses and this weed is not controlled by a single iteration of the stale seedbed technique.

In conclusion, it was shown that provided suitable conditions seeds of broom corn millet are able to emerge from ≤ 170 mm burial depths. However, in real agronomic scenario most seeds are likely to germinate only from shallower depths compared to extremities of depth investigated in this experiment. Further, with forced dormancy of the seed by deep burial then the prolific seed production of broom corn millet results in the formation of persistent seed bank which is capable of germination as soon as favourable conditions are available. In addition, the larger seed size and mass makes the factor of burial depth less inhibitory for broom corn millet seeds and allows them to germinate at deeper depths and delayed emergence. Both these facts creates problems with two common weed management technique viz. ploughing to bury seeds and the stale seed bed to promote germination and kill the resultant weed seedling.

3.5 Recommendations

Although, a wide range of burial depths were investigated in this study, it was not yet clear at what burial depth germination is completely inhibited and seedling emergence stops. The depths included in this study were decided on the base of existing knowledge of depth response to broom corn millet seeds in Canada (Bough & Cavers, 2009) and considering the unit weight of seeds. Therefore, further investigations are required to determine the threshold limit of burial on germination through both field and laboratory based trials as significant variation prevail in field and laboratory conditions (Grundy et al. 2003). The range of depths covered in this study required large volumes of soil for each soil type. Due to insufficient soil for many of the soils the experiment was not carried further with increasing depth and therefore maximum depth from which broom corn millet can emerge in New Zealand soils has not been yet been determined.

Soil physical properties except bulk density were also not known for soil types used in this experiment and it is known that soil characteristics do influence seedling emergence (Benvenuti et al. 2001). Extensive investigative study can be conducted relating parameters of soil physical properties and other factors to shed light on mechanism of depth of emergence in case of broom corn millet, as disparity in observations exist between soil types.

Considerable amount of suicidal germinations were observed with increasing depth at the end of this experiment. One explanation for this could be the duration of this experiment. Thus, in future studies duration of such experiments needs to be increased to provide adequate time for seedlings emerging from deeper burial depths.

**Chapter- 4 Comparative effect of
competition on broom corn millet and
sweet corn**

4.1 Introduction

Weed and crop competition is a complex phenomenon that is governed by various biological, environmental, and proximity factors, where proximity factors include plant density, species proportion, and spatial arrangement among individuals (Radosevich, 1987). As stated in chapter 1 of this thesis, a major concern with weeds is the reduction in crop yield and quality (Buchanan & Burns, 1970) due to competition among them for resources. Global crop losses due to weeds and other pests for the years 2001-2003 were estimated to be 50% in wheat (*Triticum spp.*) and more than 80% in cotton (*Gossipium spp.*) (Oerke, 2005). Therefore, the ability to predict the effect of weeds on yield loss is essential for an effective weed management programme which maximises yield and quality (Kropff & Spitters, 1991).

In general, crop yields reduce proportionately as weed populations increase (Knake & Slife, 1962). Weeds shorter than the crop usually compete more vigorously early in the growing season for resources close to the soil surface, while weeds taller than the crop usually reduce crop yield by competing primarily for light. Weeds emerging at the same time as crops are most competitive. However, competitiveness at the various stages of growth also depends upon the growth habit of the weed (Nelson & Nylund, 1962). Smith (1968) suggested from other researcher's work that 'the effect of weed competition varies among crops, crop varieties and weed species'. He also stated that competition from weeds is minimized with crops when there are: good crop stands, vigorous crop plants and adequate soil moisture coupled with sufficient nitrogen. A recent study by Ryan et al. (2009) also supports the observation. Their comparison, based on available data from conventional and organic trials, suggested that organic crops are better able to stand abundant weed competition compared to conventional crops. They observed that while growing at the same weed density, organic farming system produced the equivalent yield when soil fertility was managed. The observations above suggest that extensive research with a site specific approach is required while addressing the problem caused by weeds.

Factors such as relative time of emergence and density are important in predicting yield loss caused by weeds. For example, in sorghum, soybeans and corn, weeds that emerged significantly after the time of crop emergence did not cause any significant yield loss (Buchanan & Burns, 1970). Buchanan & Burns (1970) therefore suggest in their paper, quoting other's work, that relative time of emergence is important in predicting yield losses and managing weeds. It was also noticed that different crop species have different responses to a given weed free period and a species can also have different requirements for weed free periods at different times of the year (Buchanan & Burns, 1970). For example, cotton yield was drastically reduced when weed control was delayed for a prolonged period (Buchanan & Burns, 1970, Ramirez & Nieto, 1968). Maize yield can be reduced up to 10% if broom corn millet removal is delayed until two weeks after planting, and reduced up to 28% if removal is further delayed by 6 weeks (Wilson & Westra, 1991). James et al. (2000) suggested that weeds would not have an effect on final yield of maize (*Zea mays* L.) without pre-emergence herbicide treatment if they are controlled within 3 weeks of crop emergence. Further, Kropff (1988) indicated that a close relationship exists between yield loss and relative leaf cover of weeds and crops, using data from sugar beet (*Beta vulgaris*) vs. fathen (*Chenopodium album*) competition trials. They attempted to find a relationship between the leaf area of the weeds shortly after crop emergence and yield loss. It was shown that the weed's leaf area accounts for both the effect of weed density as well as the effect of length of time between crop and weed emergence.

Panicum miliaceum (broom corn millet) is an annual grass weed (Bough & Cavers, 2009). It is well known that annual weeds interfere with the normal growth processes of crop species (Buchanan & Burns, 1970). Row crops in general do not compete as well with broom corn millet as solid seeded crops because crops that are solid seeded shade the soil and young seedlings of broom corn millet more quickly (Harvey, 1979). As a result, seed production by broom corn millet was also much higher within row crops compared to solid seeded crops. O'Toole & Cavers (1983) found that broom corn millet produced 426 million seeds/ha in white beans (*Phaseolus vulgaris* L.) and 34 million seeds/ha in corn (*Zea mays* L.), compared to 1.5 million seeds/ha in barley (*Hordeum vulgare* L.). Commonly used herbicides in maize and sweet corn provide marginal to no

control for broom corn millet when applied alone. Thus, combinations of herbicides are used to control broom corn millet (Westra et al. 1990). However, it has been observed that control from these herbicide combinations may not be enough to restrict seed production and its eventual incorporation into the soil seed bank as broom corn millet germinates throughout the season (Harvey et al. 1986).

The competitive threshold of a crop has been defined as the weed density above which crop yield is reduced beyond an acceptable amount (Oliver, 1988). It has been shown for maize that weed density influences interactions between the weed and crop, and the level of eventual yield loss (Scholes et al. 1995; Fausey et al. 1997). In an experiment, densities of 10 and 20 plants m^{-2} of broom corn millet accounted for 13 and 19% yield loss respectively in corn at one site and 22 and 38% respectively at other (Wilson & Westra, 1991). A similar study by Wilson (1993) in dry beans (*Phaseolus vulgaris*) found that yield could be reduced by 12-31% when broom corn millet density was 10 plants m^{-2} . In addition, it was also recorded in the same study that yield was reduced up to 41% when removal of broom corn millet was delayed by 6 weeks.

Broom corn millet is presently an emerging threat to maize and sweet corn cultivation in New Zealand. Excluding environmental variables, weeds like broom corn millet are the main causes of yield losses in maize in New Zealand and everywhere else (Rajcan & Swanton, 2001). There is a general lack of information concerning plant density and time of emergence effects for this weed under New Zealand conditions because it is a relatively new introduction. This chapter describes the effect of competition between sweet corn and broom corn millet on early growth of both species. Plants often respond to presence or competition from neighbouring plants by accumulating more biomass in the shoots than in the roots (Kasperbauer & Karlen, 1994). As discussed before, in crop/weed competition, generally crop biomass and yield are reduced when the crop is grown in competition with weeds. Therefore, the hypothesis for this experiment was that biomass accumulation by maize would be reduced as broom corn millet density increased. We explore a wide range of ratios of crop to weed densities and attempt to identify the threshold density at which broom corn millet begins to affect aboveground maize biomass accumulation.

4.2 Methods and materials

Two separate experiments were conducted in an outdoor cage house to prevent possible damage by birds on seeds, to restrict the spread of shattered broom corn millet seeds and to provide a natural environment for growth. This nine week long study was started in second week of December, 2009 and finished in mid February, 2010. The timing of these experiments coincides with the normal time of sweet corn cropping in New Zealand.

Plants were grown in 340×340×280 mm tubs filled with potting mix (refer Table 2.1). Potting mix was used as a substrate to provide ample nutrient supply throughout the experiment. The potting mix was compressed to provide the conditions resembling soil.

The sweet corn seeds used in this experiment were TenderSweet gaucho treated variety from PGG Wrightson, Hamilton whereas broom corn millet seeds were sourced from corn growing region Gisborne in 2007-08.

Experiment 1: Seeds of broom corn millet and sweet corn were planted in a range of numerical ratios to observe the effect of degree of competition on dry matter production. Seeds of both the species were planted in excess and extra emerging seedlings were removed by hand to achieve the desired ratios of broom corn millet to sweet corn of 0:5, 1:4, 2:3, 3:2, 4:1 and 5:0. Seeds were sown in a quincunx spatial arrangement as shown in Figure 4.1. Within this pattern of distribution the allocation of sweet corn and broom corn millet was random. Each ratio was replicated six times. Plants were watered daily using sprinklers for the entire nine week duration of the experiment.

Experiment 2: Seeds of broom corn millet and sweet corn were planted in a monoculture with 14 replicates to observe the growth pattern without competition. Tubers were kept separate for both species. Seeds were planted in excess and extra seedlings were thinned as required. Soon after emergence only a single plant was kept in each tub to observe the growth rate with sufficient resources. Plants were watered using sprinklers on a daily basis and grown for eight weeks.

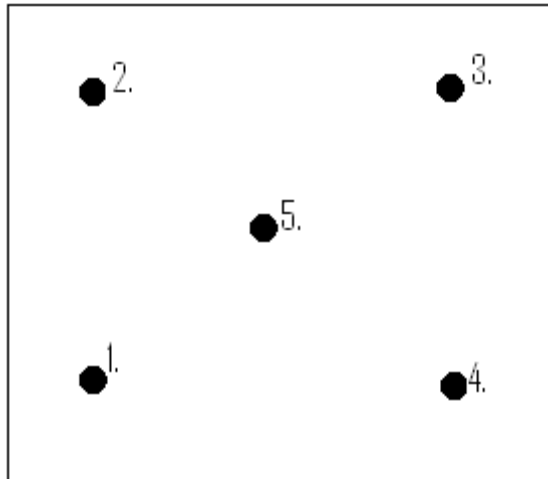


Figure 4.1 Quincunx pattern of seed distribution where each point was either of the two species, chosen randomly.

4.2.1 Data collection

Experiment 1: Three replicates of each competition regime were harvested after four weeks and the remaining replicates harvested after nine weeks. Plants were cut off at the soil surface and weighed to obtain green weight of individual plants. Subsequently, plants were then oven dried at 80°C for 48 hours and dry weight was recorded for individual plants.

Experiment 2: Commencing at two weeks after planting two replicates of broom corn millet and sweet corn were harvested each week for the next six weeks. Harvesting and data collection was performed in the same way as described above for plants grown in monoculture. Two replicates each of broom corn millet and sweet corn plant were harvested until the 8th week.

Climatic data were obtained from an automatic meteorological station located at the Ruakura Research Centre, 300 m from the experiment site, for the duration of these experiments (Station 26117, National Climate Database, National Institute of Water and Atmospheric Research Ltd, Hamilton, NZ). Temperature data were obtained for the months when the experiment was conducted as well as for the same months in the previous four years.

4.2.2 Data analysis

Data for average plant biomass from the competition experiment were analysed using a three way ANOVA, with species, planting ratio and harvest date as fixed effects.

4.3 Results

Experiment 1

Broom corn millet emerged 5 days later than sweet corn and its growth rate was lower for the duration of the experiment. By four weeks, the average aboveground dry biomass of the sweet corn plants was five times that of the broom corn millet plants, and both crop and weed had the highest per-plant biomass in the tubs with the intermediate planting ratios (Figure 4.2). Such a large difference in size was due to comparatively large size of sweet corn plants (Figure 4.3). By nine weeks the sweet corn plants were still four to five times heavier than the broom corn millet plants, although broom corn millet plants attained similar heights to the corn when they were grown together (Figures 4.2, 4.4). The effect of planting ratio differed from that at four weeks (significant ratio - week interaction, Table 4.1), with both sweet corn and broom corn millet biomass increasing with increasing ratios of broom corn millet to sweet corn (Figure 4.2). The same trends were observed when fresh biomass was considered, and there were not significant trends in shoot dry matter content with planting ratio on either date (Figure 4.2, Figure 4.5).

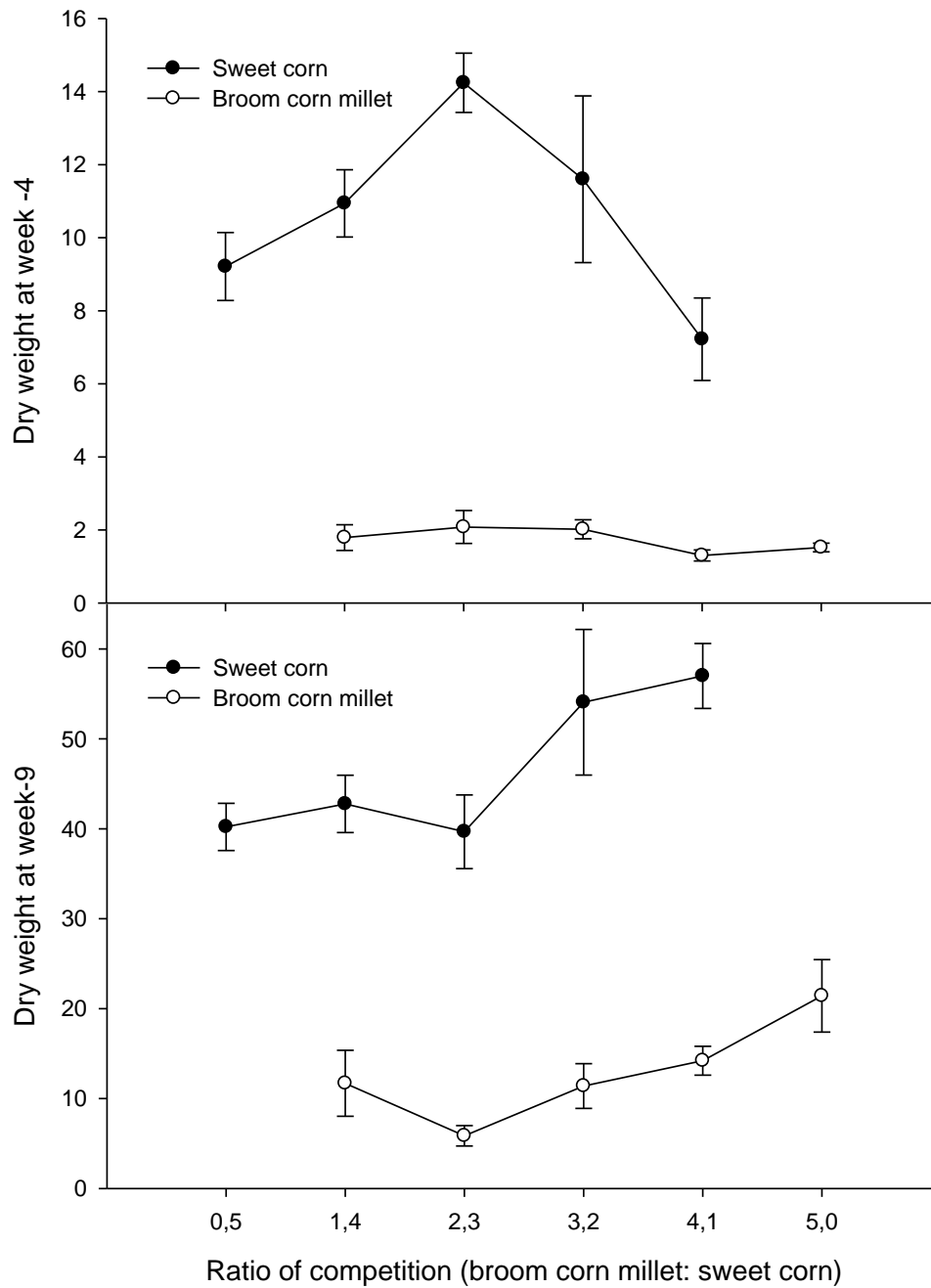


Figure 4.2 Dry weight of shoots accumulated by broom corn millet and sweet corn after 4 weeks and 9 weeks of growth, error bars = 1 SEM, n = 3.



Figure 4.3 Sweet corn and broom corn millet growing in competition at week 5. From left broom corn millet (0): sweet corn (5), followed by progressively increasing ratios of broom corn millet to sweet corn.



Figure 4.4 Broom corn millet attained fairly equal height while growing under competitive conditions.

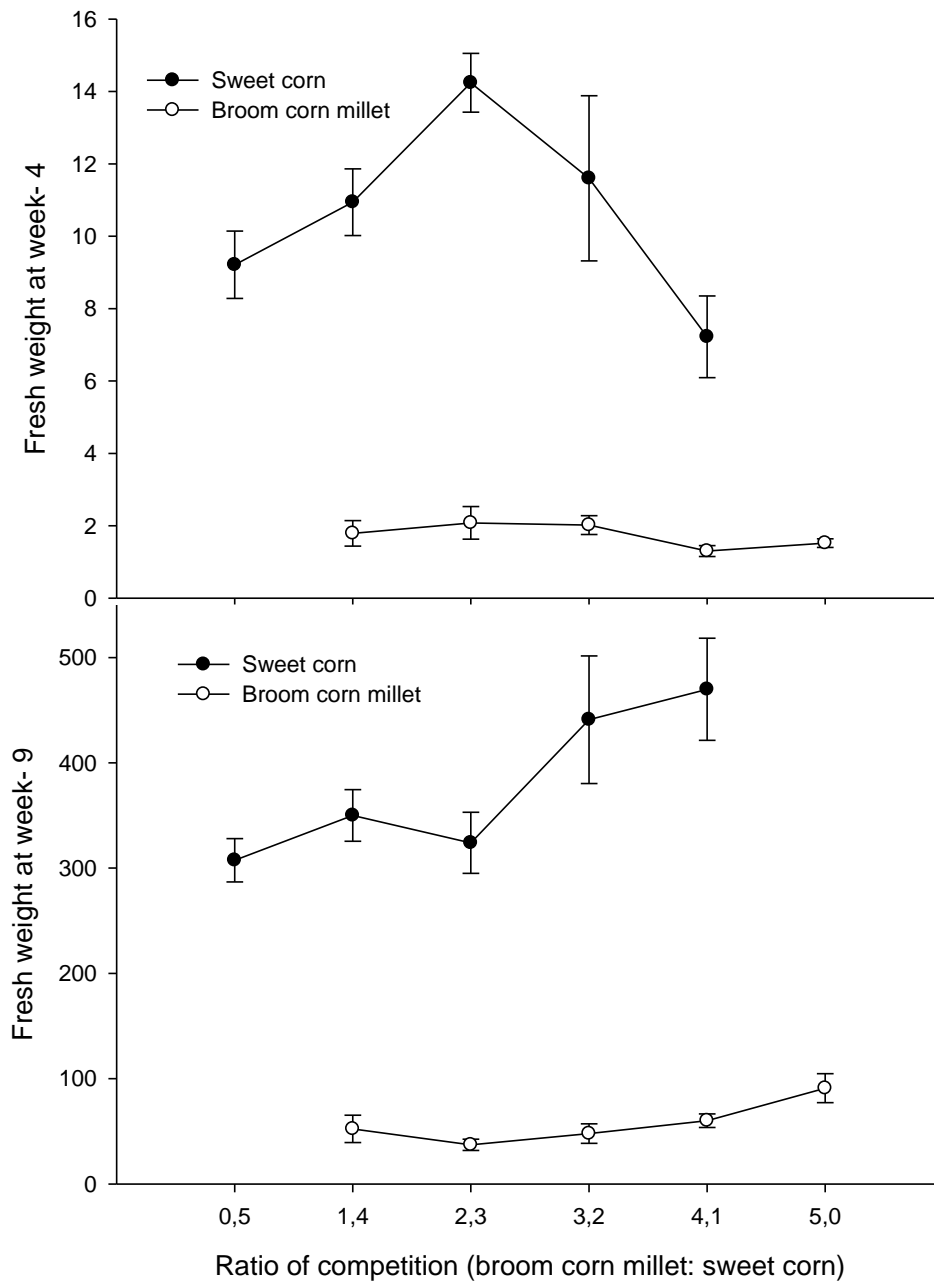


Figure 4.5 Fresh weight of shoots accumulated by broom corn millet and sweet corn after 4 weeks and 9 weeks of growth, error bars = 1 SEM, n = 3.

Table 4.1 Results of ANOVA analysis with dry biomass as the response variable.

Effect	Degrees of freedom	F	p
Species	1	165.1	<0.001
Week	1	489.9	<0.001
Ratio	4	2.6	0.05
Species-Week	1	151.5	<0.001
Species-Ratio	4	2.9	0.03
Week-Ratio	4	2.8	0.04
Species-Week-Ratio	4	2.9	0.04

Experiment 2

Above ground fresh and dry biomass produced by broom corn millet was very low until week 4 after planting. After week 4 broom corn millet biomass increased rapidly but varied greatly between individual plants (Figure 4.6).

Sweet corn also grew very slowly in first few weeks, but not as slowly as broom corn millet. After week 3, corn biomass increased steadily until the end of this experiment (Figure 4.6).

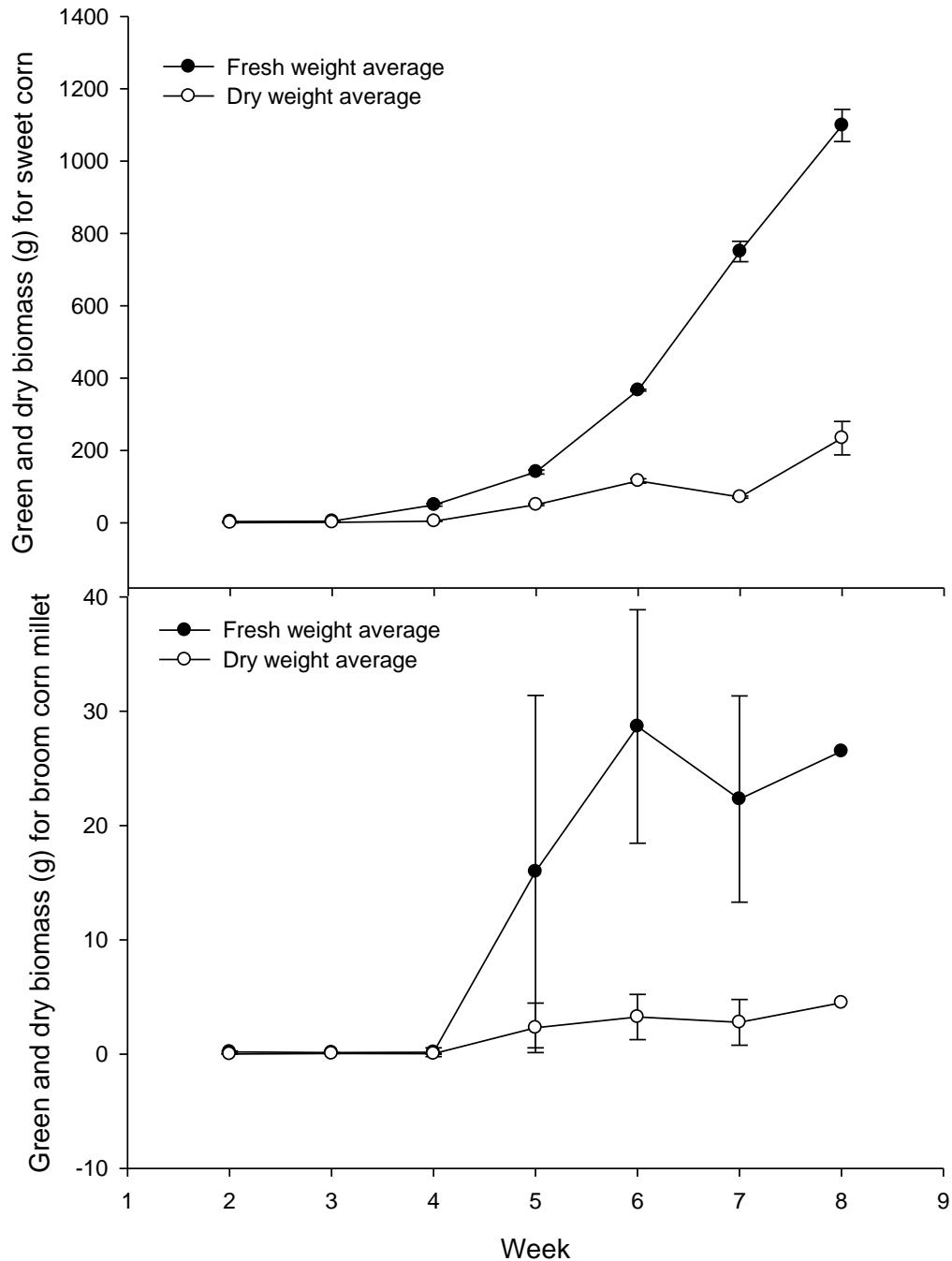


Figure 4.6 Fresh and dry shoot weight accumulated by broom corn millet grown in monoculture without competition, error bars = 1 SEM, n = 2. No error bar is presented for last data point due to insufficient seedlings for measurement.

Climatic data were extracted using CliFlo web service in the National Climate Database (CliDB) managed by New Zealand’s National Institute of Water and Atmospheric Studies (NIWA). Ambient air temperatures during December 2009 were comparatively lower than average compared to last 5 years (Table 4.2),

particularly during the two weeks after the seeds were sown for experiments 1 and 2. Nights during this period were particularly cool, with a significant number of days with minimum temperatures below 10°C (Figure 4.7). The average day time maximum was 0.5°C lower for December 2009, compared to December 2008 and 2007, whereas the average daytime minimum was at least 1.0°C lower than the previous two years. In contrast, January and February 2010 were not colder than normal. In addition, growing degree days for plants with 10°C baseline temperatures for growth were also lower compared to last two years (Figure 4.8).

Table 4.2 Meteorological observations for temperature during the period of this experiment and observations for the same period for the previous 4 years.

Month	Year	Mean Temp. (°C)	Mean Max.¹ Temp. (°C)	Mean Min.² Temp. (°C)
December	2009	16.1	21.5	10.8
	2008	16.9	22.0	11.8
	2007	17.4	22.6	12.3
	2006	14.4	19.7	9.0
	2005	18.2	22.9	13.5
January	2010	18.1	23.7	12.6
	2009	18.1	24.5	11.8
	2008	20.3	26.7	13.9
	2007	18.6	23.7	13.4
	2006	18.8	24.2	13.6
February	2010	19.9	24.7	15.0
	2009	19.5	24.9	14.1
	2008	19.1	24.9	13.3
	2007	18.6	24.6	12.5
	2006	_ ³	_ ³	_ ³

¹ Maximum

² Minimum

³ Data not available

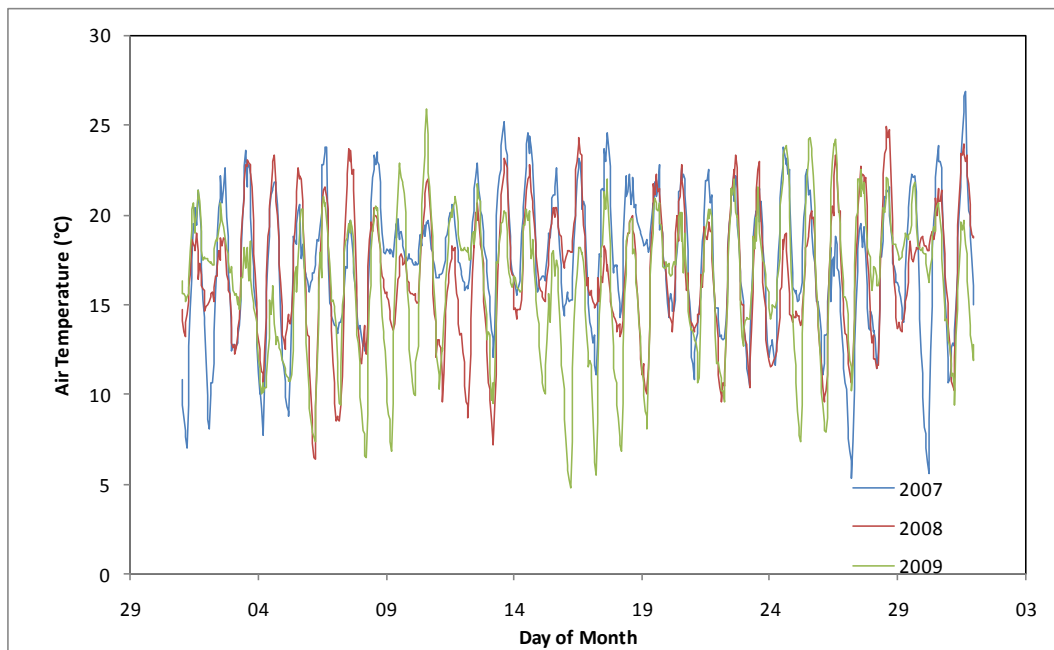


Figure 4.7 Comparison of hourly temperature for the month of December for last three years.

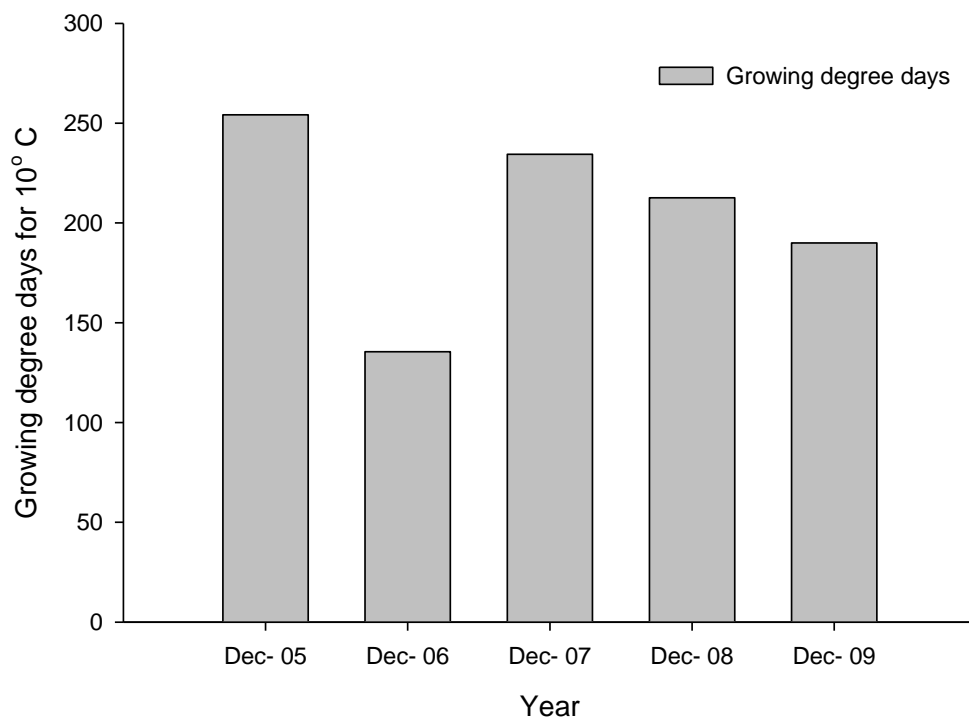


Figure 4.8 Growing degree days for last five years for plants with baseline temperature of 10°C for growth such as broom corn millet and maize.

4.4 Discussion

In this experiment, broom corn millet did not provide any significant competition to sweet corn, with corn biomass accumulation increasing when it was grown with higher proportions of broom corn millet. A higher ratio of broom corn millet did appear to reduce sweet corn growth in the first four weeks after germination, but this effect did not persist until the ninth week. In the monoculture experiment, which ran concurrently, broom corn millet grew slowly in the first few weeks, suggesting that it was unlikely to offer significant competition. This result contradicts previous observations from the field that at high densities broom corn millet competes significantly with maize (Wilson & Westra, 1991).

Comparing the results from competition and monoculture experiments, it is evident that the two species behaved differently when grown without competition from the other species. Sweet corn seedlings growing without broom corn millet in the competition experiment accumulated comparatively less biomass than sweet corn growing alone in the monoculture experiment. This suggests that competition between sweet corn seedlings caused reductions in their biomass. In contrast, broom corn millet grew faster overall in the competition experiment than the monoculture experiment. Almost three times more dry matter was produced by broom corn millet growing in competition compared to the monoculture experiment. However, broom corn millet biomass did decrease with increasing ratios of corn, suggesting competition from the larger corn plants. Overall, these results indicate that broom millet grows faster with moderate competition, compared to a wider spaced monoculture. It is generally observed in the literature that broom corn millet provides vigorous competition to sweet corn (Wilson & Westra, 1991). Growth rate and competition observed in this experiment make this assumption subject to further research in case of broom corn millet present in New Zealand.

Another possible reason for the delayed germination and seedling growth of broom corn millet could have been the lower than average temperature during the month of December in Hamilton. Seventeen days during this month had minimum temperatures below 10°C and mean temperature for this month was also

significantly lower than the previous 2 years. Broom corn millet does not germinate and grow well below 20°C (Chapter 2). Thus, the low temperature during the early growth period could have reduced the growth rate and competitive ability of broom corn millet compared to sweet corn. However, temperature alone does not explain the slower growth of broom corn millet in the monoculture experiment, compared to the competition experiment. Also, base temperature for growing degree days is suggested to be 10°C for broom corn millet and sweet corn (Anderson, 2000). However, as observed in this experiment both the species varied considerably in their response to the lower temperature during early growth period. This suggests that both the species might have different baseline temperature for growth or sweet corn variety used is more tolerant to colder climates than broom corn millet.

In the field, broom corn millet normally emerges at the same time as sweet corn in late spring and growth is simultaneous for both the species. It is well known that weeds emerging with the crop provide more intense competition (Nelson & Nylund, 1962). In such a scenario broom corn millet is known to cause yield reductions in sweet corn (Wilson & Westra, 1991). In this experiment the broom corn millet seedlings emerged more slowly and were less vigorous than the corn seedlings, despite being sown on the same date. Slower emergence and growth resulted in minimal competition between the broom corn millet and the corn. The broom corn millet found in New Zealand, and used in this experiment, is the black seeded biotype, with shattering seed heads. Previous research comparing the growth and development of broom corn millet biotypes revealed that the weedy black seeded biotype has a higher seed dormancy compared to cultivated biotypes (Eberlein et al. 1990). High levels of dormancy compared to the corn seeds, combined with the artificial sowing of dry seeds at the beginning of the experiment, could have been one of the reasons for the delayed germination of broom corn millet in this experiment.

At the planting densities used in this experiment, it is unlikely that competitive effects between seeds, known to cause delayed germination in other species (Grundy et al. 2003), contributed to the slower emergence of broom corn millet.

Research in the USA has found that weed suppressing ability and weed tolerance varies between varieties of hybrid sweet corn (Williams et al. 2007; Williams et al. 2008). Two sweet corn hybrids, GH2547, WHT2801 were more weed suppressive to broom corn millet than a third variety, Spirit (2007). Research suggests that sweet corn's ability to stand weed competition and to suppress its fitness is based on three principal factors related to canopy development, i) crop canopy development around emergence, ii) crop canopy development near canopy closure and iii) crop canopy development during reproductive phase (So et al. 2009). The sweet corn variety used in this experiment is widely used throughout New Zealand for sweet corn production. No information is available concerning the relative weed suppressing ability of this variety. It is possible that the sweet corn variety used in this experiment has a higher than normal weed suppressing ability, resulting in out-competition of the broom corn millet.

4.5 Recommendations

Broom corn millet is a relatively new grass weed in New Zealand and apart from its response to different herbicide applications (James & Rahman, 2009) its biology here has not been studied extensively. Depending upon site-specific factors, weeds as well as crops may vary in their performance from place to place (Williams et al. 2007). Hence, it is necessary to understand a weed's biology under New Zealand conditions and with New Zealand crop cultivars that may differ from overseas cultivars in their response to weed competition (Williams et al. 2007, 2008). Thus, further research should be made in either field or laboratory based trials to examine the response of different sweet corn and corn types to competition with broom corn millet. Broom corn millet has the potential to emerge as a major weed, causing significant yield loss in both corn and sweet corn (Wilson & Westra, 1991; James et al. 2010). Therefore, efforts should also be made towards either developing new corn cultivars, or encouraging use of existing cultivars, that endure weed competition better and have better weed suppressing ability, including the principal canopy development factors that confer competitive advantage over broom corn millet (So et al. 2009).

It was clearly evident from this experiment that late germinating broom corn millet does not suppress the growth and development of sweet corn. The results

suggest that the sweet corn and the broom corn millet types present in New Zealand differ in their threshold or optimum temperatures for growth, even though it is generally assumed that the two species have similar temperature requirements (Anderson, 2000). Thus, early planting of sweet corn could significantly reduce the early seedling competition and increase sweet corn yield. Further research should examine the temperature response of the broom corn millet and corn varieties present in New Zealand, and identify the best planting time to achieve maximum corn growth before the onset of significant competition from broom corn millet.

The recommended sowing density for field grown sweet corn in New Zealand is 55,000-60,000 seeds/ha. In the present competition experiment, seeds of broom corn millet and sweet corn were planted at a density equivalent to 143000 seeds/ha, which is almost three times closer to each other than they would normally be planted in field. If resources allowed, a better approach would have been attempted to conduct competition trials in larger field based plots where more normal planting densities could have been used. In this competition experiment, weed and crop planting density varied inversely with each other, such that as weed density increased, crop density decreased. This approach meant it was possible to explore a wider range of crop to weed ratios within a confined planting space. Unfortunately, large differences in initial growth between the crop and weed meant that this experimental design caused crop growth to increase with increasing weed density, because the increase in weed competition was less than the reduction in crop competition within the same pot. If field trials were initiated, it would also be appropriate to vary broom corn millet density while keeping corn planting density constant. It would also be advisable to investigate more growth parameters in future experiments as this experiment included only dry matter as a variable between the two species. These could include leaf area development, root biomass and should extend to include yield loss. The critical drawback in this experiment was the late emergence of broom corn millet. Thus, in future experiments seedlings of broom corn millet and sweet corn of the same age could be planted to avoid the factor of delayed germination.

As discussed, broom corn millet exhibited little competitive ability at an early growth stage. The result from this experiment differs from previous experiments and observations of competition by broom corn millet in the field. More research is needed in New Zealand to investigate the effect of broom corn millet competition on sweet corn growth.

**Chapter- 5 Evaluation of a controlled
seed ageing test and prediction of broom
corn millet seed longevity**

5.1 Introduction

Irrespective of their storage conditions seeds age with the passage of time and ultimately die. The definition of seed ageing given by Walters (1998) is reproduced here “The deterioration of seeds, which leads to the loss of vigour and eventually viability, is termed seed ageing”. The main factors influencing the rate of ageing are proposed to be ambient moisture and temperature and their level in the seed. It is generally assumed that higher moisture and temperature results in rapid transition towards seed death. The third proposed factor with respect to seed ageing is of seed quality which is poorly understood (Walters, 1998).

The ‘cryptobiotic’ nature of seeds means it is difficult to differentiate between alive and dead seed as they do not carry on processes like living organisms (Walters, 1998). However, considerable numbers of cellular and physiological processes have been identified and related to the process of ageing. For instance, as the seed ages its membranes become leaky, enzymes lose their catalytic activities and chromosomes accumulate mutations (Smith & Berjak, 1995). Walters (1998) reports in her review that, of 24 enzymes reportedly present in the seeds, 75% of them decrease in activity with the ageing. It is also observed that cellular membranes are the site of damage during ageing since lipid degrades during the process (Trawatha et al. 1995; Sung, 1996) and some regions of cells are more prone to damage than others (Smith & Berjak, 1995, Hoekstra et al. 1992). However, all the accumulated knowledge does not reveal the mystery about the speed of the ageing process and which seeds are more susceptible and age more rapidly compared to others, even though this knowledge has significant implications for the seed industry (Walters, 1998).

Annual weeds generally rely on prolific seed production and establishment of persistent seed banks for sustainable populations (Reuss et al. 2001). Although, to avoid the competition many annual plants show delayed germination (i.e. dormancy) resulting in age structure within the seed population. It has been well established that delayed germination in optimum conditions is a typical characteristic of aged seeds (Argerich & Bradford, 1989). Consequences of aged seed on plant performance such as seedling vigour and competitive ability are

little known for wild species. However, a recent study (Rice & Dyer, 2001) demonstrated the negative effect of aged seeds in the annual grass weed *Bromus tectorum* L. In their experiment, comparison of plants grown from aged and fresh seeds suggested that plant competitive ability was compromised by delayed germination and seedling vigour. Therefore, in weed eradication and control programmes it is critical to know the characteristics of aged seeds to evaluate the success of control measures and monitoring.

Seeds that reach the soil from a mature plant may die, germinate immediately or persist in soil for a longer period and emerge when recruited to represent the new generation of weeds. Therefore, Thompson et al. (1998) have categorised seed persistence into three groups: transient (< 1 year), short-lived (1 to 5 year) and long lived (> 5years). This categorisation represents the weedy characteristic of each species based on their seed persistence. In ecosystems weed eradication programmes cannot be declared successful until no viable seeds remain in the soil at the site of eradication (Long et al. 2008) as these seeds can germinate and again infest the site. Similarly, in arable systems prolific seed producing annual weeds such as broom corn millet (*Panicum miliaceum*) rely entirely on seed banks to establish every year. Therefore, knowledge of the size and persistence of the weedy seed bank has practical and economical implications for weed control plans. In this context knowledge of seed longevity is very important for monitoring weeds in arable farming and to achieve successful eradication by controlling newly emerged seedlings.

A considerable amount of research has been undertaken to investigate the role of various inherent characteristics of seeds as well as environmental factors to predict the persistence of seeds. However, relating the outcomes of this research to field persistence has been difficult due to weaknesses in the predictive power of these tests (Long et al. 2008). For instance, several have proposed the relation of seed persistence to its size and morphology (Bekker et al. 1998; Hodkinson et al., 1998; Moles and Westoby, 2006). The argument to this theory is that the smaller and more compact seeds easily incorporate into the soil and thus avoid predation and germination. In contrast, Moles et al. (2003) observed the opposite trend in

Australian plant species which either suggests common exceptions to this theory or seed persistence phenomenon being site specific.

Numerous attempts have also been made to predict the persistence of seeds through real time burial. A famous experiment of such nature dates back to 1879 and was completed 120 years in 2000. Seeds of some species from this trial showed viability and successfully reproduced by forming healthy propagules after 120 years (Telewski & Zeevaart, 2002). These kinds of experiments usually relate site specific factors such as soil type, soil physical properties, other soil characteristics and burial depth to seed persistence (Lewis, 1973; Egley & Chandler, 1983; James et al. 1998). However, as discussed previously changing land use of places often makes it difficult to continue such experiments. In addition, Van Mourick et al. (2005) suggest that high seed densities in buried mesh bags in such experiments may overestimate the depletion rate of the seed bank due to fungal contamination. Thus, laboratory based assays should be developed and used to match the results from the field and vice versa.

As discussed earlier, moisture and temperature are two critical factors determining seed persistence. Thus, by controlling both these variables persistence of seeds can be predicted. For *ex situ* seed storage low temperature (-20° C) and low relative humidity (RH) (15%) conditions are advocated (Smith et al., 2003), whereas vice versa conditions accelerates the ageing process. The current study is aimed to evaluate and use the laboratory based ‘Comparative seed longevity test’ (Davies & Probert, 2004) to predict the persistence of broom corn millet seeds. The technique stresses seeds in a high moisture and temperature environment and is being used at Royal Botanic Garden, Kew, U.K., and has also found to be indicative when conducted elsewhere (Long, 2007; Long et al. 2008). Further, Probert et al. (2009) published comparative seed persistence of 195 species and concluded that presence or absence of endosperm along with the climate of origin influence seed persistence.

This chapter reports on the evaluation of the ‘Controlled Ageing Test’ (CAT) to predict the persistence of broom corn millet seeds in the soil seed bank for which there is limited information on its persistence in New Zealand. The CAT is

evaluated by also using it for a broadleaf weed, nodding thistle (*Carduus nutans*), for which real time persistence data through a seed burial experiment is available. The test was then used to predict the longevity of broom corn millet seeds for which there is limited information on persistence in New Zealand.

5.2 Methods and materials

5.2.1 Real time burial experiment to investigate persistence of weed seeds

The data obtained from an ongoing real time seed burial experiment was used to evaluate the predictive power of the CAT. The experiment was set up in June 1981 by staff from Ministry of Agriculture and Fisheries Research Division, at four different locations in the vicinity of Hamilton. As described by James and Rahman, (1999, 2000, 2001, 2003) and James et al. (1998); these sites were under permanent pastures with regular grazing or mowing. From the soil types chosen Horotiu and Dunmore soils are both well drained whereas Hamilton clay loam is moderately and Rukuhia Peat soil is very poorly drained. Soil from each site was collected and heat sterilised. Ninety sets of 200 (0.44 g) of freshly collected nodding thistle seeds were prepared. The seed had a viability of 81.5%. The seed sets were mixed with the sterilised soil. For each site, 30 tubes (25 cm length of perforated and, 6 cm diameter drain pipe), were each filled with unsterilised soil from the respective site. Simultaneously, two seed/soil mixtures contained in fine nylon mesh bags (0.25 mm) were placed within the soil in each pipe at 5 cm and 20 cm depth. The tubes were buried vertically and top 2 cm of these pipes were left free of soil. Separated from soil below by fine nylon cloth, an unbagged third batch of soil seed mixture was placed on the top of the tubes. The pipes were retrieved at regular intervals up to 16 years and were last retrieved in August, 2009.

Seed viability of retrieved seeds was checked by germination in an unheated glasshouse. The contents of the retrieved nylon mesh bags were spread on a tray filled with vermiculite and separated with a paper towel. Germinated seeds were counted regularly and seedlings were removed. When there was no more germination soil was mixed thoroughly and the procedure was repeated until no

new seeds germinated (4-6 months). On completion, the soil seed mixture was washed to remove the fine particles and the remainder was checked for any ungerminated seeds (James et al., 2010).

Exponential (decay) curves were fitted to all data using the regression command in Minitab and the time taken for the viable seed to fall to both 50% and 1% of the original amount calculated from the equation for this curve. Coefficients of determination (R^2) are presented as an indication of the amount of variation accounted for by both variables.

5.2.2 Controlled ageing test

Seeds

Seeds of three weedy species were used to evaluate the predictive accuracy of the CAT. Nodding thistle, one of the species used in this experiment is an annual or, usually biennial weedy herb of pastures (Popay & Medd, 1990). Seeds of nodding thistle were collected in the year of 2006 and were stored at room temperature in a dark container. The seeds of black seeded broom corn millet biotype were collected in 2007 and stored the same as the nodding thistle seeds. White bryony (*Bryonia cretica*), an environmental weed (Trivedi et al. 2010), was used as a third species. Seeds of white bryony were also collected in 2007 and again stored in the same manner as the other two species.

Germination requirement

Viability and germination percentage for broom corn millet and nodding thistle seeds were checked prior to the CAT. All the germination tests throughout this experiment were carried out in a temperature controlled glasshouse unless stated. The seeds of broom corn millet were mixed with soil moistened with 0.2% KNO_3 and kept in a petri dish. Their germination was 86% thus meeting the minimum requirement of $\geq 85\%$ viable seed to be used for standardised aging protocol. Broom corn millet seeds used in all experiments belonged to the same seed lot. Their high viability is also evident from the experiment in chapter 2. For nodding thistle, the seeds were kept in a petri dish with filter paper at the bottom moistened

with 10 ppm gibberellic acid and 0.5% Benlate solution. Benlate solution was prepared using fungicide benomyl (0.5 g/litre) to reduce the activity of pathogenic fungi on germinating seeds. Germination for nodding thistle seeds was 88%. To overcome the dormancy of hard coated white bryony seeds, they were immersed in 70°C H₂O and left to cool down overnight to room temperature. The seeds were subsequently scarified using sandpaper and transferred into the petri dish with filter paper at the bottom. The filter paper was moistened using 10 ppm gibberellic acid solution which is a plant growth regulator (ProGibb[®] SG, Nufarm, New Zealand) (Trivedi et al. 2010). 55% of white bryony seeds germinated whereas, the seed crush test (Refer 3.2.1) suggested that 88% of the seeds were viable having an oily endosperm with cream coloured appearance. Although, the germination test method for white bryony was inadequate, they were included in the CAT, as it was comparatively easy to evaluate viability of ungerminated seeds using the seed crush test.

Controlled Ageing Test

The standardised CAT protocol used at Millennium Seed Bank Project, Royal Botanic Gardens, Kew; was followed with minor additions throughout this experiment (Davies and Probert, 2004). Seeds were counted into 12 samples of 50 and kept in open glass vials. To rehydrate or increase the moisture content of the seeds prior to the CAT were rehydrated at 47% RH and 20°C in the open glass vials which were placed over a non-saturated solution of LiCl (370 g L⁻¹) (anhydrous, analysis grade, Acros Organics, New Jersey, USA) in distilled water held in a sealed 275×275×95 mm electrical enclosure box. After a two week rehydration period seeds were transferred to a second electrical enclosure box. This box contained non-saturated solution of LiCl (300 g L⁻¹) with 60% RH and was kept in an incubator (Contherm Digital Oven) at 45±1°C. As suggested in the protocol, 40 ml H₂O was added and solution was stirred every month to maintain RH.

Sampling frequency was altered from the original protocol with one additional sampling day at Day 15. One glass vial for either broom corn millet, nodding thistle or white bryony was removed from box after 1, 2, 5, 10, 15, 20, 30, 50, 75,

100 and 125 days. On removal seeds were prepared for germination testing using the same method as the original viability evaluation. Petri dishes were checked for germination at regular intervals and germination was considered successful when the emerging radicle reached ≥ 2 mm in length. The ageing experiment with broom corn millet was conducted twice to investigate the consistency of results obtained. The seed lot used for both the experiments was the same and germination tests were conducted to check the germination percentage prior to each experiment.

For nodding thistle the seed persistence index (SPI) was calculated using Equation 1 (Bekker et al. 1998).

$$\text{SPI} = \frac{\text{No.of records for persistence} > 1 \text{ year}}{\text{Total No.of records for that species}} \quad (\text{Equation 1})$$

For nodding thistle and broom corn millet, germination (%) was plotted against time in aging (days) for both species. Logistic curves in non linear regression analysis were fitted to the acquired data using Equation 2:

$$\text{Germination (\%)} = (100 - \alpha) / 1 + e^{-\beta(t-c)} \quad (\text{Equation 2})$$

where α is the initial germination percentage, β is the rate of viability loss in aging environment, t is the accumulated time in the CAT, and c is the P_{50} value (time to reach 50% viability for seeds in ageing environment).

The P_{50} value obtained for nodding thistle seeds was calibrated by comparing it with the available real time persistence data. For nodding thistle seeds, real time persistence data is available at different burial depths. Therefore, the P_{50} value was compared with at each burial depth.

5.3 Results

Results from the real time seed burial experiment show that only 2.5% of exhumed nodding thistle buried seeds germinated from the deepest burial depth of 20 cm in Horotiu sandy loam soil. No germinations were recorded from the burial

depth of 5 cm or from the seeds near the soil surface (0-2 cm). In comparison, no germinations were observed from seeds buried at any of the depth in the Hamilton clay loam soil. In addition, no seeds were retrieved from either of the soil after washing the seed mix.

Regression analysis using the exponential function strongly suggested that the viability of seeds declines exponentially with the passage of time. It was observed that seeds at deeper burial depths survived longer than the seeds buried towards the soil surface (Figure 5.1 and 5.2).

In the CAT viability of nodding thistle seeds declined rapidly (Figure 5.3) and regression analysis suggested that the P_{50} value for nodding thistle seeds was 6.9 ± 3.1 days. This indicates that nodding thistle seeds would be short lived in the soil seed banks, probably about a year. Both the experiments for broom corn millet gave similar results each time (Figure 5.4). It was not practical to obtain a statistically derived P_{50} value for broom corn millet in either set of data as seed viability declined drastically between Day 50 and Day 75 of sampling leading to large variance in the calculated P_{50} value. Sampling from the ageing environment on Day 50 (in both the experiments) indicated that about of 50% of seeds were viable. However, the following sampling on Day 75 resulted in $\leq 2\%$ viability of seeds. Examination of the graph showing decline in seed viability (Figure 5.4) shows a plateau from 30 to 50 days followed by the sharp decline at 75 days. Therefore, in reality the P_{50} is likely to be between 30 to 50 days. A simple midpoint of that range would indicate P_{50} to be about 40 days. The P_{50} from the two experiments reported here would indicate that broom corn millet has the potential to persist in the soil seed bank for a moderately long time. The CAT for white bryony was abandoned as seed germination ceased after the Day 5 sampling interval with no apparent reason for the rapid decline in viability. Further, the seed crush test indicated that the viability of seeds was unaffected in the CAT in this very short span.

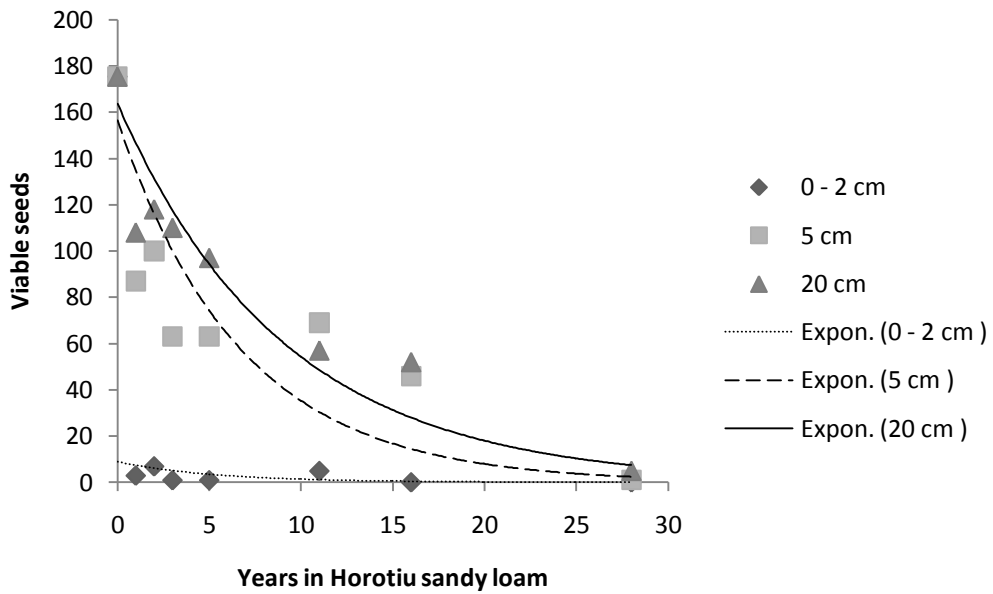


Figure 5.1 Number of seeds germinating over time after burial at various depths in Horotiu sandy loam soil, the coefficient of determination (R^2) of the fitted exponential decay curve for respective depths are: 0-2 cm- 58.3%, 5 cm- 81.6%, and 20 cm- 92.1%.

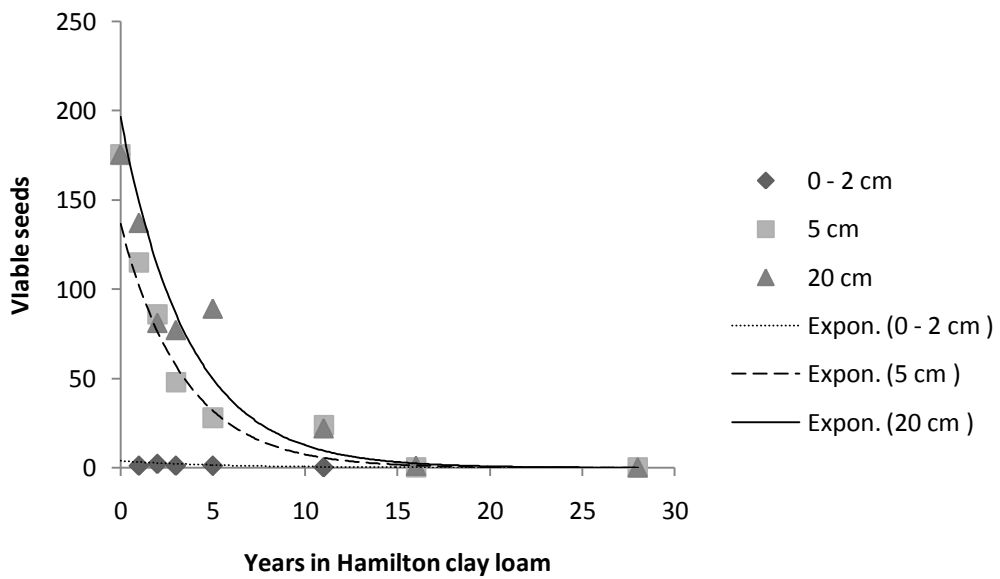


Figure 5.2 Number of seeds germinating over time after burial at various depths in Hamilton clay loam soil, the coefficient of determination (R^2) of the fitted exponential decay curve for respective depths are: 0-2 cm- 76.4%, 5 cm- 78.2%, and 20 cm- 96.3%.

Table 5.1 Predicted/observed persistence records for nodding thistle seeds buried at different locations and characteristics of respective soil type.

Literature source	Location	Site characteristics				pH	Predicted/observed persistence (years) at ≥ 10 cm burial depth
		% sand	% clay	% OC ¹			
James et al. (2010)	New Zealand ³	61	15	8.7	5.4	41.9	
Neave (2004)	Australia	- ²	- ²	- ²	- ²	21	
James et al. (1998)	New Zealand ³	54	17	19.5	5.5	32	
Burnside et al. (1996)	USA ³	24	16	49.1	4.6	33	
		16	32	4.4	5.2	9	
Popay & Medd (1990)	New Zealand	55	16	1.6	7.0	8	
		- ²	- ²	- ²	- ²	>34	

¹ Organic carbon

² No available information

³ Seeds buried in two different soil types

Table 5.2 Predicted/observed persistence records for nodding thistle seeds near the soil surface at different locations.

Literature source	Location	Predicted/observed persistence (years) at 0-2 cm burial depth in various soils
James et al. (2010)	New Zealand ¹	6.1
Neave (2004)	Australia	3.2
James et al. (1998)	New Zealand ¹	3.5
		7
		5

¹ Seeds buried in two different soil types

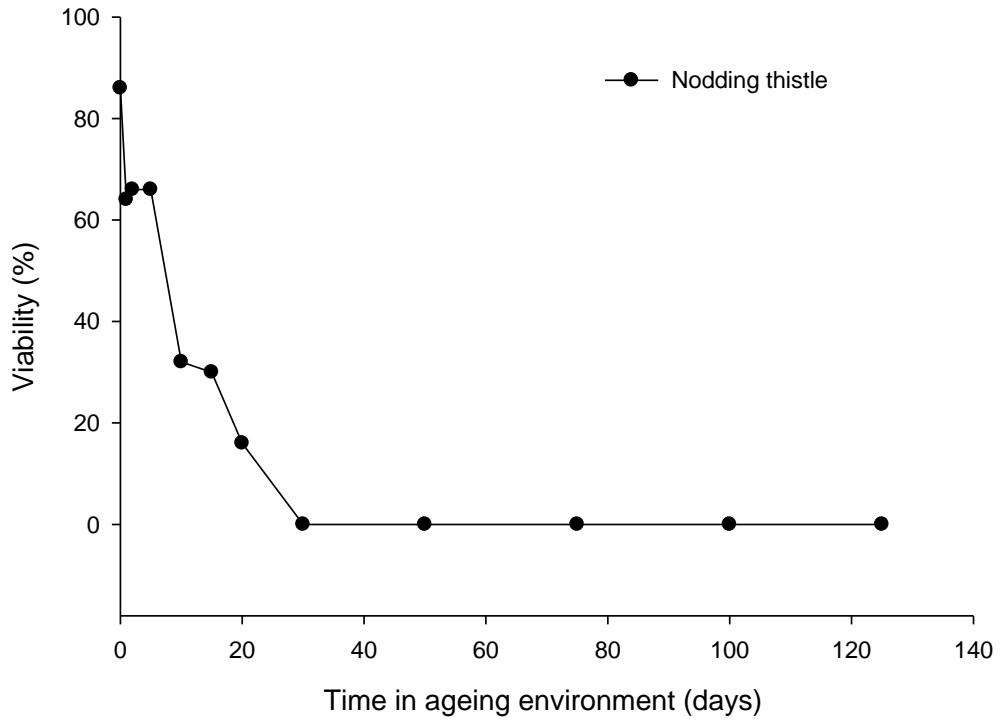


Figure 5.3 Viability (germination) of nodding thistle seeds during the CAT.

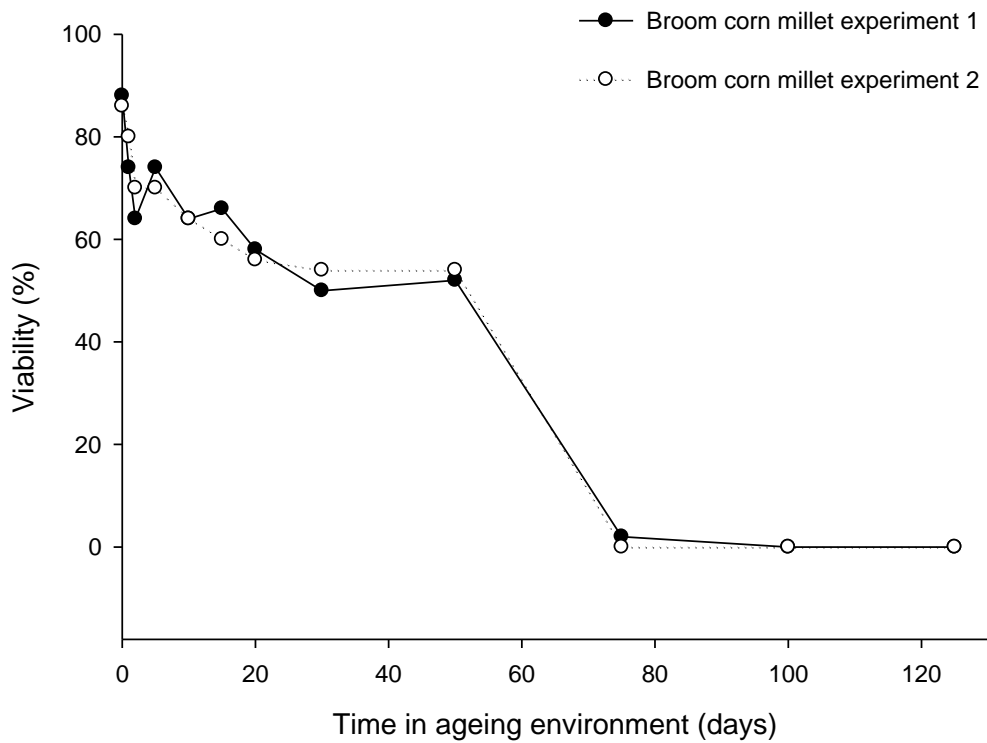


Figure 5.4 Viability (germination) of broom corn millet seeds during the CAT.

For nodding thistle seeds the equation for the fitted exponential decay line in the regression indicated that in the field experiments P_{50} value ranged from 6-11 years at 20 cm burial depth in various soils. The CAT includes only temperature and moisture as the environmental variables affecting seed persistence, therefore, the achieved P_{50} value is considered as the maximum potential persistence of seeds in the absence of other mortality factors such as germination, microbial activity and herbivory. If the field study is used to calibrate the CAT study it is then assumed that the artificially derived P_{50} of 6.9 ± 3.1 days equates to the field derived P_{50} value of 6-11 years. Thus, the CAT P_{50} value for broom corn millet of 40 days would equate to about 45 years in the field. This would also indicate that a small number of broom corn millet seeds would likely persist for more than 100 years in the seed bank.

5.4 Discussions

To describe the seed bank persistence pattern for different species Long et al., (2008) allocated the P_{50} value into one of three categories. Species with a P_{50} value < 20 days corresponded with a transient seed bank (field persistence < 1 year), P_{50} values between 20 to 50 days were described as a short-lived seed bank (field persistence between 1 to 3 years) and $P_{50} > 50$ days referred to extended persistence (> 3 years). However, long-term seed persistence is usually categorised as > 5 years (Thompson et al. 1998), no species evaluated by Long et al. (2008) met this parameter. In contrast to their results nodding thistle seeds in this study are known to survive > 5 years (Table 5.1). Therefore, in this study a short lived seed bank (P_{50} value between 20 to 50 days) is considered as field persistence between 1 to 4 years and long lived ($P_{50} > 50$ days) as > 5 years as originally suggested by Thompson et al. (1998).

The experimental P_{50} value for nodding thistle was compared to the field persistence records for the species (Table 5.1). Five available records from New Zealand (James et al. 1998; James et al. 2010; Popay & Medd, 1990) and two overseas records (Burnside et al. 1996; Neave, 2004) were used to calculate the species persistent index (SPI) nodding thistle (Equation 1). The calculated SPI value for seeds of nodding thistle species was 1, suggesting that it forms long lived seed banks. Real time long term burial experiments shows that seeds of

nodding thistle form persistent seed banks at deeper depths and short lived seed banks near the soil surface (Tables 5.1 and 5.2). This indicates that persistence of seeds declines rapidly towards soil surface (Figures 5.1 and 5.2). In contrast, in the CAT the logistic regression gave a P_{50} value of nodding thistle seeds 6.9 ± 3.1 days. According to seed bank categories described by Thompson et al. (1998) seeds of nodding thistle would form transient (< 1 year) seed bank in field, a result that was lower than the shallow seed burial in the field (Neave, 2004; James et al. 2010; James et al. 1998) (Table 3.2). This indicates a poor correlation between the CAT result and the real time seed burial experiment.

The current as well as previous studies in New Zealand with nodding thistle seeds have shown that burial depth of seeds is a significant factor affecting their persistence (James et al., 1998; James et al., 2010). Since seeds near the soil surface are more prone to loss through germination and herbivores they are found to be less persistent than seeds at deeper depths (Chambers & McMahon, 1994). Thus, even if the storage conditions post seed collection could be considered as a deteriorative factor on seed vigour, such a large discrepancy is not explained at all in case of nodding thistle seeds which would persist for considerably longer (Table 5.1) in the soil where more mortality factors prevail.

Predicted persistence of broom corn millet seeds (> 100 years) on the basis of calibrated CAT result for nodding thistle seeds is probably not an acceptable prediction. There is not much literature available on the seed persistence of broom corn millet, although, it has been observed that the black seeded biotype of broom corn millet forms a short to long lived (≥ 4 years) seed bank (Cavers et al. 1992). In that scenario, the P_{50} value for broom corn millet seeds from the CAT (40 days) may indicate its persistence potential according to seed bank categories assigned by Thompson et al. (1998). P_{50} value of 40 days is below the minimum limit for a persistent seed bank pattern. Therefore, based on the currently available information it could be argued that broom corn millet seeds should form short lived to moderately persistent seed banks.

In the first experiment broom corn millet seeds reached their P_{50} value twice, first on sampling day 30 and then between day 50 and 75. This was due to the fact that

sampling on day 50 showed viability higher than 50%. It has to be taken into account that both the P_{50} value falls under different category of seed bank. Hence, it is essential that results from CAT should be interpreted with caution. In addition, it would also be advisable to conduct the experiment with more replicates to improve the consistency of the final result.

The variability observed in above experiments also leads to another possible predictive weakness of the CAT. The sample size (50 seeds) recommended for the CAT is possibly too small as the probability of outcome is binomial (germination or no germination). For instance, if 19 germinations were observed from a given sampling interval than cumulative binomial probability of getting that result again would be 6%. Further, the distribution of probability is symmetrical; therefore, similar chances prevail for getting 31 germinations (Stat Trek, 2009). Similarly, it was observed in these experiments that a small fluctuation in germination percentage between the two experiments resulted in very broad range of P_{50} value for broom corn millet seeds, and placed broom corn millet seeds in two different seed bank categories. There, with such a small sample size it may be critical to estimate the viability in seeds removed from the CAT environment. In addition, in the CAT viability is only expressed as germination which may not be the most accurate way of determining viability in each seed. Hence, a larger sample size would be more statistically significant and indicative of viability change of seeds when compared between sampling intervals.

In addition, as discussed by Long et al. (2008), broad categories of seed persistence (< 1 year, 1 to 3 years and > 3 years) do not give flexibility to variations that may occur in nature. For instance, as observed in this experiment, the P_{50} value from broom corn millet suggests its seeds fall into the long term persistence category (> 5 year), whereas field records also suggest it forms a seed bank which survives ≥ 4 years (Cavers et al. 1992).

The nodding thistle and broom corn millet seeds used in this experiment did not have any significant germination requirement, making the assessment of viability following the CAT straightforward. However, as indicated by Long et al. (2008), it was difficult to include the species with dormant seeds such as white bryony in

the CAT. Although known to have > 85% potentially viable seeds as described through seed crush test (Sawma & Mohler, 2002), mechanisms that aid in obtaining sufficient germination were not identified for this species. In addition, with no apparent reason germination ceased when using the method developed to overcome dormancy. This exhibits a major limitation for of the CAT, as it is not readily appropriate for species exhibiting strong dormancy traits.

In addition to its germination ecology, seed persistence of white bryony is also not reported previously. The seeds have hard seed coat and larger endosperm to provide the embryo with adequate nutrition. Therefore, the morphology of the seeds indicates that seeds of white bryony may persist for significantly longer periods in seed banks. In addition, seeds of white bryony are spread by birds and plants are usually found where birds perch (Biosecurity New Zealand, 2009). Thus, it may also be possible that seeds that pass through the digestive system of birds may have comparatively short persistence than fresh seeds. Thus in such scenarios P50 value from the CAT may not put the species under appropriate seed bank category as it would not be feasible to collect the seeds post excretion by birds.

Long (2007) also examined the effect of soil type and microorganisms by incorporating them in the CAT. They created an environment of 96% RH with saturated salt solution solutions. As a result of this study it was concluded that soil type alone does not have a significant effect on seed persistence. It was evident from their study that seeds deteriorated rapidly compared to standard CAT environment. In addition, soil surface were also contaminated with mould. However, it is not clear if higher microbial activity at 96% RH or soil moisture itself would have caused this result. It is known that microorganisms inhabiting soil and participating in degradation process of biotic matter are usually mesophilic. Mesophilic bacteria and fungi differ in their optimum temperature range for growth. In this respect, optimum temperature range for mesophilic organisms is considered between 20-45°C (Madigan & Martinko, 2006). The effect of higher temperature on growth and survival of mesophiles in such experiments could bias the results. Therefore, it would be helpful to culture such

organisms from samples of CAT environment and subsequently identify them to confirm their presence.

Persistence of seeds varies as a result of different populations, soil types, temperatures, burial depths and hydration states (Bekker et al. 1998; Burnside et al. 1996; James & Rahman, 2003; Miller & Nalewaja, 1990). Therefore, in addition to other difficulties discussed concerning the predictive power of the CAT, it must be taken into account that a P_{50} value from single seed lot may not represent the species as whole (Long et al., 2008). Further, Long et al. (2008) also suggested positive linear relationship between responses in laboratory based CAT and *in situ* seed persistence of 27 northwest European species. However, their model accounted for only about 31% of the variability in data and rest of the variation was presumed to be inherent within SPI values for those species. However, for native species the inherent variation within their SPI is likely to be smaller than the variation of the SPI for weedy species which generally have more extensive range and climatic conditions (Table 5.1). Hence, for weeds such as nodding thistle the SPI value should be considered robust only if their persistence records are derived from widespread geographical locations. Similarly, the P_{50} value should also only be considered robust if it is derived from various seed lines.

In summary, the aim of this study was to evaluate the predictive power of the CAT in comparison with real time seed persistence data. Current evidence from this study questions whether accurate or indicative predictions can be made for species from a single P_{50} value from a CAT experiment. In addition, even small fluctuations of viability changed the final seed bank category for a species. Therefore, as suggested it would be advisable to conduct multiple CAT experiment with the same species using different seed lots to assess its persistence. Further, as suggested by Long et al. (2008) for a species growing in cool and dry climate, P_{50} value from the CAT may correspond to lower life span of seeds. Hence, incorporation of environmental variables in the CAT may provide more accurate predictions than with the current protocol.

Chapter- 6 Final discussion

As discussed in chapter 2 of this thesis, being a C₄ plant *Panicum miliaceum* (broom corn millet) grows better at warm temperatures. Temperatures $\geq 20^{\circ}\text{C}$ increased the germination significantly in both laboratory based experiment and in an experiment simulating the affect of soil temperature. Substrate temperature was less significant for growth than it was for germination. However, higher substrate temperature helped plants to grow vertically and accumulate significantly more biomass than at lower temperature. This indicated that at higher temperatures broom corn millet will be more competitive through its vigorous growth than at lower temperatures. The information derived from this study will enable farmers to better plan the planting of crops to reduce the impact of broom corn millet. Further, the summer climate of all sweet corn growing regions in New Zealand makes them susceptible to infestation by broom corn millet if introduced to the region.

The global average concentration of CO₂ in the atmosphere has increased from 280 parts/million in 1750 to 380 parts/million in recent times, with half of this increase occurring in the last 30 years (NIWA, 2008). In addition, due to global warming average temperature for New Zealand has also increased 0.9°C in the last 100 years. It is predicted that with current emission rates the average temperature for New Zealand will increase by up to 2°C by 2040 and by 5.1°C by 2090 (NIWA, 2008). A recent study in free-air CO₂ enrichment conditions showed that with CO₂ enrichment photosynthesis increased up to 10% in C₄ species. In addition, wild C₄ grasses were able to grow in nutrient poor sandy soil under elevated CO₂ conditions (Long et al. 2004). Therefore, it is hypothesised that along with photosynthesis and production water use also improves in C₄ species under elevated CO₂ conditions (Long et al. 2004; Ghannoum et al. 2000). Hence, as discussed increasing temperature with elevated CO₂ level up to certain limit will help C₄ plants such as broom corn millet under certain cropping conditions. Thus, if introduced into new cropping regions the weed could cause significant crop losses in coming years.

The depth of emergence experiment showed that broom corn millet is able to emerge from all the burial depths investigated (≤ 170 mm). However, seedlings did not emerge from the 170 mm burial depth from all 16 soil types tested but did

emerge from 120 mm depth in each soil type. As an annual plant, ability to emerge from such burial depths gives broom corn millet a definite advantage over the entire growing season. As suggested, it is assumed that comparatively larger seeds than other weeds with more reserves enables broom corn millet to emerge from such depths. This ability to emerge from greater depths such as 170 mm could limit the effectiveness of current practices to restrict the emergence of weed seedlings from soil seed banks. In addition, it is known that the bulk density of New Zealand cropping soil is comparatively lower than many overseas cropping systems. This may be one of the reasons which allow species such as broom corn millet to emerge from such great depths as observed in this study. Therefore, in conjunction with previous study by James et al. (2002), we suggest that observations from overseas experiments of this nature may not be applicable in New Zealand soils. Ability of broom corn millet seeds to emerge from greater than the normal ploughing depths and delayed emergence throughout the season, reduces the effectiveness of the ploughing and stale seed bed techniques employed for managing weeds. Therefore, modifications in current weed control practices or new approach will be required to reduce the impact of broom corn millet.

Results from the competition trial between sweet corn and broom corn millet at different planting ratios did not appear to reflect the field experiences of growers. In the competition trial sweet corn out-competed the broom corn millet and broom corn millet did not have any impact on the growth or biomass of sweet corn. In addition, sweet corn accumulated more biomass while growing in highest planting ratio of broom corn millet, whereas, broom corn millet responded well to moderate competition in planting ratio. It is assumed that the most limiting factor to suppress the competitive ability of broom corn millet in this study was its late emergence compared to sweet corn. Ambient temperatures significantly lower than its optimum temperature for germination and growth were recorded immediately after planting seeds of both the plants in this trial. This is considered to be a reason for the late and weak growth of broom corn millet. This further reinforces the earlier conclusion that earlier planting of sweet corn while the temperatures are cooler gives it the best chance to out compete the broom corn millet. In addition, it also indicates that increasing temperatures in New- Zealand

due to climate change will provide broom corn millet a growth advantage. A climate change scenario will enable broom corn millet seeds to emerge earlier in the growing season and thus make it a more troublesome weed.

No relationship was observed between the statistically derived P_{50} value for nodding thistle (*Carduus nutans*) from the controlled ageing test (CAT) and the real time seed burial experiment. P_{50} values for nodding thistle indicated that it forms transient seed bank (≤ 1 year), whereas real time burial data from available records in variety of conditions all suggest a persistent or long lived seed bank (> 5 year). Such a large difference between the persistence predicted from the CAT and the real time seed burial experiment is yet to be explained. Furthermore, the P_{50} value for broom corn millet from the CAT covered a broad range which included two seed bank classification categories (short term and persistent seed banks). However, the average P_{50} value for millet seeds matches its only reported persistent record indicating that it forms moderately persistent seed bank (Cavers et al. 1992). Therefore, it is advised that the outcome from the CAT must be used with caution and P_{50} value should be confirmed in multiple trials with different seed lots of the same species. These observations indicate that the CAT with a single seed lot cannot represent the persistence potential of a species as whole. Especially in the case of weeds with wide geographic distribution, environmental variations between regions may cause differences in seed persistence making a single CAT based P_{50} value redundant.

In conclusion, as observed overseas, broom corn millet has the potential to be a bad weed of arable crops in New Zealand. In addition, its arrival in New Zealand is quite recent and many aspects of its biology are unknown under New Zealand conditions. This thesis substantially increases the understanding of the biology of this weed under New Zealand conditions. The findings were: it exhibits poor growth at lower temperatures; its seeds have the ability to emerge from below normal soil inversion practices; late germinating plants appear to be much less competitive to sweet corn and it is able to survive for a medium to long time in the soil, allowing the formation of a persistent seed bank.

References

- Adams, V. M., Marsh, D. M., & Knox, J. S. (2005). Importance of seed bank for population viability and population monitoring in a threatened wetland herb. *Biological Conservation*, *124*, 425-436.
- Aderson, R. (2000). Ecology and interference of proso millet (*Panicum miliaceum*) in semi-arid corn¹. *Weed Technology*, *14*(1), 45-50.
- Al-Ani, A., Bruzau, F., Raymind, P., Sain-Ges, V., Leblank, J. M., & Pradett, A. (1985). Germination, respiration and adenylate charge of seeds at various oxygen pressures. *Plant Physiology*, *79*, 885-890.
- Albrecht, H. (2005). Development of arable weed seedbanks during 6 years after the change from conventional to organic farming. *Weed Research*, *45*, 339-350.
- Allen, P. S., & Mayer, S. E. (1998). Ecological aspects of seed dormancy loss. *Seed Science Research*, *8*, 183-191.
- Argerich, C. A., & Bradford, K. J. (1989). The effects of priming and aging on seed vigor in tomato. *Journal of Experimental Botany*, *40*, 599-607.
- Baker, R. D. (2003). Millet production. Retrieved 6/8/2009, from <http://aces.nmsu.edu/pubs/a/A-414.pdf>
- Ballare, C. L., & Casal, J., J. (2000). Light signals perceived by crop and weed plants. *Field Crops Research*, *67*(2), 149-160.
- Ballare, C. L., Scopel, A. L., & Sanchez, R. A. (1990). Far-red radiation reflected from adjacent leaves: an early signal of competition in plant canopies. *Science*, *247*(4940), 329-332.
- Ballare, C. L., Scopel, A. L., Sanchez, R. A., & Radosevich, S. R. (1992). Photomorphogenic processes in the agriculture environment. *Photochemistry and Photobiology*, *56*(5), 777-788.
- Baskin, C. C., & Baskin, J. M. (2006). The natural history of soil seed banks of arable land. *Weed Science*, *54*, 549-557.
- Baskin, J. M., & Baskin, C. C. (2004). A classification system for seed dormancy. *Seed Science Research*, *14*, 1-16.
- Bekker, R. M., Bakker, J. P., Grandin, U., Kalamees, R., Milberg, P., Poschold, P., et al. (1998). Seed size, shape and vertical distribution in soil: indicators of seed longevity. *Functional Ecology*, *12*, 834-842.

- Bekker, R. M., Bakker, J. P., Ozinga, W. A., & Thompson, K. (1998). Seed size, shape and vertical distribution in the soil: indicators of seed longevity. *Functional Ecology*, *12*(834-842), 834-842.
- Bekker, R. M., Oomes, M., & Bakker, J. P. (1998). The impact of groundwater level on soil seed bank survival. *Seed Science Research*, *8*, 399-404.
- Benvenuti, S. (1995). Soil light penetration and dormancy of Jimsonweed (*Datura stramonium* L.) seeds. *Weed Science*, *4*, 389-393.
- Benvenuti, S., & Macchia, M. (1993). Calculation of threshold temperature for the development of various weeds. *Agricoltura Mediterranea*, *123*, 252-256.
- Benvenuti, S., & Macchia, M. (1995). Hypoxia effect on buried weed seed germination. *Weed Research*, *35*, 343-351.
- Benvenuti, S., & Macchia, M. (1998). Light environment, phytochrome and germination of *Datura stramonium* L. seeds. *Environmental and experimental botany* *38*, 61-71.
- Benvenuti, S., Macchia, M., & Miele, S. (2001). Light, temperature and burial depth effects on *Rumex obtusifolius* seed germination and emergence. *Weed Research*, *41*, 177-186.
- Benvenuti, S., Macchia, M., & Miele, S. (2001). Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Science*, *49*, 528-535.
- Bewley, J. D., & Black, M. (1994). *Seeds: physiology of development and germination* (second ed.). New York: Plenum Press.
- Bhattacharyya, A. K., & De Datta, S. K. (1971). Effects of soil temperature regimes on growth characteristics, nutrition, and grain yield of IR22 rice. *Agronomy Journal*, *63*, 443-449.
- Bhowmik, P. C. (1997). Weed biology: importance to weed management. *Weed Science*, *45*, 349-356.
- Biosecurity New Zealand (2009). White bryony factsheet. Retrieved 6/11/2009 from <http://www.biosecurity.govt.nz/files/pests/white-bryony/white-bryony-factsheet.pdf>
- Blair, A. M., Martin, T., Brain, P., & Cotterill, E. G. (1991). The interaction between planting depth of four winter wheat cultivars, *Alopecurus myosuroides* Huds. and *Bromus sterilis* L. and their susceptibility to post-

- emergence applications of isoproturon and chlorotoluron. *Weed Research*, 31(5), 285-293.
- Bond, W. J., Honig, M., & Maze, K. E. (1999). Seed size and seedling emergence: an allometric relationship and some ecological implications. *Oecologia*, 120, 132-136.
- Bough, M. A., Colosi, J. C., & Cavers, P. B. (1986). The major weedy biotypes of proso millet (*Panicum miliaceum*). *Canadian Journal of Botany*, 64, 1188-1198.
- Bough, N., & Cavers, P. B. (2009). Proso Millet- Factsheet. Retrieved 11/7/2009, 2009, from <http://www.omafra.gov.on.ca/english/crops/facts/87-0.25.htm>
- Boyd, H. W. (1971). Manganese toxicity to peanuts in autoclaved soil. *Plant and soil*, 34, 133-144.
- Bray, C. M. (1995). Biochemical processes during the osmopriming of seeds. In J. Kigel & G. Galili (Eds.), *Seed development and germination* (pp. 767-789). New York: Marcel Dekker.
- Bridges, D. C. (1994). Impact of weeds on human endeavours. *Weed Technology*, 8(2), 392-395.
- Buchanan, G. A., & Burns, E. R. (1970). Influence of weed competition in cotton. *Weed Science*, 18(1), 149-154.
- Buhler, D. D. (2002). Challenges and opportunities for integrated weed management. *Weed Science*, 50(3), 273-280.
- Buhler, D. D., Hartzler, R. G., & Forcella, F. (1998). Weed seed bank dynamics: implications to weed management. *Journal of Crop Production*, 1(145-168).
- Burgess, R. W., & Powell, A. A. (1984). Evidence for repair processes in the invigoration of seeds by hydration. *Annals of Botany*, 53, 753-757.
- Burnside, O. C., Wilson, R. G., Weisberg, S., & Hubbard, K. G. (1996). Seed longevity of 41 weed species buried 17 years in eastern and Western Nebraska. *Weed Science*, 44(1), 74-86.
- Carpenter, J. L., & Hopen, H. J. (1985). A comparison of the biology of wild and cultivated proso millet (*Panicum miliaceum*). *Weed Science*, 33, 795-799.

- Cavers, P. B., & Bough, M. A. (1985). Proso millet (*Panicum miliaceum* L.): a crop and a weed. In J. White (Ed.), *Studies on plant demography* (pp. 143-155). Orlando: Academic Press.
- Cavers, P. B., Kane, M., & O'Toole, J. J. (1992). Importance of seedbanks for establishment of newly introduced weeds- a case study of proso millet (*Panicum miliaceum*). *Weed Science*, 40(4), 630-635.
- Chambers, J. C., & MacMahon, J. A. (1994). A day in life of a seed: movements and fates of seeds and their implications for natural and managed systems. *Annual Review of Ecology and Systematics*, 25, 263-292.
- Chancellor, R. J. (1982). Dormancy in weed seeds. *Outlook on agriculture*, 11(2), 87-93.
- Chantre, G. R., Sabbatini, M. R., & Orioli, G. A. (2008). Effect of burial depth and soil water regime on the fate of *Lithospermum arvense* seeds in relation to burial time. *Weed Research*, 49, 81-89.
- Coolbear, P., Francis, A., & Grierson, D. (1984). The effect of low-temperature pre-sowing treatment on the germination performance and membrane integrity of artificially aged tomato seeds. *Journal of Experimental Botany*, 35, 1609-1617.
- Cruttwell McFadyen, R. E. (1998). Biological control of weeds. *Annual Review of Entomology*, 43, 369-393.
- Csontos, P., & Tamas, J. (2003). Comparison of soil seed bank classification systems. *Seed Science Research*, 13, 101-111.
- Cunningham, D. C., Woldendorp, G., Burgess, M. B., & Barry, S. C. (2003). *Prioritising sleeper weeds for eradication: Selection of species based on potential impacts on agriculture and feasibility of eradication*, Canberra: Bureau of Rural Sciences.
- Cussans, G. W., Raudonius, S., Brain, P., & Cumberworth, S. (1996). Effect of depth of seed burial and soil aggregate size on seedling emergence of *Alopecurus myosuroides*, *Galium aparine*, *Stellaria media* and wheat. *Weed Research*, 36, 133-141.
- Davies, H., & Probert, R. J. (2004). Protocol for comparative seed longevity testing. In (The Millennium Seed Bank, The Royal Botanic Gardens, Kew.: Wakehurst Place, West Sussex UK.).

- Debeaujon, I., Leon- Kloosterziel, K. M., & Koornneef, M. (2000). Influence of the testa on seed dormancy, germination, and longevity in *Arabidopsis*. *Plant physiology*, *122*, 403-413.
- Dippery, J. K., Tissue, D. T., Thomas, R. B., & Strain, B. R. (1995). Effects of low and elevated CO₂ on C₃ and C₄ annuals. I. Growth and biomass allocation. *Oecologia*, *101*, 13-20.
- Drew, M. C. (1992). Soil aeration and plant root metabolism. *Soil Science*, *154*(4), 259-268.
- Eberlein, C. V., Lurvey, E. L., Miller, T. L., & Michael, J. L. (1990). Growth and development wild-proso millet (*Panicum miliaceum*) biotypes. *Weed Technology*, *4*(2), 415-419.
- Edwards, T. L., Crucifix, M., & Harrison, S. P. (2007). Using the past to constrain the future: how the palaeorecord can improve estimates of global warming. *Progress in Physical Geography*, *31*, 481-500.
- Egley, G. H., & Chandler, J. M. (1983). Longevity of weed seeds after 5.5 years in the Stoneville 50-year buried-seed study. *Weed Science*, *31*, 264-270.
- Ehleringer, J. R., Sage, R. F., Flanagan, L. B., & Pearcy, R. W. (1991). Climate change and evolution of C₄ photosynthesis. *Trends in Ecology and Evolution*, *6*(3), 95-99.
- Fantroussi, S., Verschuere, L., Verstraete, W., & Top, E. M. (1999). Effect of Phenylurea herbicides on soil microbial communities estimated by analysis of 16S rRNA gene fingerprints and community-level physiological profiles. *Applied and Environmental Microbiologist*, *65*(3), 982-988.
- Fausey, J. C., Kells, J. A., Swinton, S. M., & Renner, K. A. (1997). Giant foxtail (*Setaria faberi*) interference in nonirrigated corn (*Zea mays*). *Weed Science*, *45*(256-260).
- Fenner, M. (1985). *Seed ecology*. New York: Chapman and Hall.
- Forcella, F. (1992). Prediction of weed seedlings densities from buried seed reserves. *Aspects of Applied Biology*, *69*, 151-162.
- Forcella, F. (2003). Debiting the seed bank: priorities and predictions. *Aspects of Applied Biology*, *69*, 151-162.

- Forcella, F., Eradat-Oskoul, K., & Wagner, S. W. (1993). Application of weed seedbank ecology to low-input crop management. *Ecological Applications*, 3(1), 74-83.
- Froud-Williams, R. J., Chancellor, R. J., & Drennan, D. S. H. (1984). The effect of seed burial and soil disturbance on emergence and survival of arable weeds in relation to minimal cultivation. *Journal of Applied Ecology*, 21, 629-641.
- Garcia-Huidobro, J., Monteith, J. L., & Squire, G. R. (1982). Time, temperature and germination of pearl millet (*Pennisetum typhoides* S. & H.). *Journal of Experimental Botany*, 33(2), 288-296.
- Gardestrom, P., & Edwards, G. E. (1983). Isolation of mitochondria from leaf tissue of *Panicum miliaceum*, a NAD- Malic enzyme type C₄ plant. *Plant physiology*, 71, 24-29.
- Gavito, M. E., Curtis, P. S., Mikkelsen, T. N., & Jokobsen, I. (2001). Interactive effects of soil temperature, atmospheric carbon dioxide and soil N on root development, biomass and nutrient uptake of winter wheat during vegetative growth. *Journal of Experimental Botany*, 52, 1913-1923.
- Ghannoum, O., von Caemmerer, S., Ziska, L. H., & Conroy, J. P. (2000). The growth response of C₄ plants to rising atmospheric CO₂ partial pressure: a reassessment. *Plant, Cell and Environment*, 23, 931-942.
- Gonzales- Zertuche, L., Vazquez- Yanes, C., Gamboa, A., Sanchez- Conrado, M. E., Aguilera, P., & Orozco- Segovia, A. (2001). Natural priming of *Wigandia urens* seeds during burial: effect on germination growth and protein expression. *Seed Science Research*, 11, 27-34.
- Grundy, A. C., Mead, A., & Bond, W. (1996). Modelling the effect of weed- seed distribution in the soil profile on seedling emergence. *Weed Research*, 36, 375-384.
- Grundy, A. C., Mead, A., & Burston, S. (2003). Modelling the emergence response of weed seeds to burial depth: interactions with seed density, weight and shape. *Journal of Applied Ecology*, 40, 757-770.
- Guo, P., & Al- Khatib, K. (2003). Temperature effect on germination and growth of redroot pigweed (*Amaranthus retroflexus*), Palmer amaranth (*A. plameri*), and common waterhemp (*A. rudis*). *Weed Science*, 51, 869-875.

- Harris, P. (1988). Environmental impact of weed-control insects. *Bioscience*, 38(8), 542-548.
- Harvey, R. G. (1979). Wild proso millet: Serious new weed threat. *Crops Soils Management*, 31(6), 10-13.
- Harvey, R. G., McNevin, G. R., Albright, J. W., & Kozak, M. E. (1986). Wild proso millet (*Panicum miliaceum*) control with thiocarbamate herbicides on previously treated soils. *Weed Science*, 34(5), 773-780.
- Hendry, G. A. F., Thompson, K., Moss, C. J., Edwards, E., & Thorpe, P. C. (1994). Seed persistence: a correlation between seed longevity in the soil and ortho-dihydroxyphenol concentration. *Functional Ecology*, 8(658-664).
- Hillhorst, H. W. M., & Karssen, C. M. (2000). Effect of chemical environment on seed germination. In M. Fenner (Ed.), *Seeds: The Ecology of Regeneration in Plant Communities* (pp. 293-310). Wallingford, UK: CABI Publishing.
- Hodkinson, D. A., Askew, A., Thompson, K., Hodgson, J. G., Bakker, J. P., & Bekker, R. M. (1998). Ecological correlates of seed size in the British flora. *Functional Ecology*, 12, 762-766.
- Hoekstra, E. A., Crowe, J. H., & Crowe, L. M. (1992). Germination and ion leakage are linked with phase transitions of membrane lipids during imbibition of *Typha latifolia* pollen. *Physiologia plantarum*, 84, 29-34.
- Hoekstra, F. A., Golovina, E. A., & Buitink, J. (2001). Mechanism of plant desiccation tolerance. *Trends in Plant Science*, 6(9), 431-438.
- Hollingsworth, R. (2000). War of the weeds. *New Zealand Wilderness*.
- Holzner, W., & Numata, M. (Eds.). (1982). *Biology and Ecology of weeds*. Boston, USA: Dr. W. Junk Publishers.
- Houghton, J. T., Callander, B. A., & varney, S. K. (1992). *Climate change 1992*. Cambridge: Press Syndicate of the University of Cambridge.
- ISTA (1991). *In Zurich: International Rule for Seed Testing*: International Seed Testing Association.
- ISTA (2003). ISTA handbook on seedling evaluation. In R. Don (Ed.). Basserdorf, Switzerland: The International Seed Testing Association.
- Jackson, S. T. (2007). Looking forward from the past: history, ecology and conservation. *Frontiers in Ecology and the Environment*, 5(9), 455.

- Jacobsohn, R., Greenberger, A., Katan, J., Levi, M., & Alon, H. (1980). Control of Egyptian broomrape (*Orbanche aegyptiaca*) and other weeds by means of solar heating of the soil by polyethylene mulching. *Weed Science*, 28(3), 312-316.
- James, T. K., & Rahman, A. (1999). Survival of giant butter cup seeds buried at different depths in four soils. *New Zealand Plant Protection*, 52, 234-239.
- James, T. K., & Rahman, A. (2000). Longevity of buried ragwort seed in four soil. *New Zealand Plant Protection*, 53, 253-257.
- James, T. K., & Rahman, A. (2001). *Longevity of buried Cirsium arvense seed in four New Zealand soils*. Paper presented at the 18th Asian-Pacific Weed Science Society Conference, Beijing, China.
- James, T. K., & Rahman, A. (2003). Survival of scotch thistle seed buried at three depths in four New Zealand soils. *New Zealand Plant Protection*, 56, 113-117.
- James, T. K., & Rahman, A. (2009). Efficacy of pre-emergence herbicides on three annual grass weeds in different soils. *New Zealand Plant Protection*, 62, 356-362.
- James, T. K., Rahman, A., & Mellsop, J. (2000). Weed competition in maize crop under different timings for post-emergence weed control. *New Zealand Plant Protection*, 53, 269-272.
- James, T. K., Rahman, A., & Trivedi, P. (2010, In Press). *Broom corn millet (Panicum miliaceum): A new menace for maize and sweet corn growers in New Zealand*. Paper to be presented at the 17th Australasian Weeds Conference, Christchurch.
- James, T. K., Rahman, A., & Trivedi, P. (2010). Germination of seed from five broadleaf weeds after 28 years burial in two soils. *New Zealand Plant Protection*, 63, In Press.
- James, T. K., Rahman, A., Wardle, D. A., & Bonner, K. I. (1998). Survival of nodding thistle (*Carduus nutans*) seed buried at different depths in four soils. *New Zealand Plant Protection*(51), 33-37.
- James, T. K., Rahman, A., Webster, T., & Waller, J. (2002). Emergence of weeds as affected by vertical seed distribution in arable soils. *New Zealand Plant Protection*, 55, 213-217.

- Jessep, C. T. (1990). Aspects of the biology of nodding thistle (*Carduus nutans* L.) in Canterbury, New Zealand. *New Zealand Journal of Agricultural Research* 33, 173-183.
- Jianhua, Z., & McDonald, M. (1997). The saturated salt accelerated aging test for small-seeded crops. *Seed Science and Technology*, 25(1), 123-131.
- Jones, J. S., & Yamazaki, T. (1974). Genetic background and fitness of allozymes. *Genetics*, 78, 1185-1189.
- Karam, D., Westra, P., Nissen, S. J., Ward, S. M., & Figueiredo, J. E. F. (2004). Genetic diversity among proso millet (*Panicum miliacem*) biotypes assessed by AFLP technique. *Planta Daninha*, 22(2), 167-174.
- Kasperbauer, M. J., & Karlen, D. L. (1994). Plant spacing and reflected far-red light effects on phytochrome- regulated photosynthate allocation in corn seedlings. *Crop Science*, 34(1564-1569).
- Katan, J. (1981). Solar heating (solarization) control of soil born pests. *Annual Review of Phytopathology*, 19, 211-236.
- Kenny, G. (2001). *Climate change: Likely impacts on New Zealand agriculture*. Wellington: Ministry for the Environment.
- Khan, M., Cavers, P. B., Kane, M., & Thompson, K. (1996). Role of the pigmented seed coat of proso millet (*Panicum miliaceum* L.) in imbibition, germination and seed persistence. *Seed Science Research*, 7, 21-25.
- Kigel, J., Lior, E., & Zamir, L. (1992). Biology of reproduction in the summer annual weed *Euphorbia geniculata* Ortega. *Weed Research*, 32, 317-328.
- Kivilaan, A., & Bandurski, R. S. (1981). The one hundred- year period for Dr. Beal's seed viability experiment. *American Journal of Botany*, 68(9), 1290-1292.
- Knake, E. L., & Slife, F. W. (1962). Competition of *Setaria faberii* with corn and soybeans. *Weeds*, 10, 26-29.
- Kok, L. T. (2001). Classical biological control of nodding and plumeless thistles. *Biological Control*, 21, 206-213.
- Kremer, R. J. (1993). Management of seed banks with microorganisms. *Ecological Applications*, 3, 42-52.
- Kremer, R. J. (2000). *Combination of microbial and insect biocontrol agents for management of weed seeds*. Paper presented at the Proceedings of the X International Symposium on Biological Control of Weeds, Montana, USA.

- Kropff, M. J. (1988). Modelling the effect of weeds on crop production. *Weed Research*, 28, 465-471.
- Kropff, M. J., & Spitters, C. J. T. (1991). A simple model of crop loss by weed competition from early observations on relative leaf area of weeds. *Weed Research*, 31, 97-105.
- Kurstjens, D. A. G., & Kropff, M. J. (2001). The impact of uprooting and soil-covering on the effectiveness of weed harrowing. *Weed Research*, 41, 221-228.
- Lafold, G. P., & Baker, R. J. (1986). Effects of genotype and seed size on speed of emergence and seedling vigour in nine spring wheat cultivars. *Crop Science*, 26, 341-346.
- Lamour, A., & Lotz, A. P. (2007). The importance of tillage depth in relation to seedling emergence in stale seedbeds. *Ecological Modelling*, 20(I), 536-546.
- Landcare Research (2009). Weeds in New Zealand Retrieved 24/8/2009, from <http://www.landcareresearch.co.nz/education/weeds/>
- Lee, W. G., Williams, P. A., & Cameron, E. (1999). Managing urban weeds and pests. *New Zealand Plant Protection*, 52, 43-58.
- Leegood, R. C. (2002). C₄ photosynthesis: principles of CO₂ concentration and prospects for its introduction into C₃ plants. *Journal of Experimental Botany*, 53(369), 587-590.
- Leishman, M. R., & Westoby, M. (1998). Seed size and shape are not related to persistence in soil in Australia in the same way as Britain. *Functional Ecology*, 12, 480-485.
- Lenz, T. I., & Facelli, J. M. (2005). The role of seed limitation and resource availability in the recruitment of native perennial grasses and exotics in a South Australian grassland. *Austral Ecology*, 30, 684-694.
- Lewis, J. (1973). Longevity of crop and weed seeds: survival after 20 years in soil. *Weed Research*, 13, 179-191.
- Liebman, M., & Dyck, E. (1993). Crop rotation and intercropping strategies for weed management. *Ecological Applications*, 3(1), 92-122.
- Long, R. L. (2007). *Predicting weed seed persistence: towards a technique for rapid and reliable assessment*. The University of Queensland.

- Long, R. L., Panetta, F. D., Steadman, K. J., Probert, R., Bekker, R. M., Brookes, S., et al. (2008). Seed persistence in the field may be predicted by laboratory-controlled ageing. *Weed Science*, 56, 523-528.
- Long, S. P. (1983). C₄ photosynthesis at low temperature. *Plant, Cell and Environment*, 6(4), 345-363.
- Long, S. P., Ainsworth, E. A., Rogers, R., & Ort, D. R. (2004). Rising atmospheric carbon dioxide: plants face the future. *Annual Review of Plant Biology*, 55, 591-628.
- Lu, H., Yang, X., Ye, M., Liu, K., Xia, Z., Ren, X., et al. (2005). Millet noodles in late Neolithic area. *Nature*, 437, 967-968.
- Madigan, M. T., & Martinko, J. M. (2006). *Brock biology of microorganisms* (11 ed.). New Jersey: Pearson Prentice Hall
- Mayr, E. (1942). *Systematic and origin of species*. New York: Columbia University Press.
- McGill, C. (2009). Interim report: Seed germination of *Panicum miliaceum*. Massey University.
- McLaren, R. G., & Cameron, K. C. (2002). *Soil Science- Sustainable production and environmental protection* (Second ed.). Melbourne: Oxford University Press.
- Menzel, C. M., Simpson, D. R., & Winks, C. R. (1987). Effect of temperature on growth, flowering and nutrient uptake of three passionfruit cultivars under low irradiance. *Scientia Horticulturae*, 31(3-4), 259-268.
- Meyers, S. P., Neson, C. J., & Horrocks, R. D. (1984). Temperature effects on imbibition, germination and respiration of sorghum seeds. *Field Crops Research*, 8, 135-142.
- Milberg, P., Andersson, L., Elfverson, C., & Regner, S. (1996). Germination characteristics of seeds differing in mass. *Seed Science Research*, 6, 191-197.
- Miller, S. D., & Nalewaja, J. D. (1990). Influence of burial depth on wild oat (*Avena fatua*) seed longevity. *Weed Technology*, 4, 514-517.
- Moles, A. T., Warton, D. I., & Westoby, M. (2003). Seed size and survival in soil in arid Australia. *Austral Ecology*, 28, 575-585.
- Moles, A. T., & Westoby, M. (2006). Seed size and plant strategy across the whole life cycle *Oikos*, 113, 91-105.

- Moore, D. R., & Cavers, P. B. (1985). A comparison of seedling vigour in crop and weed biotypes of proso millet (*Panicum miliaceum*). *Canadian Journal of Botany*, 63(9), 1659-1663.
- Mudroch, A. J., & Ellis, R. H. (1992). Longevity, viability and dormancy. In M. Fenner (Ed.), *Seeds- the ecology of regeneration in plant communities* (pp. 183-214). Wallingford: CAB International.
- Mumford, J. D. (1982). Perceptions of losses from pests to arable crops by some farmers in England and New Zealand. *Crop Protection*, 1(3), 283-288.
- Neave, M. J. (2004). *Seed longevity of Carduus nutans in Australia: consequences for weed management*. Paper presented at the 14th Australian Weeds Conference, Wagga Wagga.
- Nelson, D. C., & Nylund, R. E. (1962). Competition between peas grown for processing and weeds. *Weeds*, 10, 224-229.
- NIWA (2008). Climate change- Projections for New Zealand. Retrieved 26/4/2010, from http://www.niwa.co.nz/data/assets/pdf_file/0007/75958/IPCC_08_report_02s.pdf
- NIWA (2010). CliFlo National Climate Database. National Institute of Water and Atmospheric Research.
- Obeid, M., Machin, D., & Harper, J. L. (1967). Influence of density on plant to plant variations in fiber flax (*Linum usitatissimum*). *Crop Science*, 7, 471-473.
- Oerke, E. (2005). Crop losses to pests. *The Journal of Agricultural Science*, 144(1), 31-43.
- Oliver, L. R. (1988). Principals of weed threshold research. *Weed Technology*, 2, 398-403.
- O'Toole, J. J., & Cavers, P. B. (1983). Input to seed banks of proso millet (*Panicum miliaceum*) in Southern Ontario. *Canadian Journal of Plant Science*, 63, 1023-1030.
- Panetta, F. D. (2004). Seed banks: the bane of the weed eradicator. In B. M. Sindel & S. B. Johnson (Eds.), *14th Australian Weed Conference*. Sydney: Weed Science Society of New South Wales.
- Pareja, M. R., & Staniforth, D. W. (1985). Seed-soil characteristics in relation to weed seed germination. *Weed Science*, 33, 190-195.

- Patterson, D. T. (1995). Effects of environmental stress on weed/crop interaction. *Weed Science*, *43*, 483-490.
- Pearcy, R. W., Tumosa, N., & Williams, K. (1981). Relationships between growth, photosynthesis and competitive interactions for a C₃ and C₄ plant. *Oecologia*, *48*, 371-376.
- Picman, J., & Picman, A. K. (1984). Autotoxicity in *Parthenium hysterophorus* and its possible role in control of germination. *Biochemical Systematics and Ecology*, *12*, 287-292.
- Pitterman, J., & Sage, R. F. (2000). Photosynthetic performance at low temperature of *Bouteloua gracilis* Lag., a high-altitude C₄ grass from the Rocky Mountains, USA. *Plant Cell Environment*, *23*, 811-823.
- Popay, A. I., & Medd, R. W. (1990). The biology of Australian weeds 21. *Carduus nutans* L. ssp *nutans* *Plant Protection Quarterly*, *5*(1), 3-13.
- Porter, J. R., & Gawith, M. (1999). Temperatures and the growth and development of wheat: a review. *European Journal of Agronomy*, *10*, 23-36.
- Powell, J. R., & Richmond, R. C. (1974). Founder effects and linkage disequilibria in experimental populations of *Drosophila*. *Proceedings of the National Academy of Sciences of the United States of America*, *71*(5), 1663- 1665.
- Probert, R. J. (2000). The role of temperature in the regulation of seed dormancy and germination. In M. E. Fenner (Ed.), *Seeds the ecology of regeneration in plant communities* (pp. 261-292). Wallingford, UK: CABI Publishing.
- Probert, R. J., Daws, M. L., & Hay, F. R. (2009). Ecological correlates of *ex situ* seed longevity: a comparative study on 195 species. *Annals of Botany*, *104*, 57-69.
- Radosevich, S. R. (1987). Methods to study interaction among crops and weeds. *Weed Technology*, *1*(3), 190-198.
- Rahman, A., & James, T. K. (1993). *Patterns of weed seedling emergence in two New Zealand soils*. Paper presented at the 8th EWRS Symposium, Braunschweig.
- Rahman, A., James, T. K., Bourdot, G., & Grbavac, N. (1998). Weed seed bank estimation, spatial distribution, decline and potential for predicting future weed populations. *Plant Protection Quarterly*, *13*(3), 117-122.

- Rahman, A., James, T. K., Mellso, J., & Grbavac, N. (2000). Effect of cultivation methods on weed seed distribution and seedling emergence. *New Zealand Plant Protection*, 53, 28-33.
- Rajcan, I., & Swanton, C. J. (2001). Understanding- maize weed competition: resource competition, light quality and whole plant. *Field Crops Research*, 71, 139-150.
- Ramirez, F. A., & Nieto, J. H. (1968). The critical periods of competition between weeds and winter cotton in the irrigated valley of Mochis, Sins, Mexico. *Weed Science Society of America*, Abstract p. 152.
- Raymond, P. A., Al- Ani, A., & Pradet, A. (1985). ATP production by respiration and fermentation, and energy charge during aerobiosis and anaerobiosis in twelve fatty and starchy germination seeds. *Plant Physiology*, 79, 879-884.
- Reddy, V. G., Upadhyaya, H. D., & Gowda, C. L. L. (2007). Morphological characterisation of world's proso millet germplasm collection. *An open access journal published by International Crop research Institute for Semi- Arid Tropics*, 3(1).
- Relyea, R. A. (2005). The impact of insecticides and herbicides on the biodiversity and productivity of aquatic communities. *Ecological Applications*, 15(2), 618-627.
- Reuss, S. A., Buhler, D. D., & Gunsolus, J. L. (2001). Effects of soil depth and aggregate size on weed seed distribution and viability in a silt loam soil. *Applied Soil Ecology*, 16(3), 209-217.
- Rice, K. J., & Dyer, A. R. (2001). Seed aging, delayed germination and reduced competitive ability in *Bromus tectorum*. *Plant Ecology*, 155, 237-243.
- Riemens, M. M., Groeneveld, R. M. W., Lotz, L. A. P., & Kropff, M. J. (2007). Effects of three management strategies on the seedbank, emergence and the need for hand weeding in an organic arable cropping system. *Weed Research*, 47, 442-451.
- Rubin, B., & Benjamin, A. (1984). Solar heating of the soil: Involvement of environmental factors in the weed control process *Weed Science*, 32, 138-142.
- Russell, E. W. (1988). The temperature of the soil. In A. Wild (Ed.), *Soil Conditions and Plant Growth* (Eleventh ed., pp. 293-297). Essex: Longman Scientific & Technical.

- Ryan, M. R., Smith, R. G., Mortensen, D. A., Teasdale, J. R., Curran, W. S., Seidel, R., et al. (2009). Weed–crop competition relationships differ between organic and conventional cropping systems. *Weed Research*, 49(6), 572-580.
- Sage, R. F. (2002). Variation in the k_{cat} of rubisco in C₃ and C₄ plants and some implications for photosynthetic performance at high and low temperature. *Journal of Experimental Botany*, 53, 609-620.
- Sage, R. F., & Monson, R. K. (Eds.). (1999). *C₄ Plant Biology*. California: Academic Press.
- Savidge, J. A. (1987). Extinction of an island forest avifauna by an introduced snake. *Ecology*, 68, 660-668.
- Sawma, J. T., & Mohler, C. L. (2002). Evaluating seed viability by an unimbibed seed crush test in comparison with the tetrazolium test. *Weed Technology*, 16, 781-786.
- Schaal, R. (1991). Estimation in generalized linear models with random effects. *Biometrika*, 78(4), 719-727.
- Schafer, D. E., & Chilcote, D. O. (1970). Factors influencing persistence and depletion in buried seed populations. II. The effect of soil temperature and moisture. *Crop Science*, 10, 342- 345.
- Scholes, C., Clay, S. A., & Brix-Davis, K. (1995). Velvetleaf (*Abutilon theophrasti*) effect on corn (*Zea mays*) growth and yield in South Dakota. *Weed Technology*, 9, 665-668.
- Schulze, E.-D., Ellis, R., Schulze, W., Trimborn, P., & Ziegler, H. (1996). Diversity, metabolic types and $\delta^{15}\text{C}$ carbon isotope ratios in the grass flora of Namibia in relation to growth form, precipitation and habitat conditions *Oecologia*, 106, 352-369.
- Schwaegerle, K. E., & Schaal, B. A. (1979). Genetic variability and founder effect in the pitcher plant *Sarracenia purpurea* L. *Evolution*, 33(4), 1210-1218.
- Seiwa, K., Watanabe, A., Saitoh, T., Kannu, H., & Akasaka, S. (2002). Effects of burying depth and seed size on seedling establishment of Japanese chestnuts, *Castanea crenata*. *Forest Ecology and Management*, 164, 149-156.
- Shadbolt, C. A., & Holm, L. G. (1956). Some quantitative aspects of weed competition in vegetable crops. *Weeds*, 4(2), 111-123.

- Shea, K., Kelly, D., Sheppard, A. W., & Woodburn, T. L. (2006). Context-dependent biological control of an invasive thistle. *Ecology*, 86(12), 3174-3181.
- Smith Jr., R. J. (1968). Weed competition in rice. *Weed Science*, 16(2), 252-255.
- Smith, M. T., & Berjak, P. (1995). Deteriorative changes associated with the loss of viability of stored desiccation-tolerant and desiccation sensitive seeds. In J. Kigel & G. Galili (Eds.), *Seed development and germination*. New York: Marcel Dekker.
- Smith, M. T., & Berjak, P. (1995). Deteriorative changes associated with the loss of viability of stored desiccation-sensitive seeds. In J. Kigel & J. Galili (Eds.), *Seed development and germination*. New York: Marcel Dekker.
- Smith, R. D., Dickie, J. B., Linington, S. H., Pritchard, H. W., & Probert, R. J. (Eds.). (2003). *Seed Conservation- Turning Science into Practice*. Kew, UK: Royal Botanic Gardens.
- So, Y. F., Williams II, M. M., Pataky, J. K., & Davis, A. S. (2009). Principal canopy factors of sweet corn and relationships to competitive ability with wild-proso millet (*Panicum miliaceum*). *Weed Science*, 57, 296-303.
- Stat Trek (2009). Binomial distribution calculator. from <http://stattrek.com/Tables/Binomial.aspx>
- Stewart, D. W., Dwyer, L. W., & Carrigan, L. L. (1998). Phenological temperature response of maize. *Agronomy Journal*, 90(1), 73-79.
- Stoller, E. W., & Wax, L. M. (1973). Periodicity of germination and emergence of some annual weeds. *Weed Science*, 21, 574-580.
- Sung, J. M. (1996). Lipid peroxidation and peroxide scavenging in soybean seeds during aging. *Physiologia plantarum*, 97, 85-89.
- Sutherland, S. (2004). What makes a weed a weed: life history traits of native and exotic plants. *Oecologia*, 141, 24-39.
- Tanksley, S. D., & McCouch, S. R. (1997). Seed bank and molecular maps: Unlocking genetic potential from wild. *Science*, 277(5329), 1063-1066.
- Telewski, F. W., & Zeevaart, J. A. D. (2002). The 120-YR period for Dr. Beal's seed viability experiment. *American Journal of Botany*, 89(8), 1285-1288.
- Terpstra, R. (1995). Dormancy of seeds of shepherd's purse in alternating wet and dry, compressed aggregated soil: a laboratory experiment. *Journal of Applied Ecology*, 32, 434-444.

- Thompson, K. (1987). Seeds and seedbank. *New Phytology*, 106(Suppl.), 23-34.
- Thompson, K., Bakker, J. P., Bekker, R. M., & Hodgson, J. G. (1998). Ecological correlates of seed persistence in soil in the north-west European flora. *Journal of Ecology*, 86, 163-169.
- Thompson, K. (1992). The functional ecology of seed banks. In M. Fenner (Ed.), *Seeds: the Ecology of Regeneration in Plant Communities*. Wallingford, UK: CAB International.
- Thompson, K., Band, S. R., & Hodgson, J. G. (1993). Seed size and shape predict persistence in soil. *Functional Ecology*, 7, 553-563.
- Tilman, D. (1999). Global environmental impacts of agricultural expansion: The need for sustainable and efficient practices. *Proceedings of the National Academy of Sciences*, 96, 5995-6000.
- Tilman, D., Cassman, K. G., Matson, P. A., Naylor, R., & Stephen, P. (2002). Agriculture sustainability and intensive production practices. *Nature*, 418, 671-677.
- Tissue, D. T., Griffin, K. L., Thomas, R. B., & Strain, B. R. (1995). Effect of low and elevated CO₂ on C₃ and C₄ annuals. II. Photosynthesis and biochemistry. *Oecologia*, 101, 21-28.
- Trawatha, S. E., TeKrony, D. M., & Hildebrand, D. F. (1995). Soybean lipoxygenase mutants and seed longevity. *Crop Science*, 35, 1415-1422.
- Trivedi, P. D., James, T. K., & Clearwater, M. J. (2010). *Methods of scarification to overcome dormancy in Bryonia cretica subsp. Dioica (Jacq.) Tutin (white bryony) seeds*. Poster presented at the NZBIO Conference, Auckland.
- Turner, D. W., & Lahav, E. (1985). Temperature influences nutrient absorption and uptake rates of banana grown in controlled environments. *Scientia Horticulturae*, 26(4), 311-322.
- Van Mourik, T. A., Stomph, T. J., & Mudroch, A. J. (2005). Why high seed densities within buried mesh bags may overestimate depletion rates of soil seed banks. *Journal of Applied Ecology*, 42, 299-305.
- Venable, L. D. (1989). Modelling the evolutionary ecology of seed banks. In M. A. Leck, V. T. Parker & R. L. Simpson (Eds.), *Ecology of soil seed banks* (pp. 67-97). London: Academic Press.

- Vertucci, C. W. (1989). The effect of lower water contents on physiological activities for seeds. *Physiologia Plantarum*, 77, 172-176.
- Vertucci, C. W., & Farrant, J. M. (1995). Acquisition and loss of desiccation tolerance. In J. Kigel & G. Galili (Eds.), *Seed development and germination*. New York: Marcel Dekker.
- Veteli, L. O., Kuokkanen, K., Julkunen-Tiitto, R., Roininen, R., & Tahvanainen, J. (2002). Effects of elevated CO₂ and temperature on plant growth and herbivore defence chemistry. *Global Change Biology*, 8, 1240-1252.
- Vleeshouwers, L. M. (1996). Modelling the effect of temperature, soil penetration resistance, burial depth and seed weight on pre-emergence growth of seeds. *Annals of Botany*, 79, 553-563.
- Vleeshouwers, L. M. (1996). Modelling the effect of temperature, soil penetration resistance, burial depth and seed weight on pre-emergence growth of weeds. *Annals of Botany*, 79, 553-563.
- Walters, C. (1998). Understanding the mechanisms and kinetics of seed aging. *Seed Science Research*, 8, 223-244.
- Walters, C., Hill, L. M., & Wheeler, L. J. (2005). Dying while dry: Kinetics and mechanism of deterioration in desiccated organism. *Integrative and Comparative Biology*, 45, 751-758.
- Ward, J. K., Myres, D. A., & Thomas, R. B. (2008). Physiological and growth response of C₃ and C₄ plants to reduced temperature when grown at low CO₂ of the last ice age. *Journal of Integrative Plant Biology*, 50(11), 1388-1395.
- Wardle, D. A., Nicholson, K. S., & Rahman, A. (1993). Influence of age on the allelopathic potential of nodding thistle (*Carduus nutans* L.) against pastures grasses and legumes. *Weed Research*, 33, 69-78.
- Warr, S. J., Thompson, K., & Kent, M. (1993). Seed banks as a neglected area of biogeographic research: a review of literature and sampling techniques. *Progress in Physical Geography*, 17(3), 329-347.
- Watt, M. S., Whitehead, D., Mason, E. G., Richardson, B., & Kimberley, M. O. (2003). The influence of weed competition for light and water on growth and dry matter partitioning of young *Pinus radiata*, at a dryland site *Forest Ecology and Management*, 183, 363-376.

- Westra, P., Wilson, R. G., & Zimdahl, R. G. (1990). Wild-proso millet (*Panicum miliaceum*) control in Central Great Plains irrigated corn (*Zea mays*). *Weed Technology*, 4(2), 409-414.
- Wiese, A. M., & Binning, L. K. (1987). Calculating the threshold temperature of development for weeds. *Weed Science*, 35, 177-179.
- Williams II, M. M., Boydston, R. A., & Davis, A. S. (2007). Wild proso millet (*Panicum miliaceum*) suppressive ability among three sweet corn hybrids. *Weed Science*, 55, 245-251.
- Williams II, M. M., Boydston, R. A., & Davis, A. S. (2008). Differential tolerance in sweet corn to wild-proso millet (*Panicum miliaceum*) interference. *Weed Science*, 56, 91-96.
- Williams, R. D., & Hoagland, R. E. (1982). The effects of naturally occurring phenolic compounds on seed germination. *Weed Science*, 30(2), 206-212.
- Willson, F. M., & Traveset, A. (2000). The ecology of seed dispersal. In M. Fenner (Ed.), *Seeds: The Ecology of Regeneration in Plant Communities* (2nd ed., pp. 85-110). Wallingford, UK: CABI Publishing.
- Wilson, R. G. (1993). Wild proso millet (*Panicum miliaceum*) interference in dry beans (*Phaseolus vulgaris*). *Weed Science*, 41, 607-610.
- Wilson, R. G., & Westra, P. (1991). Wild proso millet (*Panicum miliaceum*) interference in corn (*Zea mays*). *Weed Science*, 39, 217-220.
- Wuest, S. (2007). Vapour is the principal source of water imbibed by seeds in unsaturated soils. *Seed Science Research*, 17, 3-9.
- Young, J. A., Kay, B. L., George, H., & Evans, R. A. (1980). Germination of three species of *Atriplex*. *Agronomy Journal*, 72, 705-709.
- Zhang, Z., Zhao, M., Lu, H., & Faiia, A. M. (2003). Lower temperature as the main cause of C₄ plant declines during the glacial periods on the Chinese Loess Plateau. *Earth and Planetary Science Letters*, 214, 467-481.
- Ziska, L. H. (2000). The impact of elevated CO₂ on yield loss from a C₃ and C₄ weed in field grown soybean. *Global Change Biology*, 6, 899-905.

Appendices

Appendix 1: Laboratory test results for various soils

Appendix 2: Species notes

Appendix 3: Poster publication

Trivedi, P. D., James, T. K., & Clearwater, M. J. (2010, March).
Methods of scarification to overcome dormancy in *Bryonia cretica* subsp. *Dioica* (Jacq.) Tutin (white bryony) seeds.
Poster presented at the NZBIO 2010 Conference, Auckland.

Appendix 4: Publication 1

James, T. K., Rahman, A., & Trivedi, P. (2010). Germination of seed
from five broadleaf weeds after 28 years burial in two soils.
New Zealand Plant Protection, 63, (In Press).

Appendix 5: Publication 2

James, T. K., Rahman, A., & Trivedi, P. (2010). Broom corn millet
(*Panicum miliaceum*): A new menace for maize and sweet
corn growers in New Zealand. *Proceedings of the 17th
Australasian Weeds Conference*, (In press).

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Any interpretation or recommendations are prepared independently by your consultant

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Soil Lab Test Results								
Sample Name	Soil Group	pH Acidity / Alkalinity	Ca Calcium MAF QT	P Olsen Phosphate µg/ml	K Potassium MAF QT	S(SO ₄) Sulphate Sulphur ppm	Mg Magnesium MAF QT	Na Sodium MAF QT
McLeod Rd	Sedimentary	6.9	15	51	22	16	108	56
Flemming Pdk BSL	Sedimentary	5.1	11	117	15	23	24	5
Brookfield Rd	Sedimentary	5.6	8	36	16	8	27	5
Lawn Rd	Sedimentary	5.7	12	45	27	6	43	5
Valley Rd	Sedimentary	7.0	25	89	28	54	25	15
Stock Rd	Sedimentary	5.2	6	9	6	26	27	7
Corner Brookfield & Gilberson Rd	Sedimentary	5.9	13	63	34	26	42	6
Washpool Stn	Sedimentary	4.6	4	49	7	17	12	9
Flemming Pdk FSL	Sedimentary	5.3	8	66	6	6	19	4
New Plymouth	Sedimentary	6.1	4	7	2	254	35	9
Wanganui	Sedimentary	5.9	9	99	41	20	41	6
Seddon	Sedimentary	5.9	11	27	5	6	34	6
Waikato	Sedimentary	6.0	12	52	15	49	24	6
Peat	Peat	5.3	8	37	5	61	15	5
Gisborne	Sedimentary	7.0	17	30	5	6	25	9

[†] Indicates tests which are not IANZ Registered.

[‡] Indicates Subcontracted Tests

Signed

Brent Miller: Soil & Fert Team Leader



RUAKURA RESEARCH CENTRE, PO Box 281, East Street, HAMILTON

While all care is taken with analyses, we will not accept any responsibility for their resulting use. Source references for Plant Nutrition Status, Animal Health Status and footnotes are obtainable upon request. IANZ accreditation does not apply to comments or graphical representations. These results have been obtained from the sample 'as received' at the laboratory and may not be representative of the bulk material. This report may not be reproduced except in full.
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Form No: 6303675

Sampled: 11-Dec-2009

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Page 2 of 2

Any interpretation or recommendations are prepared independently by your consultant

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Property Name Attn: Pathik Trivedi; Plant Protection

Soil Lab Test Results		
Sample Name	OC ^{TS}	OM ^{TS}
	Organic Carbon %	Organic Matter %
McLeod Rd	2.5	4.4
Flemming Pdk BSL	5.6	9.6
Brookfield Rd	2.3	3.9
Lawn Rd	3.5	6.0
Valley Rd	5.0	8.6
Stock Rd	4.1	7.0
Corner Brookfield & Gilberson Rd	2.9	5.0
Washpool Stn	3.6	6.2
Flemming Pdk FSL	2.2	3.7
New Plymouth	4.7	8.1
Wanganui	3.9	6.8
Seddon	3.1	5.4
Waikato	6.3	10.8
Peat	29.1	50.1
Gisborne	1.8	3.1

1. Soil Mg levels of 20 or less will limit animal pasture Mg requirements and may cause metabolic disorders during calving/lambing. A pasture analysis is recommended.
2. For Ca levels of 5 or less pasture levels may be low at calving or lambing - a pasture analysis is recommended.
3. For K levels greater than 12 a pasture sample should be submitted to check for the availability of Mg Ca and Na for animals.

Test Units and Test Methods			
Test	Unit	Unit Description	Test Method
pH			1:2.1 V/V Water Slurry: Electrode determination
Ca	MAF QT	QT = µg per mL ± 113.6	Ammonium Acetate Extraction: AA determination
P	µg/ml	ppm volume basis	Olsen Extraction: Colorimetry
K	MAF QT	QT = µg per mL ± 18.2	Ammonium Acetate Extraction: AA determination
S(SO ₄)	ppm	mg S per kg	KH ₂ PO ₄ Extraction: Ion Chromatography
Mg	MAF QT	QT = µg per mL ± 4.55	Ammonium Acetate Extraction: AA determination
Na	MAF QT	QT = µg per mL ± 4.55	Ammonium Acetate Extraction: AA determination
OC	%	g C per 100g (dry wgt)	Sub-contracted. Solid sample combusted, CO ₂ produced measured
OM	%	g per 100g (dry wgt)	Calculation: OC x 1.724 (Van Bemmelen factor)

Species notes

Nodding thistle (*Carduus nutans*)

Native to Europe, Siberia, Asia Minor and North Africa *Carduus nutans* (nodding thistle) (Kok, 2001) has been introduced and naturalised in North and South America, Australia and New Zealand (Popay & Medd, 1990, Shea et al. 2005). This annual or usually biennial herb has also been declared as noxious weed in parts of Australia (Popay & Medd, 1990) and in New Zealand (Jessep, 1990). In New Zealand, plant is considered as an important economical weed of pastures where it occupies pasture otherwise available to livestock (Wardle et al. 1993). Therefore, numbers of biological control agents have been released to control this species (Shea & Kelly, 2004).

Nodding thistle grows well in regions where winters are cool with adequate warmth as well as rain during autumn and spring (Popay & Medd, 1990). Herb initially forms a prostrate rosette with undissected leaves with soft spines on margin. Branched tap roots of plants which can penetrate 40 cm or more makes plant more tolerant to drought than other plants of pasture. Nodding thistle is also a prolific seed producer as up to 20,000 seeds per plant have been recorded and significant proportion of fresh seeds is viable. In addition, its seed forms persistent seed bank making it a notorious weed to control and eradicate. However, its susceptibility to common herbicides makes it comparatively easy to control if timing of application is accurate (Popay & Medd, 1990).

White bryony (*Bryonia cretica*)

Native to Europe, West Asia and North Africa white bryony was introduced into New Zealand as garden plant. Found in diverse habitats such as hedges, fence lines, rank grass, native forest, scrub, paddocks and exotic plantations, this climber smothers and shades everything it grows on as it can climb up to six metres. It is already a serious threat to native and desired exotic plants and a potential threat to the economy and landscape value. In addition, its fruit and tubers contain toxic alkaloids. Hence, it has recently been declared as an unwanted organism under the Biosecurity Act 1993 and targeted for eradication under the National Interest Pest initiative led by MAF Biosecurity New Zealand in

partnership with regional councils and the Department of Conservation. Primary dispersion of white bryony is through seed which is aided by birds. However, their shoots also form perennial tuber after dying in autumn which helps in regeneration of plant (Biosecurity New Zealand, 2009).

References:

Biosecurity New Zealand. White bryony factsheet. Retrieved 24/9/2009, from <http://www.biosecurity.govt.nz/files/pests/white-bryony/white-bryony-factsheet.pdf>

Jessep, C. T. (1990). Aspects of the biology of nodding thistle (*Carduus nutans* L.) in Canterbury, New Zealand. *New Zealand Journal of Agricultural Research* 33, 173-183.

Kok, L. T. (2001). Classical biological control of nodding and plumeless thistles. *Biological Control*, 21, 206-213.

Popay, A. I., & Medd, R. W. (1990). The biology of Australian weeds 21. *Carduus nutans* L. ssp *nutans* *Plant Protection Quarterly*, 5(1), 3-13.

Shea, K., Kelly, D., Sheppard, A. W., & Woodburn, T. L. (2006). Context-dependent biological control of an invasive thistle. *Ecology*, 86(12), 3174-3181.

Wardle, D. A., Nicholson, K. S., & Rahman, A. (1993). Influence of age on the allelopathic potential of nodding thistle (*Carduus nutans* L.) against pastures grasses and legumes. *Weed Research*, 33, 69-78.

Methods of scarification to overcome dormancy in *Bryonia cretica* subsp. *dioica* (Jacq.) Tutin (White bryony) seeds

White bryony - what is the problem?

An **exotic environmental weed** capable of **smothering and shading** trees and other objects up to six metres. Berries and tubers contain **toxic alkaloids**.

A **serious threat** to desired exotic plantations and natives and ultimately to economy and landscape values.

Currently **present at three locations** in New Zealand: along the Rangitikei River near Mangaweka, and around Aria and Mokaiti Valley in the King Country.

Undergoing eradication by MAF Biosecurity New Zealand as an unwanted organism.



Why look at seed germination?

Seeds of white bryony are about 5x3 mm in size and have a **hard seed coat** forming a physical dormancy which inhibits their germination.

The **ability to break dormancy** and understanding of germination criteria are required to study other aspects of its biology in the laboratory, such as:

- longevity, using Accelerated Ageing technique
- growth pattern
- time to maturity.

Knowledge of **germination behaviour** and **longevity** of seeds has significant influence on:

- the efficacy and cost of weed eradication
- the frequency and duration of site monitoring.



Red berries turned red and attract birds, aiding seed dispersal.

The challenge

To develop scarification techniques to overcome dormancy



All experiments done in sealed petri dishes

✗	Standard germination method: untreated seeds either on filter paper or in soil, moistened with 0.2% KNO ₃	
✗	Seed treatment methods	Followed by standard germination method , no improvement
✗	Soaking seeds in cold water (3°C to 6°C) for 48 hours.	
✗	Puncturing seed coats with needle	
✗	Treating seeds in concentrated H ₂ SO ₄ for different time periods (5-45 minutes)	
?	Sandpaper-scarified seeds immersed in water of different elevated temperatures (initially 60°C to 100°C), then cooled down to room temperature overnight	
✓	Untreated seeds	Seeds kept on filter paper moistened with gibberellic acid concentrations ranging from 1 ppm to 10 ppm (instead of KNO ₃)
✓	Sandpaper-scarified seeds	Seeds kept on filter paper moistened with gibberellic acid concentrations ranging from 1 ppm to 10 ppm (instead of KNO ₃)
✓	Sandpaper-scarified seeds	Seeds immersed in water of different elevated temperatures (initially 60°C to 100°C), then cooled down to room temperature overnight
		Seeds kept on filter paper moistened with gibberellic acid concentrations ranging from 1 ppm to 10 ppm (instead of KNO ₃)

From all the methods outlined above, only two proved to be significantly successful in overcoming the dormancy barrier in white bryony.

Treatment of white bryony seeds with 10 ppm gibberellic acid concentration, or water at 70°C followed by 10 ppm gibberellic acid, resulted in 55% germination in 15 days using seeds with healthy appearance (seed crushing test, Sawma and Mohler, 2002).

Maximum germination with the standard germination tests was 15%.

Both the successful methods include treatment with gibberellic acid, suggesting its role in the dormancy-breaking phenomenon along with scarification of hard seed coat, by sandpaper with or without elevated water temperatures.

The next step

Knowledge obtained from these experiments allows biologists to understand and study the various aspects of the ecology of this targeted weed. The information will help to further manage eradication and control by setting limits to potential germination from the soil seedbank.

With reliable germination, the Accelerated Ageing technique (Davies and Probert, 2004) can be used to determine likely persistence of seed in the soil.

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References:
Davies, H. and Probert, R. J. (2004). Protocol for comparative seed longevity testing. In (The Millennium Seed Bank, The Royal Botanic Gardens, Kew, Wakehurst Place, West Sussex UK).

Sawma, T. J. and Mohler, L. C. (2002). Evaluating seed viability by an undisturbed seed crush test in comparison with the tetrazolium test. Weed Technology 16: 781-786.

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Farming, Food and Health. First
Te Ahuwhenua, Te Kai me te Whai Ora. Tuatahi



Germination of seed from five broadleaf weeds after burial for up to 28 years in two soils

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Abstract Some herbaceous broadleaf weed species are major weeds of pastures and are difficult to manage with ongoing re-infestation from the persistent soil weed seedbank. In this study, seeds from five weed species were collected in 2009 from two sites where they had been buried at three depths since 1981. The species were Scotch thistle (*Cirsium vulgare*), Californian thistle (*Cirsium arvense*), nodding thistle (*Carduus nutans*), ragwort (*Jacobaea vulgaris*) and giant buttercup (*Ranunculus acris*). None of the seeds were viable after being buried for 28 years in a clay soil, while in a sandy soil seeds of the three thistle species remained viable when buried at 200 mm depth. It is estimated that these seeds may remain viable for up to 66 years.

Keywords buried seed, seed longevity, weed seed, burial depth, soil seedbank, Californian thistle, nodding thistle, Scotch thistle, ragwort, giant buttercup.

INTRODUCTION

The adage 'one year's seeding makes seven year's weeding', which refers to the persistence of viable seed in the soil, has been shown to be true for many weedy species, particularly when they are buried below their normal emergence zone (Benech-Arnold et al. 2000; Benvenuti et al. 2001). In fact, evidence shows that the seed of some weed species persists for more than seven times that 7 years (Telewski & Zeevaert 2002). Seed dormancy is a survival mechanism utilised by many weeds, especially broadleaf species (Burnside et al. 1996), and many farmers have reported weeds, such as ragwort (*Jacobaea vulgaris*) and nodding thistle (*Carduus nutans*), emerging in large numbers after ploughing when no plants have been present for several years.

The traditional method for studying the

longevity and persistence of weed seeds in the soil seedbank is to bury fresh seed in known locations and then dig them up some years later and determine their ability to germinate (e.g. Dawson & Bruns 1975). This was also the method chosen here to study the longevity of seeds of five serious pasture weeds in 1981. Ragwort, nodding thistle, Scotch thistle (*Cirsium vulgare*), Californian thistle (*C. arvense*), and giant buttercup (*Ranunculus acris*) have all been present in New Zealand for more than one hundred years and were most likely introduced in contaminated grass seed brought into the country by early settlers. These five weeds have a reputation for being difficult to manage as well as inducing significant loss of production in pastures (e.g. Harvey & Bourdôt; Bourdôt & Saville 2002; Moore et al. 1989).

Results from the first 16 years after the 1981 seed burial experiment have been reported for individual species (James et al. 1998; James & Rahman 1999, 2000, 2001, 2003). Originally, seed was buried in four different soils in the vicinity of Hamilton. In 2009 all the sites were revisited to remove more seed for evaluation. Unfortunately two of the sites had been destroyed in the interim. This paper summarises the data on the seed viability of these five weed species after 28 years burial at the two remaining sites.

MATERIALS AND METHODS

The two seed burial sites were located on the Horotiu sandy loam and Hamilton clay loam soils in permanent pasture that was regularly grazed or mown (Table 1). Both soils are of volcanic origin; the Horotiu soil is derived from water-deposited material resulting in a free draining coarse textured soil, while the Hamilton soil has a finer texture and is less free draining, being derived from air-deposited material. During autumn 1981 fresh seed of the five test species was collected, cleaned and tested for germination. Approximately 250 seeds of ragwort (0.06 g), giant buttercup (0.31 g) and Californian thistle (0.26 g) and 200 seeds of Scotch thistle (0.49 g) and nodding thistle (0.44 g) were weighed out in preparation for burial.

In June 1981, 90 batches of weighed seed of each of the five species were individually buried at each of the two sites. Each batch was mixed with heat-sterilised soil (60 g) collected from the site and 60 batches were placed in fine nylon mesh bags (0.25 mm mesh). Thirty tubes for each site (250 mm length of perforated, 60 mm diameter plastic drainpipe) were filled with non-sterilised soil from the site. During this process, two seed/soil bags were placed in each tube at 50 mm and 200 mm from the top end of the pipe. The top 20 mm of the pipe was left free of soil and an unbagged seed/soil mixture was placed there, separated from the soil below by a layer of fine nylon mesh but uncovered at the top. The pipe sections were then buried vertically in the appropriate soil type with their tops flush with the soil surface. The tubes were arranged in a regular 10 x 3 matrix at 200 mm centres.

At 2–4 month intervals for the first 3 years after burial, emerged seedlings were counted and removed from the tops of the pipe sections. After 1, 2, 3, 5, 11, 16 and 28 years, three randomly selected pipes were retrieved from each site in June or July and the viable seed from each depth counted. Seed viability was determined by germination in an unheated glasshouse. The contents of each nylon bag and the 0–20 mm layer were spread thinly (2–4 mm) on paper towels laid over damp vermiculite in a tray. At approximately monthly intervals, emerged seedlings were counted and removed. The soil was then thoroughly mixed and the procedure repeated until no further seedlings emerged (4–6 months). On completion of the final incubation the seed/soil mixtures were washed to remove fine particles and then dry sieved to remove large particles. The remaining soil was searched for ungerminated seeds.

Exponential (decay) curves were fitted to all data using the regression command in Minitab and the time taken for the viable seed to fall to 1% of the original amount calculated from the equation for this line. Coefficients of determination (R^2) are presented as an indication of the amount of variation accounted for by both variables.

RESULTS AND DISCUSSION

The viability of the original seed, as determined by the Official Seed Testing Station, Palmerston North, was 80% for Scotch thistle, 81.5% for nodding thistle, 70% for Californian thistle, 43% for giant buttercup and 69% for ragwort. This value was used to determine the theoretical initial number of viable seeds (Time 0, Table 2) buried at each depth. This number was higher than subsequent germination after 1 year for all species except giant buttercup where the low initial germination of just 43% appears to be incorrect as germination in year 1 was as high as 75%.

Results for all the five species show that seed viability was affected most by the burial depth, with a rapid decline in emergence of seed in the 1–20 mm burial zone compared with a much extended seed viability at a depth of 200 mm. Seeds of Scotch thistle and giant buttercup buried

Table 1 Some characteristics of the two soils in which seeds were buried.

Soil	% sand	% clay	% OC ¹	pH	CEC ¹ meq/100g	Field capacity (% v/v)
Horotiu sandy loam	61	15	8.7	5.4	37.4	44.8
Hamilton clay loam	29	31	4.6	5.6	28.2	36.8

¹OC = Organic carbon; CEC = Cation exchange capacity.

in the top 20 mm of the soil mostly disappeared within the first year, with seeds germinating only occasionally in the next few years (Table 2). Nodding thistle seed in the surface layer suffered a fate similar to the above two species, although a very small number was found viable until year 11 (Table 2). In contrast, Californian thistle and ragwort were found to have significant numbers of viable seed in the top 20 mm for up to 5 years; even after 11 years there were a few viable seeds

remaining (Table 2). For all the species, however, the seeds placed in the surface layer lost their viability at a faster rate than at the 50 and 200 mm depths. No seeds were found in the soil after the germination incubation period showing that all viable seeds had germinated and the remaining seeds had disappeared, probably through decay.

Some of the Scotch thistle, nodding thistle and giant buttercup seeds placed in the top layer germinated in the field, mostly during the first

Table 2 Number of seeds germinating over time after burial at different depths in two soils, the coefficient of determination (R²) of the calculated exponential decay curve and the predicted time (years) for seed viability to decline to 1%.

Burial time (years)	Horotiu sandy loam			Hamilton clay loam		
	Burial depth (mm)			Burial depth (mm)		
	1-20	50	200	1-20	50	200
Scotch thistle						
0	159	159	159	159	159	159
1	3	76	120	0	69	134
2	3	79	125	1	57	104
3	2	49	120	0	51	106
5	0	56	85	0	25	100
11	1	57	71	0	17	29
16	0	18	74	0	5	5
28	0	0	15	0	0	0
Time to 1%	3.6	16.9	62.2	1.2	15.1	16.1
R ² (%)	49.8	81.3	90.9	83.6	91.8	92.8
nodding thistle						
0	163	163	163	163	163	163
1	3	87	108	1	115	137
2	7	100	118	2	86	81
3	1	63	110	1	48	77
5	1	63	97	1	28	89
11	5	69	57	0	24	22
16	0	46	52	0	0	1
28	0	1	5	0	0	0
Time to 1%	6.1	30.7	41.9	3.2	10.4	14.7
R ²	58.3	81.6	92.1	76.4	78.2	96.3

Burial time (years)	Horotiu sandy loam			Hamilton clay loam		
	Burial depth (mm)			Burial depth (mm)		
	1-20	50	200	1-20	50	200
Californian thistle						
0	175	175	175	175	175	175
1	51	157	181	49	142	135
2	34	113	172	18	77	108
3	31	112	139	16	66	124
5	25	87	134	11	34	97
11	4	82	92	0	9	15
16	1	37	101	0	2	14
28	0	2	20	0	0	1
Time to 1%	12.8	32.9	65.9	5.5	14.3	25.4
R ²	98.3	91.0	90.9	95.3	97.9	97.6
giant buttercup						
0	108	108	108	108	108	108
1	0	64	118	1	66	172
2	1	6	107	1	6	115
3	0	5	96	1	13	123
5	0	6	47	0	4	52
11	0	4	41	1	1	18
16	0	1	19	0	0	1
28	0	0	0	0	0	0
Time to 1%	1.0	12.9	18.2	3.3	8.1	15.8
R ²	45.7	88.5	84.1	40.4	91.6	97.0
ragwort						
0	172	172	172	172	172	172
1	98	159	185	80	156	196
2	74	138	138	69	149	148
3	55	133	127	37	159	161
5	43	116	130	44	160	179
11	13	53	81	3	4	6
16	0	8	13	0	0	0
28	0	0	0	0	0	0
Time to 1%	9.8	16.6	17.2	9.0	9.9	10.1
R ²	82.6	90.2	86.6	91.3	88.2	86.5

2 years but this accounted for less than 20% of the initial amounts of viable seed (James et al. 1998; James & Rahman 1999, 2003). Thus nearly 80% of the viable seed placed in the top 20 mm remained unaccounted for, which suggests that under normal field conditions much of the seed of these species that falls to the ground is lost within the first year unless it is quickly buried. This rapid disappearance of the seed from the top layer of soil, particularly in the case of giant buttercup,

means that long term control of this weed should be readily attainable if seed production is eliminated before the plants are killed. The case was markedly different for Californian thistle and ragwort, however, where significant numbers of seeds continued to germinate, particularly over the first 5 years but also up to 11 years, confirming the longer viability of seed from these two than the other three species. This relatively long persistence of viable seed of Californian thistle

and ragwort in the surface layer poses serious management problems for these weeds. Even if additions of fresh seed to the soil are eliminated, the remaining viable seed in the seedbank could still produce infestations for more than 10 years.

Seed buried at 200 mm depth remained viable for a longer period than at the shallower depth of 50 mm. Nodding thistle appeared to be the only species for which after 16 years there was little difference in seed viability due to burial depth. Deep burial has been found by many researchers to induce dormancy rather than suicide germination in most weed species (e.g. Dawson & Bruns 1975; Benvenuti et al. 2001). The ability to retain viability longer at the greater depth of 200 mm (cf. 50 mm) was most obvious in the case of giant buttercup and much less so in the case of ragwort. This further highlights the difficulty in managing a weed like ragwort where seed remains viable longer than many other species on the surface layer as well as when buried in deeper layers of the soil.

Two species, giant buttercup and ragwort, which were found to have small numbers of viable seed after 16 years of burial in the Horotiu soil, did not have any viable seed at any depth in either soil type after 28 years (Table 2). The three thistle species, viz. Scotch, nodding and Californian, which had some viable seed present at 50 and/or 200 mm depths after 16 years of burial in both soil types, were found to have some viable seed even after 28 years in the Horotiu soil (Table 2). In contrast, no viable seed were noted for any species in the Hamilton soil, except for a single Californian thistle seed buried at the 200 mm depth (Table 2). The additional data obtained at 28 years enabled new fitted exponential decay curves to be calculated and compared with those presented in earlier publications for the 16-year data set (James et al. 1998, James & Rahman 1999, 2000, 2001, 2003). Generally the fitted exponential decay curves over the 28 years were good descriptions of the data, as evident from high R^2 values (Table 2). However, the fit for ragwort was not as good as for other species because it tended to persist without loss of viability for about 5 years after which the viability declined rapidly. This could be related to the seed size as ragwort has the smallest seed of the species

evaluated. From the 28-year data it would appear that the seed viability of these weed species was not as long in most cases as was predicted based on our 16-year results. As the predictions were mostly extrapolations from the 16-year data, it is quite plausible that our estimates of the longevity of seed buried at the 50 and 200 mm depths were over-estimated. For example, James et al. (1998) estimated the longevity of nodding thistle to be 6, 79 and 67 years at 0–20, 50 and 200 mm depths respectively in the Horotiu soil. Based on the data presented here, these estimates have been revised to 6, 31 and 42 years respectively due to a greater decline in seed viability between 16 and 28 years than originally predicted from the 16-year data.

Seeds of all three thistle species retained greater viability in the Horotiu sandy loam soil compared to the Hamilton clay loam soil. The Hamilton soil has a heavier texture, lower water holding capacity (Table 1) and is more prone to drying. All these characteristics are likely to be associated with the more rapid depletion of the weed seedbank as they aid scarification of the seed coat allowing imbibition to commence and the seed then either germinates or decays. In the absence of scarification processes, several species were shown to retain 90% viability after 110 years when stored at ambient temperature in sealed containers (Steiner & Ruckebauer 1995).

Although the results of seed viability after 28 years burial presented here show shorter longevity of buried seed, it is probably of little comfort to farmers as 1 years seeding could still result in the need for 10 to 66 years weeding, depending on the weed species and soil type.

REFERENCES

- Benech-Arnold RL, Sanchez RA, Forcella F, Kruk BC, Ghersa MM 2000. Environmental control of dormancy in weed seedbanks in soil. *Field Crops Research* 67: 105-122.
- Benvenuti S, MacChia M, Miele S 2001. Quantitative analysis of emergence of seedlings from buried weed seeds with increasing soil depth. *Weed Science* 49: 528-535.

- Bourdôt GW, Saville DJ 2002. Estimating economic losses due to pasture weeds. *New Zealand Plant Protection* 55:106-110.
- Burnside OC, Wilson RG, Weisberg S, Hubbard KG 1996. Weed longevity of 41 weed species buried 17 years in eastern and western Nebraska. *Weed Science* 44: 74-86.
- Dawson JH, Bruns VF 1975. Longevity of barnyard grass, green foxtail, and yellow foxtail seeds in soil. *Weed Science* 23: 437-440.
- Harvey IC, Bourdôt 2001. Giant buttercup (*Ranunculus acris* L.) control in dairy pasture using a mycoherbicide based on *Sclerotinia sclerotiorum*. *New Zealand Plant Protection* 54: 120-124.
- James TK, Rahman A, Wardle DA, Bonner KI 1998. Survival of nodding thistle (*Carduus nutans*) seed buried at different depths in four soils. *Proceedings of the 51st New Zealand Plant Protection Conference*: 33-37.
- James TK, Rahman A 1999. Survival of giant buttercup seed buried at different soil depths in four soils. *Proceedings of the 52nd New Zealand Plant Protection Conference*: 234-239.
- James TK, Rahman A 2000. Longevity of buried ragwort seed in four soils. *New Zealand Plant Protection* 53: 253-257.
- James TK, Rahman A 2001. Longevity of buried *Cirsium arvense* seed in four New Zealand soils. *Proceedings of the 18th Asian-Pacific Weed Science Society Conference*: 33-38.
- James TK, Rahman A 2003. Survival of Scotch thistle seed buried at three depths in four New Zealand soils. *New Zealand Plant Protection* 56: 113-117.
- Moore WB, Doyle CJ, Rahman A 1989. Economics of controlling *Carduus nutans* on grazed pasture in New Zealand. *Crop Protection* 8: 16-24.
- Steiner AM, Ruckebauer P 1995. Germination of 110-year-old cereal and weed seeds, the Vienna Sample of 1877. Verification of effective ultra-dry storage at ambient temperature. *Seed Science Research* 5: 195-199.
- Telewski FW, Zeevaart JAD 2002. The 120-yr period for Dr. Beal's seed viability experiment. *American Journal of Botany* 89: 1285-1288.

Broom corn millet (*Panicum miliaceum*): a new menace for maize and sweetcorn growers in New Zealand

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Summary Broom corn millet (*Panicum miliaceum*) is an aggressive annual grass weed that is rapidly establishing in many maize and sweet corn crops in New Zealand. It has a C4 photosynthetic pathway and can reach up to 2 m high in crops. It is readily identified by its quick germination, rapid and vigorous growth, wide leaves (up to 2 cm wide), hairy stem and distinctive large black seed that can persist in the soil for a number of years. The seed can germinate within days of ripening and its large size gives it a growth advantage over all other grasses and many broadleaf weeds. From our research we have preliminary results on: (1) time and depth of emergence in New Zealand soils; (2) survival of the seed in maize and grass silage stack, pasture balage, and sweetcorn wilter; and (3) the efficacy of several pre-emergence herbicides used in maize crops. In pot experiments all herbicides were less effective against this weed than other annual grass weeds, such as summer grass (*Digitaria sanguinalis*) and rough bristle grass (*Setaria verticillata*).

Keywords Broom corn millet, *Panicum miliaceum*, grass weeds, sweetcorn, maize.

INTRODUCTION

Broom corn millet (*Panicum miliaceum* L.) is a recent grass weed currently undergoing rapid range expansion and causing problems for many arable farmers. This weed possibly had multiple entry points to New Zealand according to herbaria records (Anon. 2010). It was first recorded in Auckland and Palmerston North in 1961 and in a railway yard in a Northland granary in 1967. The first two infestations were probably from birdseed and the second from imported grain where it was a contaminant. More recently it was found in Ararimu, in a choumollier (*Brassica oleracea*) crop (most likely contaminated seed), and Taihape, where bird seed was thrown out, in 1975. Another infestation from birdseed occurred in Wellington in 1999, where it was found outside an aviary. There are also records from Hastings (1976) and Otago (1988) but unfortunately these records do not give details of the environment in which it was found or how the seed got there.

Also there are four voucher specimens, three from Gisborne (1995, 2001 and 2004) and one from

Hawke's Bay (1998) – all found in sweetcorn or maize crops. These latter records are important to farmers as they indicate the move from point of introduction into the production sector. There is no official record but anecdotal evidence suggests that it established in Marlborough about the same time. Gisborne and Hawke's Bay are sufficiently close to each other and these records could have come from the same introduction. However, neither is close to most of the earlier records and Marlborough is even more distant from them. Therefore it can be concluded that within two decades there have been multiple introductions of broom corn millet into New Zealand by a variety of pathways.

What we do know, however, is that once in the production sector this weed was rapidly spread from one field to the next via agricultural equipment (Westra *et al.* 1990). Although it is likely to be spread by cultivation and other equipment, the main culprit was sweetcorn harvesters. So much so that in one district the weed was colloquially named after the largest processing company of the region.

BIOLOGY

Broom corn millet is a fast-growing summer annual grass that can grow more than 2 m tall in crops. It is readily identified by its quick germination, rapid and vigorous growth, wide leaves (up to 2 cm wide), hairy stem and distinctive large black seed. It has a C4 photosynthetic pathway, which means its growth rate is determined mainly by temperature rather than light or moisture. It is grown as a crop (proso millet) for animal and bird feed in many northern hemisphere countries. The New Zealand infestation matches the description for the black seeded wild type (wild proso millet), which is also a serious weed in many countries (Wilson and Westra 1991).

Broom corn millet germinates during the warmer months of October to March and its germination appears to not be constrained by day length. Wiese and Binning (1987) found the threshold temperature for germination of broom corn millet to be 6.9°C cf. 9.7°C for barnyard grass (*Echinochloa crus-galli*), 6.0°C for fathen (*Chenopodium album*) and 10.0°C for redroot (*Amaranthus retroflexus*) other major weeds of maize and sweet corn. However, this threshold temperature for broom corn millet appears to be in

disagreement with local evidence. Results from a germination plate experiment carried out at Massey University showed the lowest germination temperature to be 13°C (1 seed) while 15-20% of seed germinated at 15°C. Germination still occurred at the maximum temperature of 35°C (C. McGill, unpublished data). These temperatures better match casual observations from the field.

Broom corn millet seed has no dormancy and is able to germinate within days of shattering under the right conditions. It has been noted to emerge within a few days of soil disturbance in the field. In a depth of emergence study where we placed broom corn millet seed at depths of 30–170 mm in 16 different soils collected from the major sweetcorn growing regions, Poverty Bay, Hawke's Bay, Manawatu and Marlborough, broom corn millet emerged from the shallowest depths after 5 days in light soil while it took 10–14 days to emerge from the deeper depths. It was able to emerge from 170 mm in seven of the soils but emerged from 120 mm in all soils.

The ability of broom corn millet to emerge quickly as well as from deep within the soil is due to its large seed size. Thousand seed weight for this species is 4.35 g, cf. yellow bristle grass (*Setaria pumila*) 2.41 g, barnyard grass (*Echinochloa crus-galli*) 2.27 g, rough bristle grass (*Setaria verticillata*) 1.35 g, smooth witchgrass (*P. dichotomiflorum*) 0.75 g and summer grass (*Digitaria sanguinalis*) 0.61 g (T. James, unpublished data). James *et al.* (2002) have previously shown the relationship between seed size and depths from which they can emerge.

Broom corn millet also has the ability to set seed in a very short time if under stress. Normally it would grow into a multi-tillered plant producing thousands of seeds, but we found in both pot and field trials that when under stress from severe competition, lack of water or nutrient resources or of late germination (autumn), broom corn millet plants were able to set seed with 5 weeks of emergence, much quicker than the other C4 grass weeds in the experiments.

MANAGEMENT

From observations of trials and discussions with growers and industry representatives it appears that broom corn millet is not a difficult weed to kill but it is a difficult weed to manage. Due to its spread via sweetcorn harvesting equipment, broom corn millet is mainly a weed of this crop. Sweetcorn is slower to establish than maize, requiring a longer critical weed-free period. Planting time also influences the critical weed-free period, with early planted sweetcorn (early November) requiring twice

as long a weed-free period than that planted in late December (Williams II 2006).

Broom corn millet populations as low as five plants m⁻² have resulted in 5% yield loss, while 20–40 plants m⁻² have given up to 50% yield loss (Williams II *et al.* 2008a & b). However, this crop competition is highly influenced by the relative time of emergence of the weed to the crop, and regularly irrigated sweet corn crops are generally more resistant to competition than those reliant on natural rainfall (Williams II *et al.* 2008a & b).

In pot experiments we found that pre-emergence herbicides were less effective against the large seeded broom corn millet than other annual grasses (James *et al.* 2009). The herbicides evaluated included alachlor, metolachlor, dimethanamid, two formulations of acetochlor, and proprietary mixes of acetochlor with atrazine or metribuzin, and each was tested in representative soils from Waikato, Bay of Plenty and Poverty Bay as well as in a high organic matter, peat-based soil. The problem in controlling broom corn millet with pre-emergence herbicides was not only the lack of long term control (Shenk *et al.* 1990) but also their low initial efficacy soon after application. There are two likely reasons for this. Firstly, the quick emergence of broom corn millet may enable the plant to emerge before the herbicide has been fully activated with rain, irrigation or incorporation into the soil. Secondly, if the seed is near the surface it is possible that the emerging coleoptile simply does not absorb sufficient chemical to kill the plant before it emerges. Thus with either mechanism, some of the seeds that are near the surface and emerge quickly are not adequately controlled.

With pre-emergence herbicides often failing to adequately control this weed, growers are looking for post-emergence herbicide options. Broom corn millet is able to germinate over a long period of time and as a C4 weed it is more likely to germinate as temperatures rise rather than due to a soil disturbance. Thus the timing of postemergence applications is critical and frequently a single application is insufficient. Recent trials have shown that nicosulfuron gives excellent control of this weed as long as it is applied before or soon after tillering commences and the plants do not get too large (Williams *et al.* 2000). However, this can often require a second application if there is a further germination flush. To avoid two applications of nicosulfuron, many growers are using mesotrione for early post-emergence weed control. This herbicide gives excellent control of the broadleaf weeds but often only partial control of broom corn millet. It does, however, slow the development of

plants, which assists with subsequent control by a later application of nicosulfuron.

An efficient management strategy for control of a weed must address the reproduction phase of its life cycle. This is critical in the case of broom corn millet for several reasons. The seed is more persistent in the soil than most other grass species and growers have reported the weed appearing after ploughing more than 5 years since it was last seen in that field. Khan *et al.* (1991) concluded that the black colouration of broom corn millet seed aided persistence of the seed in the soil and found that these seeds were still viable after 5 years in the soil. We are currently verifying this through a buried seed trial in which we have buried seed at two depths in eight locations throughout New Zealand. The longevity of the seed in the soil makes it imperative to minimise seed set and to minimise or eliminate spread between fields.

Minimising seed set involves making the best use of all the tools available to control the weed. Thus in addition to herbicides, stale seed beds and post-emergence cultivation should also be considered when designing a management programme. In the stale seed bed technique, the seedbed remains untilled for about 2 weeks prior to sowing and the emerging weeds are controlled soon after sowing with an application of glyphosate. The glyphosate can be applied in a tank mix with a pre-emergence herbicide. This method is one way to control the broom corn millet seedlings, which germinate early and are not adequately controlled by the pre-emergence herbicide.

Although broom corn millet is found in many regions of New Zealand, it is usually confined to fields that have been or currently are in sweet corn production. This is mostly due to the seed being spread from field to field via the sweet corn harvesting equipment. Also, the sweet corn residue is frequently removed for animal fodder and it is possible that the seed could easily be carried along with it. The greatest danger is if the residue is fed out fresh and no steps are taken to remove the seed. However, our recent studies have shown some effective ways of killing broom corn millet seed. McCains Foods pass the whole sweetcorn plant through a wilter on arrival at the processing plant, which is a steam heated, 15 m long, enclosed, conveyor belt. Test packets of seed were passed through the wilter in the absence of sweet corn (2 min duration) and with sweetcorn (7 min duration). Ten separate packets of seed, each containing 200 broom corn millet seeds, were passed through the wilter and subsequent germination tests showed that all seed were killed. Similar packets of seed placed in ensiled maize and ensiled grass were also all

killed within 3 months as was seed placed in wrapped balage. Placement within the stack or bale, adjacent to the plastic lining or wrapper or towards the centre, produced similar results. This shows that there are effective means of killing the seed before using the sweetcorn residue as animal feed.

DISCUSSION

Although a recent problem for New Zealand farmers, broom corn millet is a serious weed in many countries around the world. With our maritime climate and the well distributed rainfall, it has the potential to spread more rapidly. The current weed management practices for maize and sweet corn crops are not conducive for its containment. Our research programme, in cooperation with maize growers and the industry, has provided much needed information on broom corn millet biology under local growing conditions. This information will be utilised in devising practical and effective management strategies for this weed. Although some pre-emergence herbicides showed better efficacy than others, none provided effective control of broom corn millet. Future research needs to concentrate on post-emergence herbicide options and the most appropriate time to use them. In addition, any other measures that reduce the size of the soil seedbank should be included in management plans for this weed.

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REFERENCES

- Anonymous. (2010). New Zealand Virtual Herbarium. <http://www.virtualherbarium.org.nz>. Accessed January 2010.
- James, T.K. and Rahman, A. (2009). Efficacy of pre-emergence herbicides on three annual grass weeds in different soils. *New Zealand Plant Protection* 62, 356-362.
- James, T.K., Rahman, A., Webster, T. and Waller, J. (2002). Emergence of weeds as affected by vertical seed distribution in arable soils. *New Zealand Plant Protection* 55, 213-217.
- Khan, M., Cavers, P.B., Kane, M. and Thompson K. (1996). Role of the pigmented seed coat of proso millet (*Panicum miliaceum* L.) in imbibition, germination and seed persistence. *Seed Science Research* 7, 21-25.
- Shenk, M.D., Braunworth, W.S., Fernandez, R.J., Curtis, D.W., McGrath, D. and William, R.D.

- (1990). Wild-proso millet (*Panicum miliaceum*) control in sweet corn (*Zea mays*). *Weed Technology* 4, 440-445.
- Westra, P., Wilson, R.G. and Zimdahl, R.L. (1990). Wild-proso millet (*Panicum miliaceum*) control on central Great Plains irrigated corn (*Zea mays*). *Weed Technology* 4, 409-414.
- Wiese, A.M. and Binning, L.K. (1987). Calculating the threshold temperature of development for weeds. *Weed Science* 35, 177-179.
- Williams, B.J. and Harvey, R.G. (2000). Effect of nicosulfuron timing on wild-proso millet (*Panicum miliaceum*) control in sweet corn (*Zea mays*). *Weed Technology* 14, 377-382.
- Williams II, M.M. (2006). Planting date influences critical period of weed control in sweet corn. *Weed Science* 54, 928-933.
- Williams II, M.M., Boydston, R.A. and Davis, A.S. (2008a). Differential tolerance in sweet corn to wild-proso millet interference. *Weed Science* 55, 91-96.
- Williams II, M.M., Boydston, R.A. and Davis, A.S. (2008b). Crop competitive ability contributes to herbicide performance in sweet corn. *Weed Research* 48, 58-67.
- Williams II, M.M. and Lindquist, J.L. (2007). Influence of planting date and weed interference on sweet corn growth and development. *Agronomy Journal* 99, 1066-1072.
- Wilson, R.G. and Westra, P. (1991). Wild proso millet (*Panicum miliaceum*) interference in corn (*Zea mays*). *Weed Science* 39, 217-220.