Characteristics of Black Medic (*Medicago lupulina* L.) Seed Dormancy Loss in Western Canada

by

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Abstract

Characteristics of Black Medic (*Medicago lupulina* L.) Seed Dormancy Loss in Western Canada

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Cover crops are an important innovation in sustainable cropping systems. The successful use and management of black medic (*Medicago lupulina* L.) as a self-regenerating cover crop requires a better understanding of its physical seed dormancy. In order to break this seed dormancy, it appears that a low temperature 2-stage seed softening process is required. However, whether or not this 2-stage process is required for black medic seed softening in Western Canada is unclear. Also, the influence of the presence of a companion crop, medic population type, seed burial depth and seed production environment on black medic production and seed softening is unknown. Field and controlled environment studies were established in 2003 and 2004 in an effort to address these questions.

The results from a field study conducted in different prairie environments showed that although, seed production environment, the presence of a companion crop, and medic population affected the growth and development of the black medic plants, they did not affect initial seed dormancy. A second field study tested the effect of seed production environment, seed burial depth and population on seed softening. Results indicated that there was an effect of population on summer seed softening, which suggested that there were differences in seed dormancy between a population of black medic that had been subjected to selection pressures (e.g., herbicides, competition) for 12

years versus one that had not (Foundation stock). Therefore, this suggests that some genetic drift had occurred within the population. The results also indicated that there was an effect of seed burial depth on seed softening, with more seed softening occurring for the buried seed during winter/spring and more for the surface seed during summer, and these differences appeared to be somewhat linked to differences in soil temperature.

Results of this field study suggested that black medic in Western Canada goes through a 2-stage softening process. Hence, a controlled environment study was established to test this hypothesis. Results from both studies confirmed that a 2-stage softening process is required for black medic softening in Western Canada. Stage 1 requirements appear to be met by exposing seed to temperatures between –5°C and 5°C for at least 4 weeks, while exposure of the seed to a low fluctuating temperature (e.g. 15/6°C) for a short period of time (i.e., approximately 4 days) appears to meet stage 2 requirements.

In summary, this research has provided us with valuable information about black medic seed softening under Western Canadian conditions, which will hopefully lead to a better understanding of how to best manage and utilize black medic as a self-regenerating cover crop in a Western Canadian cropping system.

Chapter 1: Literature Review

1.1 Cover Crops

Cover crops are typically low growing plants that are grown in association with a main crop. Cover crops fill either a temporal or spatial gap that has been left bare by the main crop (Lal et al. 1991). Annual cover crops are usually seeded at the beginning of the growing season or once the main crop has been established; the successful cover crops are those that continue to grow once the main crop has been harvested, thereby providing benefits throughout the fall. The dry matter produced by the cover crop can either be used as feed for livestock or as a green manure (Stopes et al. 1996).

Cover crops are an important innovation in sustainable crop production systems. In the past few decades, environmental concerns over excessive fertilizer and pesticide use and soil erosion have lead to renewed interest in cover crops (Hartwig and Ammon 2002). Cover crops can also "perennialize" the annual cropping system to more thoroughly utilize water, nutrient and light resources (Nason, University of Manitoba, pers. comm.). Since cover crops are usually grown along with a cash crop, the farmer is able to receive in one growing season both an income from a cash crop and the benefits associated with having a cover crop in the cropping system.

1.1.1 Positive Impacts of Cover Crops

Benefits of cover crops have been documented for centuries (Hartwig and Ammon 2002). For example, in ancient Greece and Rome, the use of legume cover crops, such as vetch (*Vicia* species) and lupines (*Lupinus* species), to enhance soil fertility and crop production was well documented (Hargrove and Frye 1987). Today, there are

several other reasons for including cover crops in cropping systems. For example, species such as hairy vetch (*Vicia villosa*) and annual ryegrass (*Lolium multiflorum*) are able to suppress weed growth through competition and/or allelopathy (Brandsaeter and Netland 1999; Lal et al. 1991). In some instances, cover crops can provide improved disease control by, for example, preventing the splashing of fungal spores (e.g. common smut (*Ustilago maydis*)) from the soil surface to the leaves of the host crop (Hartwig and Ammon 2002). Cover crops have also been noted to provide insect control by directly interfering with the pest insect (e.g. inhibiting the movement of the insect to the host plant) or by providing habitat for predatory insects (Hartwig and Ammon 2002; Verhallen et al. 2003).

Other benefits of cover crops include: soil moisture retention, improved soil structure, increased soil organic matter, reduced soil erosion and reduced runoff (Lal et al. 1991; Worsham 1991; Hartwig and Ammon 2002). Zhu et al. (1989) found that winter cover crops (common chickweed (*Stellaria media L.*), Canada bluegrass (*Poa compressa L.*), and downy brome (*Bromus tectorum L.*)) established in soybeans reduced spring soil erosion and runoff by an average of 93% and 47%, respectively, compared to when the soybeans were grown without a cover crop. Overall, due to the many benefits that cover crops can offer, their use can indirectly improve crop yields and reduce the need for chemical inputs (Power and Koerner 1994).

Currently, cover crops are being used across Canada in several applications. In drier areas, such as southern Alberta, cereal cover crops (e.g. fall rye (*Secale cereale*)) have been successfully used for soil conservation and weed suppression during the fallow phase of a crop rotation (Moyer et al. 2000). In wetter areas of the prairies, such as

southern Manitoba, legume cover crops (e.g. red clover (*Trifolium pratense*)) are being grown after the crop has been harvested in order to provide some late season production by taking advantage of available heat and moisture, and are also being used as an alternative to fallow (Thiessen Martens et al. 2001). In Ontario, a variety of different legume, broadleaved and grass cover crops are being used for their abilities to reduce soil erosion, improve soil structure and fertility, reduce pest populations, improve water management and for many other reasons (Verhallen et al. 2003).

1.1.2 Negative Impacts of Cover Crops

Although cover crops are generally considered to be beneficial to a cropping system, there are often a number of negative aspects associated with their use. For example, additional management, interference with crop establishment, cooler soil temperature and less predictable crop fertilizer requirements have been cited as some problems associated with using cover crops (Teasdale 1996). Some cover crops are quite competitive and may outcompete the main crop for resources, which in turn may result in yield losses. For example, Thiessen Martens et al. (2001) showed that the presence of an alfalfa (*Medicago sativa*) or red clover (*Trifoloum pratense*) cover crop decreased winter wheat and rye grain yields slightly (3.4 to 3.8%). Also, even though the cost of cover crop seed has been shown to be equal to the value of the benefits received (Mallory et al. 1998), the monetary cost of the seed can still often be a deterrent to farmers (Brandsaeter and Netland 1999).

1.1.3 A Cover Crop Ideotype for the Canadian Prairies

A cover crop ideotype is one that provides all the aforementioned benefits, and at the same time minimizes the associated problems. Most importantly, a cover crop for a particular system should have a low maintenance cost and should not outcompete the main crop for resources (Brandsaeter and Netland 1999). It is also essential that the cover crop species chosen is well adapted to the local climate (Brandsaeter and Netland 1999). Zhu et al. (1991) suggest that an ideal cover crop species should have a relatively short height to minimize interference with the main crop and, in the case of self-regenerating species, should be a prolific seed producer.

1.2 Self-Regenerating Cover Crops

The idea behind self-regenerating cover crops is that the farmer only has to seed this crop once and it will regenerate on its own from seed in the seed bank each subsequent year. This allows the farmers to get the yearly benefits of having a cover crop without having the yearly cost of reseeding it.

Self-seeding cover crops have been used for decades in many places around the world, in both Mediterranean and temperate climates. In Australia, self-regenerating *Trifolium* and *Medicago* legume species have been used in the ley-farming system since the 1950s (Puckridge and French 1983). Ley-farming is a system that integrates livestock and crop production by having a rotation of cereal crops with annual legume pasture phases that regenerate from seed at the start of each pasture phase (Walsh et al. 2001). This ley farming system has been shown to increase soil fertility, provide high-quality pasture and increase production from crops and livestock (Puckridge and French 1983).

However, a major requirement of these systems is that the pasture crop can maintain a high enough seedbank level to allow regeneration from seed at the start of each pasture phase (Walsh et al. 2001).

In Scandinavia, a number of self-regenerating legumes have been used as winter annual cover crops to help suppress weeds and provide other benefits to the cropping system (Brandsaeter and Netland 1999; Enache and Ilnicki 1990). In this temperate climate, winter survival for many annual cover crop species is very low or inconsistent, and therefore it is important that they be able to regenerate each year from seed (Moomaw 1995). On the Canadian prairies, one self-regenerating species drawing particular interest is *Medicago lupulina*, due to its proven ability to regenerate well in this region of the world (Braul 2004). The first Northern Great Plains researcher to work on *Medicago lupulina* was Jim Sims of Montana State University (Entz, University of Manitoba, pers. comm.).

1.3 *Medicago lupulina*

1.3.1 Biology

Medicago lupulina, commonly known as black medic, has been used for decades in many cropping systems around the world. Black medic is a low growing, short-lived perennial, biennial or annual species (Sims et al. 1985; Turkington and Cavers 1979). This plant is an obligate self-pollinated species, and therefore interbreeding among individuals is not likely (Sidhu 1971). In Mediterranean climates, black medic usually germinates during the first fall rains and grows rapidly under the warm and moist conditions, with flowering being completed in spring (Rumbaugh and Johnson 1986).

Since black medic has some frost resistance, it is sometimes used as a winter annual in temperate climates (Brandsaeter et al. 2000). However, in order to take advantage of its reseeding ability, *M. lupulina* is best used as a summer annual in these environments. In general, although seedlings can emerge throughout the growing season, the greatest number of seedlings appear in spring (Turkington and Cavers 1979).

Black medic has been noted to be a prolific seed producer, often out producing many other annual *Medicago* species (Rumbaugh and Johnson 1986). In pastures, the seedbank size for black medic has been found to be approximately 600 to 2000 seeds per m^2, though estimates of the seedbank size vary (Pavone and Reader 1982). This available seed reserve gives black medic its self-regenerating ability. Since seed cost often limits the value of a particular cover crop species, it is important that the natural reseeding ability of black medic is understood and exploited (Moomaw 1995).

1.3.2 Origin and Distribution

The exact origin of black medic is unknown, but it is believed to be native to Western Asia, Eastern Europe or the Mediterranean (Turkington and Cavers 1979; Sidhu 1971). Black medic was likely introduced to North America in the 1600s as either a contaminant in alfalfa seed or as a constituent of general pasture mixtures, and was subsequently naturalized throughout most of North America (Turkington and Cavers 1979; De Haan et al. 1997; Sidhu 1971).

Black medic is typically found in 'disturbed' areas such as roadsides, riverbanks, lawns, and fields in both temperate and subtropical regions, such as North America, Asia, North Africa and Europe (Sidhu 1971; Turkington and Cavers 1979). This species has

even been documented in weed surveys done for fields in Manitoba (Ominski et al. 1999) and Ontario (Thomas and Dale 1991; Frick and Thomas 1992). Black medic is adapted to a wide range of environmental conditions, as noted by its widespread distribution (Turkington and Cavers 1979). Although black medic grows best in moister soils and at cooler temperatures than other annual *Medicago* species, it is also considered to be resistant to drought stress (Foulds 1978; Rumbaugh and Johnson 1986). In general, black medic is best adapted to well-aerated soils of calcareous origin (Blaser and Stokes 1946).

In the 1980s, the cultivar 'George' black medic was developed using seed collected from naturalized plants adapted to Montana conditions and was subsequently registered as the first North American *M. lupulina* cultivar (Sims et al. 1985).

1.3.3 Genetic Plasticity

Black medic's adaptability to a wide range of environmental conditions is attributed to the genetic plasticity of this species. *M. lupulina* is considered to have high morphological and phenotypic diversity both within and between populations, and therefore many varieties and accessions of this species can be found (Turkington and Cavers 1979; Sidhu 1971). A single population of black medic can consist of a number of different genotypes and phenotypes in varying proportions and these proportions can be shifted under different environmental stresses (Rumbaugh and Johnson 1986; Sidhu 1971).

Norman et al. (2002b) suggested that for different legume species, physical seed dormancy (i.e. hardseededness) might shift in response to environmental selection. More specifically, seed dormancy will tend to be highest in populations subjected to conditions

that reduce the chance of successful reproduction. For example, Norman et al. (2002b) reported that for *Trifolium tomentosum* and *Trifolium campestre* the level of hardseededness decreased with decreasing grazing intensity. In general, ecological theory predicts that as conditions become more favourable for the plant (i.e. less grazing pressure), dormancy of a given species should decrease (Cohen 1967; Brown and Venable 1986).

With this in mind, other stresses associated with different cropping systems may also result in a shift in the hardseededness of a particular legume population. For example, exposure to pre-emergence herbicides may result in a shift in the population towards a phenotype that emerges later in the growing season. Later emergence would allow the seedlings to escape the pre-emergence herbicides. In order to facilitate later emergence, the proportion of seeds in a population that normally breaks dormancy (i.e. softens) during the pre-emergence period would be shifted towards the cohort of seeds that have a longer lasting dormancy, thereby enabling the population to break dormancy and successfully establish after the herbicide has been applied.

Such a response is believed to be the case for a population of black medic, which we denote as 'selected George'. In 1992, this population of black medic was established from certified 'George' black medic seed on a no-till farm near Goodrich, North Dakota. Over the years, this population has regenerated successfully every year, even though it has been subjected to continuous grain crop competition and a number of yearly herbicide applications. However, it appears that this selection pressure has resulted in a shift from more spring recruitment to more mid-summer recruitment (Entz, University of

Manitoba, pers. comm.). This could be due to a shift in the seed softening and dormancy nature of this population of black medic in order to escape the herbicide applications.

1.3.4 Agricultural Use

Although *Medicago lupulina* is often considered to be a weed (Turkington and Cavers 1979; Frick and Thomas 1992; Thomas and Dale 1991), it is also considered by many to be a valuable legume species for use as a green manure crop and pasture or forage species (Stopes et al. 1996; Power and Koerner 1994; Rumbaugh and Johnson 1986). On the Canadian prairies, the use of black medic in cropping systems is somewhat limited. Those who have included black medic in their cropping systems are often organic farmers who rely on this self-regenerating species to help with weed and erosion control, and to provide an annual source of nitrogen to the cropping system (Miller, Liseux, Saskatchewan, pers. comm.). Although there are other cover crops that may be able to provide greater weed control, nitrogen accumulation etc., black medic is a favourable choice for farmers because unlike most annual cover crop species, there is no annual reseeding cost.

As a legume cover crop species, black medic is considered to be less successful at accumulating dry matter and nitrogen than other species. For example, in one study conducted in the UK, the accumulation of nitrogen and dry matter was observed for black medic, *Trifolium pratense* (red clover) and *Melilotus alba* (white clover). At the end of 25 months, the results for black medic, red clover and white clover were, 20,400, 25,400 and 25,000 kg/ha of dry matter, respectively, and 459, 741 and 592 kg/ha of nitrogen, respectively (Stopes et al. 1996). In a study in Nebraska, it was shown that black medic

could produce up to 3430 kg/ha of dry matter in a year, but that annual dry matter yields tended to vary (Power and Koerner 1994). In contrast, Rumbaugh and Johnson (1986) found that black medic had a superior ability to produce ground cover in the years following establishment compared to 30 other annual *Medicago* species, such as *M. laciniata*, *M. murex* and *M. scutellata*. In general, black medic may provide better ground cover than other species, but will often produce less dry matter, which means less forage or green manure will be available.

As a pasture plant, black medic is considered to provide a high quality forage, though of a lesser quantity than others species, such as hairy vetch (*Vicia villosa* Roth.) and berseem clover (*Trifolium alexandrinum* L.) (Fraser et al. 2004). Zhu et al. (1996) noted that among annual *Medicago* species, black medic was one of the species producing the highest forage crude protein concentration levels and, it produced forage with quality levels similar to that of alfalfa. In Wyoming, black medic was the best at producing high quality forage for late season (November) grazing compared with other medic species, sweetclover (*Melilotus alba*) and alfalfa (Alford et al. 2003).

Since black medic usually produces less biomass than other cover crops, it is generally considered to be relatively uncompetitive with the main crop, especially compared to other cover crop species (Moomaw 1995; Moynihan et al. 1996). Also, black medic tends to grow more aggressively later in the season, which also helps it avoid causing yield losses to the main crop (Alford et al. 2003). In a number of cases, black medic was grown with soybeans (*Glycine max*), corn (*Zea mays*) and other cash crops without causing any reduction in the yield of the cash crop (Moomaw 1995; Alford et al. 2003). When barley (*Hordeum vulgare* L.) was the main crop, medics were found to

increase barley yields by 9% in one location and decrease yields by 76% in another location (Moyniham et al. 1996). When black medic was used as a green manure crop, it has been shown to improve grain and protein yields, nitrogen uptake and water use efficiency of the following year's spring wheat crop (Sims et al. 1985). Although black medic can sometimes outcompete the main crop, steps can be taken to help control early season growth, such as applying pre-seeding herbicides, which allow the main crop to successfully establish and grow.

As for weed control, although black medic produces less biomass and is less competitive than other species, such as *M. polymorpha* and hairy vetch, it is often just as effective at suppressing weed growth (De Haan et al. 1997). Moyniham et al. (1996) found that black medic was able to reduce the fall weed biomass in a barley crop by an average of 65% and Hartl (1989) concluded that under field conditions, undersowing a winter wheat crop with *M. lupulina* was useful in helping control late season weed development.

1.4 Seed Dormancy

1.4.1 Primary Dormancy

The key to the success of black medic as a self-regenerating legume is its seed dormancy mechanism, hardseededness, which allows it to form long-lived seedbanks (Cavers 1995). Hardseededness is a form of physical dormancy related to the seed coat, which prevents water from entering the seed, thereby preventing germination from occurring (Norman et al. 2002a). Hardseededness is very common in legume species and is considered to be by far the most important dormancy mechanism in the plant genus

(Zeng 2001). *Lupinus*, *Trifolium*, *Stylosanthes*, *Macroptilium*, *Medicago* and many other legume species have all been shown to possess this physical dormancy mechanism (Quinlivan 1961; McDonald 2000).

For annual legume populations, dormancy plays two important roles; it spreads the risk of mortality associated with germination between seasons and it also increases the likelihood that germination occurs at the optimal time within a season (Philippi 1993; Norman et al. 2002b). In Australia, the presence of summer and early autumn rains often results in many legume seeds (e.g. *Trifolium subterraneum* (subterranean clover)) germinating but dying shortly afterwards due to the absence of follow-up rains (Zeng 2001). In order to reduce losses from such a 'false break' in the season, seed dormancy in legume seeds has developed to prevent the germination of seeds until the time when favourable germination conditions are present (Zeng 2001).

Since the optimal time for seed dormancy loss will vary among environments and cropping systems, over time, selection pressures may shift the seed softening pattern of a population so that they coincide with this optimal time. For *M. lupulina*, the variation in hardseededness that exists between seeds allows some seeds to soften and germinate immediately while others may take a number of years before dormancy is broken (Sidhu and Cavers 1977). Norman et al. (2002b) point out that this genetic variability must exist if natural selection is to shift the hardseededness of populations in response to environmental changes. An understanding of the dynamics of seedbanks and the mechanism by which hardseededness operates is important if we are to understand the adaptation of annual legumes to their environment (Zeng 2001; Norman et al. 2002b).

1.4.2 Secondary Dormancy

If favourable growing conditions are not present when primary dormancy is broken, the seed can revert back to dormancy. This is referred to as secondary dormancy. In adapted species, secondary dormancy is induced in a period preceding the season with environmental conditions unsuitable for plant survival (Benech-Arnold et al. 2000). The presence of secondary dormancy allows seeds to become dormant if conditions are not favourable, thereby allowing the seedling a better chance of survival and reproduction. For example, for a summer annual species, dormancy should be broken in late winter or early spring to allow for spring recruitment when adequate moisture is available, competition is low and temperatures are moderate. As summer approaches, non-dormant seed that has not yet germinated may re-induce dormancy (secondary dormancy) in order to avoid the harsher conditions of summer (e.g. more competition, moisture limitations, extreme temperatures), when survival is less likely. Seed that enters secondary dormancy will remain dormant until the appropriate dormancy breaking conditions are present during the following season.

1.5 Hardseededness

According to Benech-Arnold et al. (2000), dormancy is an internal condition of the seed that impedes its germination under otherwise adequate hydric, thermal and gaseous conditions. One type of dormancy is physical dormancy or hardseededness, which is usually the result of an impermeable seed coat preventing water from entering the seed, thereby preventing the seed from germinating (Norman et al. 2002a). The impermeability of the seed coat can be caused by several parts of the testa, including the

waxy cuticle, the suberin, and the thick-walled palisade (i.e. lignified macrosclerid) and osteosclerid layers (Bewley and Black 1994; Sidhu and Cavers 1977). In legumes, there is a structure called the lens that functions as the 'water gap' for the seed and, along with the entire testa, controls the movement of water into the seed (Zeng 2001; Baskin 2003). In mature legume seeds, the macrosclerids in the lens are initially closed, but once the appropriate environmental signal is received these cells pull apart. This breaks dormancy by allowing water to enter the seed (Baskin 2003). Chemical processes may also be involved in the softening of the legume seed coat, but at this point little is known about these processes (Zeng 2001).

The dormancy of black medic and other legume species can vary depending on the environment under which the seed matures and the genotype of the parent plant (Sidhu and Cavers 1977). Seeds that mature under less optimal conditions will develop an impermeable seed coat, which prevents the seed from germinating until dormancy is broken (Sidhu and Cavers 1977). For example, the hardseededness of alfalfa tends to vary depending on the conditions, with only 20% hardseed in warmer climates, such as California, and 40 to 50% hardseed in cooler climates, such as the USA Pacific Northwest (Fairey and Lefkovitch 1991). On the Canadian prairies, the hardseededness of alfalfa has been found to be between 70 and 96% (Acharya et al. 1999; Leggatt 1927).

For black medic, the percent of seed that becomes dormant after development is often over 90% (Moomaw 1995; Pavone and Reader 1982; Sidhu 1971). Initially, the seed of black medic is non-dormant and able to germinate if a suitable microhabitat is available. This was observed in a field trial in Manitoba in 2004 where field grown medic plants produced seed in the fall that subsequently germinated (Thiessen Martens,

University of Manitoab, pers. comm.). However, if germination doesn't occur, eventually some unknown chemical change in the testa makes it impermeable to water and the seed will not germinate until this seed coat is 'broken' (Sidhu and Cavers 1977). In some cases, black medic seed may remain dormant for over 6 years (Roberts and Feast 1973; Roberts and Feast 1972).

1.6 Seed Softening

Seed softening, or dormancy breaking, occurs when the impermeable seed coat of the legume is 'broken', and therefore water is allowed to enter the seed causing germination. Seed softening usually occurs during the time preceding the period with favourable conditions for seedling growth and development (Benech-Arnold et al. 2000). For a summer annual species adapted to the Canadian prairies, this softening period is in the late winter and early spring. For black medic, the amount of softening that occurs in the springtime can vary. For example, Pavone and Reader (1982) found that over 60% of black medic seeds were still dormant by spring of the first year, with only 30 to 40% of the natural seed bank of black medic germinating in a single year. However, hardseededness can last even longer under some natural conditions. Van Assche et al. (2003) found that 92% of the black medic seed placed at 2 cm in the soil was still dormant after 2.5 years.

It has also been noted that black medic seed tends to exhibit more softening and emergence in the second year than in the first year after establishment or seed development (Van Assche et al. 2003). Roberts and Feast (1973) found this to be true under cultivated soils. In another study, Sims and Slinkard (1991) found that black medic

established poorly in the first summer but produced good stands in the following summers.

In summary, the exact conditions involved in the seed softening of black medic remain unknown. However, it is important that we understand the conditions involved so that we can better estimate the impact that different cropping practices, such as zero-tillage, or different conditions, such as a lack of snow cover or an unseasonably cold winter, may have on seed softening, and in turn, the yearly recruitment and long-term persistence of this self-regenerating cover crop species. The following sub-sections will discuss the hypothesized role that temperature, moisture and depth of seed burial may play in the seed softening of *Medicago lupulina.*

1.6.1 Temperature

Temperature is often cited as a major factor or cue involved in breaking seed physical dormancy. This is especially true in temperate environments, where temperature is usually the seasonally limiting factor (Benech-Arnold et al. 2000). A number of experiments have been performed over the years to investigate the effect of temperature on seed softening of legumes in temperate regions. In one such experiment, Moomaw (1995) tried freezing and thawing black medic seed for 7 days in order to break dormancy, but still observed 96% hardseed at the end of the experiment. The effect of cold temperatures on seed softening was also studied by exposing black medic to different cold temperatures (-5°C and 1°C) for 23 and 218 days followed by 7 days at 20°C (Sidhu 1971). Again, these treatments did not result in significant seed softening, and in fact the exposure to the –5°C temperature for the 218 days caused a 39% decrease

in seed viability.

In another experiment, Sidhu (1971) investigated the germination percentage of unscarified black medic seeds after being exposed for 25 days to a number of different alternating (0/10°C, 10/20°C, 10/30°C, 20/30°C, 20/35°C, 25/35°C) and constant (1°C, 5°C, 10°C, 15°C, 20°C, 25°C, 30°C and 35°C) temperatures. The results showed that none of these temperature treatments were effective at softening the seed by more than 5%. Sidhu (1971) also studied the effect of storing black seed on the soil surface of a cultivated field for 281 days compared to storing it in a storage room at 20°C on seed softening. He found that the exposure to the field conditions resulted in a 39 to 75% softening of the different populations of black medic seed, which was significantly higher than the softening of the seed kept at 20°C (1 to 6%).

Van Assche et al. (2003) working in Belgium investigated the effect of temperature on the seed softening of a number of legume species, including *M. lupulina.* In one experiment, black medic seed that was stored for 2 months at 20°C and then subjected to different temperatures (30°C, 23°C, 10°C, 20/10°C, 5°C (8wks) then transferred to 23°C) had germination rates of 0.7%, 4.3%, 2.3%, 4.3% and 6%, respectively. These results indicate very little softening due to these temperature regimes. They also found that when seeds were chilled (5°C) for 8 weeks and then subjected to 23°C, 10°C, 20/10°C and 15/6°C treatments, the germination percentages were substantially higher (5.2%, 5.9%, 52.6%, 65.3%, respectively) than for the seed that wasn't chilled. Notably, the greatest amount of softening occurred when the chilling period was followed by the fluctuating temperature treatment, 15/6°C.

Van Assche et al. (2003) also conducted a field study, in which seeds were placed in nylon bags and buried at 2 cm, with samples being removed at regular intervals over 2 years and tested for germination under different temperature regimes (30/20°C, 20/10°C, 15/6°C and 23°C). This study showed that the greatest germination occurred when the seeds were removed during the end of winter (February) or in early spring (March) and subjected to the 15/6°C temperature, and, to a lesser extent, the 20/10°C temperature (Van Assche et al. 2003). Overall, they found that under natural conditions, *M. lupulina* showed a marked seasonal cycle of germination, with high germination percentages in spring and very low percentages in other seasons (Van Assche et al. 2003). It was also noted that both a chilling and a low fluctuating temperature stage were required in order to break dormancy. Research performed on other hardseeded legume species, such as red, white, alsike (*Trifolium hybridum*) and sweetclover has also shown that a low temperature period followed by a period of low alternating temperatures is required in order to cause dormancy loss (Leggatt 1927).

Research performed in Australia has also shown the importance of temperature in the softening of the hard seed coat of legumes, such as subterranean clover, *Ornithopus compressus* (yellow serradella), *Medicago tribuloides* (barrel medic) and many other species (Taylor 1981; Quinlivan 1961). In order to break seed dormancy, a 2-stage process has been shown to be required for most annual legume species. The first stage in this process is a high temperature phase (e.g. 60°C), which weakens the structure of the testa, forming 'latent soft seeds' (Taylor 1981). The second stage is a short period (3 to 7 days) of high alternating temperatures (e.g. 60/15°C), which finally breaks the dormancy of the seed. Seed softening of a seed population is often a 2-stage process with one factor,

such as temperature, reducing the degree of dormancy in the population, while a second factor, such as fluctuating temperatures, regulates the time at which the dormancy is terminated (Benech-Arnold et al. 2000; Taylor 1981).

Does a 2-stage process also exist for temperate legumes such as *Medicago lupulina*? From the results of the research performed by Sidhu (1971) and Van Assche et al. (2003), it appears that a 2-stage seed softening process is also required for the softening of black medic when grown in a temperate environment. However, unlike the Australian 2-stage process, which involves exposure to high temperatures, followed by high fluctuating temperatures, under temperate environments, stage 1 is believed to consist of a "chilling" period (5°C), while stage 2 is a low fluctuating temperature stage (15°C/6°C). This theory of the 2-stage process for temperate environments is supported by field observations, as we tend to find the greatest percentage of black medic germination in the springtime when low fluctuating temperatures follow the cold temperatures of winter (Van Assche et al 2003; Braul 2004). During the chilling phase, the sensitivity of the seed to temperature fluctuations increases and if this chilling period is followed by a period of low fluctuating temperatures, dormancy will be broken (Van Assche et al. 2003). From the initial research performed by Moomaw (1995), Sidhu (1971) and Van Assche et al. (2003), it is obvious that only a very small amount of softening will occur if the conditions for these stages are not met. Therefore, such a 2-stage process appears to be a plausible mechanism for black medic seed softening.

Although this 2-stage process appears to be applicable for black medic in Western Canada, the actual stage 1 temperature suggested (5°C) does not correspond with the temperatures actually experienced in this region during the winter. On the Canadian

prairies, winter and early spring soil temperatures typically range from –5°C to 0°C (see Table 1.1) (Environment Canada 2004a), which is colder than the 5°C "chilling" temperature suggested by Van Assche et al. (2003). However, Braul (2004) observed significant softening of black medic under field conditions in Manitoba. Therefore, temperatures ranging from –5°C to 0°C may also constitute "chilling" temperatures.

Table 1.1 Average soil temperatures (5 cm depth) at Winnipeg (airport station) and Lethbridge (Agriculture and Agri-Food Canada station) during the winter and early spring

	Month				
	December	January	February	March	April
Winnipeg (°C)	-3.0	-5.0	-4.7	-2.3	1.1
Lethbridge (°C)	-2.5	-3.5	-2.4	0.7	5.5

Source: Environment Canada (2004a)

Braul (2004) also observed that in Manitoba, black medic appeared to respond to both the Mediterranean and temperate 2-stage softening processes. Early in the season, some seed softening occurred when the black medic was exposed to the chilling temperatures of winter followed by the fluctuating temperatures of spring (i.e. temperate 2-stage process), while later in the season, elevated temperatures followed by cooler fluctuating temperatures resulted in some seed softening (i.e. Mediterranean 2-stage process). In order to more clearly illustrate this phenomenon, Braul (2004) proposed a conceptual model of black medic seed softening during the early and late season

softening periods (Figure 1.1). Although this late season, high temperature softening appears to occur in temperate environments, the majority of black medic softening occurs in spring.

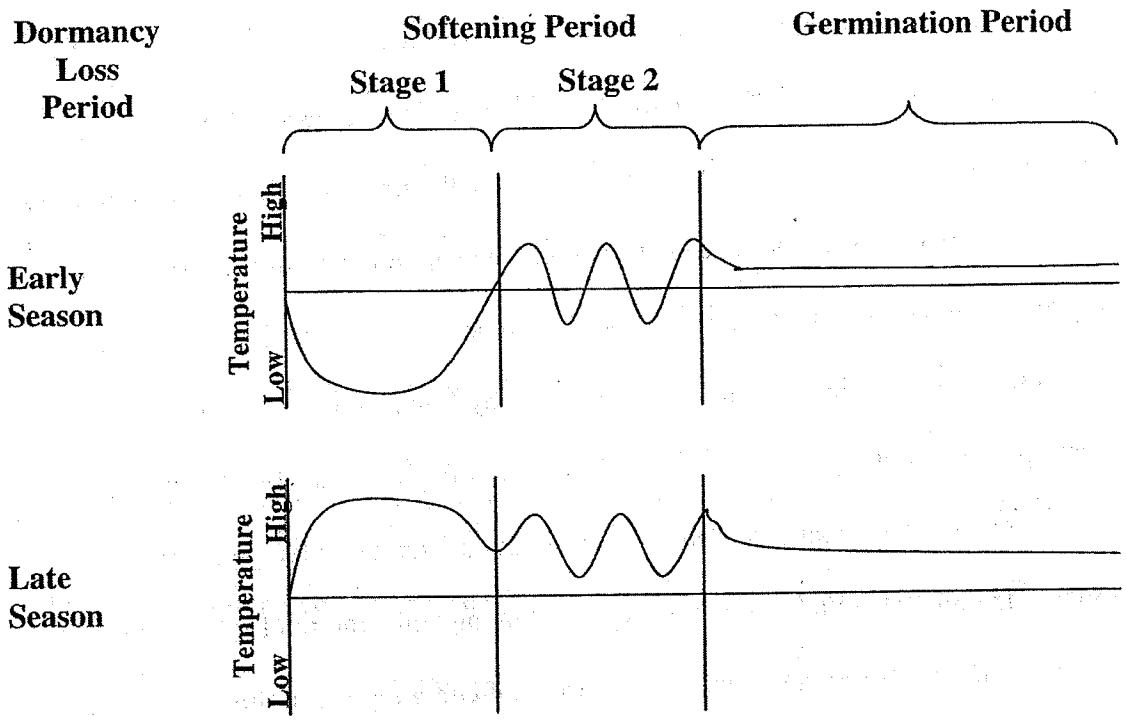


Figure 1.1 Conceptual model of the black medic two-stage softening process during early and late season dormancy loss periods (Braul 2004).

Since, the 2-stage process appears to occur in very different environments (i.e. Australia, Belgium, Western Canada), it appears to require different initial temperature cues. In Australia, the temperature cues are related to the time of year when suitable temperature and moisture will be present, thereby preventing a 'false-break' from occurring and the young seedlings from drying out. In Belgium and Western Canada, the temperature cues are present in order to prevent seeds from germinating in late summer

or fall, when competition and moisture stress is high and when the seedlings do not have enough time to reproduce before a killing frost. In each environment the temperature cues are present in order to ensure that the seeds germinate and grow during the most favourable time of the year. These cues allow the legume species to adapt to particular environments, which each call for different cues.

1.6.2 Moisture

Although temperature appears to be the major factor controlling seed softening in black medic, it has been proposed that moisture may also play a role (Benech-Arnold et al. 2000). Both Zeng (2001) and Van Assche et al. (2003) have found that legumes often need some moisture present in order to progress through stage 2 of the softening process. This was also observed in a lab experiment by Braul (2004), which showed that as moisture increased, germination increased from 23 to 49%. This moisture requirement for stage 2 of the softening process is logical from an adaptation perspective since it would be unfavourable for the seed to soften if there was no moisture available to allow the seed to successfully germinate and emerge. However, some research has shown that the presence of moisture during the overwintering of black medic seed may actually result in less softening due to interference with the physiological state of the seed, preventing it from responding to temperature changes (Sidhu 1971). In conclusion, it appears that the role of moisture in the softening of black medic seed is not yet thoroughly understood.

1.6.3 Depth of Seed Burial

Depth of seed burial has also proven to be a factor indirectly contributing to the

loss of seed dormancy for many legume species. This is because the depth of the seed in the soil can affect the amount of moisture available, the amount of light reaching the seed, and, more importantly, the temperature to which the seeds are exposed. As previously stated, moisture may affect the softening of black medic seed, and since the amount of moisture usually varies with depth, softening may also vary. As for the impact of depth on the amount of light reaching the seed, black medic is believed to germinate equally well in light and darkness (Van Assche et al. 2003), and therefore any differences between buried and surface seeds should be due to temperature or other factors, such as moisture, not the presence or absence of light.

As for temperature, since deeper buried seed will likely experience higher winter temperatures than seed near the soil surface (Environment Canada 2004a), this may affect whether or not the seed experiences the "chilling" conditions that are required for stage 1 softening. Also, since burial typically dampens the temperature extremes and the fluctuation of temperatures experienced by the seed (Taylor and Ewing 1996), it is reasonable to expect that the deeper buried seed won't receive the temperature fluctuations necessary to meet the stage 2 requirements, and therefore will result in less softening.

The response to seed burial appears to vary between species. For example, in Australia, the seed softening of *Trifolium subterraneum* (subterranean clover) decreases with increasing depth of burial, the seed softening of *Medicago polymorpha* (burr medic) and *Medicago truncatula* (barrel medic) is unaffected by burial depth, and *Trifolium spumosum*, *Trifolium clypeatum* and *Ornithopus compressus* (yellow serradella) tend to soften more when buried (Taylor and Ewing 1996; Taylor and Revell 2002; Zeng 2001).

It is likely that these differences in response are to do with differences in the temperature, moisture and light requirements for each of these species.

As for black medic, the effect of burial depth on seed softening also appears to vary. In one study, the amount of seed softening that occurred in the field did not vary between reduced tillage (seed on surface) and conventional tillage (seed buried) treatments (Frick and Thomas 1992). Roberts and Feast (1973) also found that over a 6 year period there was no difference between the percent emergence of black medic under cultivated and undisturbed soil conditions. In contrast, in an experiment by Roberts and Feast (1972), the percent dormant seed remaining at the 2.5, 7.5 and 15 cm soil depths after five years was found to be 6, 12, and 17%, respectively, for the cultivated treatment and 12, 21, 29%, respectively, for the undisturbed treatment (Roberts and Feast 1972). Therefore, dormancy of black medic seed increased with depth, regardless of the disturbance present. However, in a study by Sidhu (1971) it was found that the percent germination for seeds that had been overwintered at 0, 7.5, 15 and 30 cm soil depths was 11, 19, 32, and 26%, respectively, indicating that the greatest amount of softening occurred at the greater depths (15 and 30 cm). Sidhu (1971) also reported that seed that was overwintered 30 cm above the soil surface resulted in percent germinations ranging from 4.2 to 11.5, which was significantly lower than germination (26.7 to 86.5%) of seeds that were overwintered at 7.5 cm below the surface.

The reasons for this variation in the response of black medic to seed burial depth may be as follows. One reason is that since depth only indirectly affects seed softening (i.e., through temperature modification), it is not the actual depth that is important but the conditions, such as moisture and temperature, found at that depth that are important. For

example, a depth of 3 cm in one environment/location may produce an equal softening response as a depth of 0 cm in another environment/location if the actual seed softening factors, particularly temperature, are similar at these two depths. Another reason for the variation in the optimal depth for black medic seed softening may be that although the seed may soften at a certain depth, unless favourable germination conditions are present, the seed may revert to secondary dormancy. In this regard, the germination or non-germination of the seed may bias the seed softening data.

It is important that we understand the effect of seed burial on seed softening because different cropping practices, such as tillage, can influence the position of the seed in the soil, and therefore could potentially influence the rate and magnitude of black medic seed softening. However, before any conclusions can be made about the impact of burial on black medic seed softening more research needs to be done. Also, in order to ensure the accuracy of the seed softening data, this research needs to be conducted under controlled conditions (i.e. pouches). The Australian's have been using "pouch technology" for years in order to control the conditions to which their seeds are exposed (Norman et al. 1998; Taylor and Revell 2002). These pouches are usually made from a fine mesh material (e.g. fiberglass, cotton etc.), which allows the seed to have contact with the soil, without any of them escaping. For each experiment, according to the requirements, the pouches, each containing a certain number of legume seeds, are placed at the appropriate location in the soil and are secured into place until the time of sampling. This methodology allows the experiment to be conducted under field conditions, while still maintaining some control over the study.

1.7 Summary and Objectives

Cover crops are an important innovation in sustainable crop production systems. The successful use of black medic as a cover crop in grain production systems requires a better understanding of how different environmental conditions, particularly temperature, affect its dormancy and its yearly capacity to regenerate. The key to the success of black medic as a self-regenerating legume is its seed dormancy mechanism, hardseededness. In order to 'soften' this hardseed coat, it has been suggested that a 2-stage seed softening process is required. Unlike the high temperatures that are required for the softening of legume species in a Mediterranean climate such as that in Australia (e.g. Stage 1: 20 to 60°C, Stage 2: 60/15°C), in temperate climates (e.g. Belgium), a series of low temperature stages (e.g. Stage 1: 5°C, Stage 2: 15/6°C) appear to be required. A preliminary study in Manitoba suggests that a similar 2-stage process may be required for black medic in Western Canada (Braul 2004). However, the exact conditions involved in the seed softening of black medic in Western Canada remain unknown. In order to better characterize these conditions, a field experiment and a controlled environment experiment were established. The objectives of these experiments were to answer the following questions:

A) *Field experiment*

1) What influence does seed production and softening environment have on hardseededness and seed softening?
2) When will seed softening occur during the year?
3) What is the effect of soil burial depth on seed softening?

4) What are the differences in plant growth and development and seed dormancy between the two populations: 'selected George' and 'foundation George'?

5) What are the differences in plant growth and development and seed dormancy between medic plants seeded alone and medic plants seeded with wheat?

B) *Controlled environment experiment*

1) What temperature(s) meet(s) the stage 1 ("chilling'' stage) requirements: 5, -5°C or –23°C?

2) What is the minimum exposure time required for stage 1 softening?

Chapter 2: Factors Affecting Black Medic (*Medicago lupulina*) Production and Seed Softening

2.1 Introduction

In order to better understand the seed dormancy breaking of black medic, a two-part field experiment was established. This field experiment involved a seed production study and a seed softening study. The objective of the seed production study was to investigate the effect of seed production location, medic population, and the presence of a companion crop (wheat) on the growth and development of the black medic. Specific objectives of this study were to:

1) Determine the influence of seed production environment on growth, development and initial hardseededness of black medic
2) Determine the differences in plant growth and development and initial hardseededness of two black medic populations
3) Determine the differences in plant growth and development between medic plants grown alone and in the presence of a wheat main crop

However, the main purpose for the seed production study was to produce seed for the seed softening study. For the seed softening study, the questions being investigated were:

1) What influence do seed production and softening environments have on seed softening?
2) When will seed softening occur during the year?
3) What is the effect of soil burial depth on seed softening of black medic?
4) What are the differences in seed softening characteristics of black medic populations?

5) What is the effect of germination temperature regime (20°C vs. 15/6°C) on seed softening of black medic?

2.2 Methods and Materials

2.2.1 Seed Production Study

2.2.1.1 Site Descriptions

The seed production study was conducted in the summer of 2003 at three locations in Western Canada: University of Manitoba Research Station at Winnipeg, Manitoba; Agriculture and Agri-Food Canada Research Station at Indian Head, Saskatchewan; Agriculture and Agri-Food Canada Research Station at Lethbridge, Alberta. These three sites each represented different environments. Typically, Winnipeg has a more sub-humid climate (514 mm annual precipitation), while Indian Head is considered to be dry sub-humid (447 mm annual precipitation) and Lethbridge is considered to be semi-arid (386 mm annual precipitation) (Environment Canada 2004a).

The soil in Winnipeg is a Riverdale silty clay, while the soil in Indian Head is an Indian Head heavy clay and the soil in Lethbridge is a sandy clay loam. A soil sample was obtained in July 2003 from each of the three sites and the mineral content (N, P, S, K) and pH for the 0-6 cm and 6-24 cm depths at each site are presented in Appendix A.

The experimental design was a randomized complete block design (4 replicates) with population ('selected George', 'foundation George') as the factor in Indian Head and Lethbridge, and population and the presence/absence of a wheat companion crop (medic only, medic/wheat) being the factors in Winnipeg. The purpose of the wheat companion crop was to observe if there were any significant differences in plant

development (e.g., plant height) between medic growing alone compared to medic growing with a companion crop.

2.2.1.2 Preparation

Two seed populations were used in this study: 'foundation George' and 'selected George'. 'Foundation George' seed was obtained from the Timeless Seeds Company in Conrad, Montana in the spring of 2003 and was stored at room temperature at the University of Manitoba until its use. 'Selected George' seed, which was originally produced from certified 'George', was produced in 2002 in Goodrich, North Dakota under a flax crop (Braul 2004). After harvest, this seed was stored in a grain bin until February 2003, at which point it was separated from the flax crop and placed in a paper sack in a non-heated shed. In April 2003, this seed was transported to Winnipeg and stored at room temperature. Both seed populations were cleaned by hand in order to remove any foreign material, including weed seeds, as well as any dead or immature black medic seed.

Since black medic has a hard seed coat, the seed needed to be scarified before it was seeded. The medic seed was scarified by placing it between two pieces of sandpaper and, with a consistent amount of pressure being applied, rubbing the pieces together a certain number of times. For each population, the optimal number of times was determined by testing the germination of the seeds after they were rubbed for a set number of times. If not enough rubbing occurred, the hardseed coat would remain intact and germination would not occur, but if excessive rubbing occurred, the seeds would be damaged and successful germination would not occur. In general, the seed was rubbed

approximately 60 times with the sandpaper, which resulted in 90 and 75 percent germination for the 'selected George' and 'foundation George' seed, respectively.

2.2.1.3 Seeding

In late May, which is the typical emergence time for black medic in a regenerating system, the black medic seed was seeded into field plots. Before seeding, the black medic was inoculated with Nitragin® alfalfa/clover powder inoculant by placing the seed in an envelope along with some inoculant and shaking the envelope until all the seeds were coated. The black medic was then broadcast seeded, at a rate of 5 kg/ha of viable, non-dormant seed, by sprinkling the seed evenly across the plot surface by hand. Since each plot was 2m by 6m, this translated into 6.6 g of 'selected George' seed and 7.6 g of 'foundation George' seed being used per plot.

2.2.1.3.1 Winnipeg

On May 27th 2003, the soil at the Winnipeg site was cultivated and wheat (cv: AC Barrie) was seeded at a rate of 70 kg/ha into the appropriate plots. Following this, the black medic was hand-seeded and the seeds were incorporated into the soil using a harrow. In order to promote germination and establishment, the plots were watered after seeding and on a number of subsequent occasions until the wheat and medic crops became successfully established. In order to control grassy weeds, on July 4th the medic/wheat plots were sprayed with Achieve 80 DG (tralkoxydim) at a rate of 250 g/ha of product and the medic only plots were sprayed with Poast Ultra (sethoxydim) at a rate of 0.5 L/ha of product. Hand-weeding and hoeing was used to control the broadleaved

weeds.

2.2.1.3.2 Indian Head

On May 20th 2003, the black medic was hand-seeded into no-till soil and incorporated into the soil using a rake. The plots were watered with a sprinkler, when deemed necessary, until the black medic emerged. In order to support the growth of the black medic plants, the plots were hand-weeded on a regular basis and were sprayed for grasshoppers on June 28th with Lorsban (chlorpyrifos) at a rate of 1 L/ha of product.

2.2.1.3.3 Lethbridge

On May 22nd 2003, the black medic was seeded into no-till soil and incorporated into the soil using a rake. The plots were watered with a watering can a number of times, but the black medic did not successfully establish and reseeding occurred on June 19th. In order to provide a better seedbed and to improve seed-soil contact, the plot area was cultivated three times before reseeding and after reseeding the seed was packed into the soil using a packer. The plots were hand-weeded/hoed in order to control the weeds and Sevin (carbaryl) was sprayed twice in order to help control the grasshoppers. However, due to unfavourable growing conditions (i.e., moisture stress and grasshoppers), the black medic plants did not survive at Lethbridge.

2.2.1.4 Plant Measurements

Phenological development, plant density and height, dry matter and mean seed weight were observed and recorded for the *M. lupulina* plants throughout the growing

season. The difference in the development of the two populations at the different locations was measured by recording the dates of 50% emergence, 50% flowering and 50% pod formation. In late June, plant density was measured at each location. At Indian Head, the plant stand in each plot was quite variable, and therefore 5 - 1/10m^2 quadrats were used per plot for these measurements compared to the 2 - 1/4 m^2 quadrats per plot that were used to assess the more uniform stand at Winnipeg. In September, in order to determine differences in medic growth between the different treatments, plant height was measured for 10 randomly chosen plants in each plot. At harvest, dry matter samples were taken (2 - 1/4m^2 quadrats per plot), forced-air dried and weighed. These samples were then threshed in order to obtain seed for the calculation of the mean seed weight. Mean seed weight was determined for each treatment by randomly choosing 100 seeds from each treatment replicate and calculating the overall mean weight per seed.

2.2.1.5 Environmental Monitoring

At each location, a HOBO (Model #H20-001) Water Temp Pro logger (Onset Computer Corporation, Bourne, MA) was placed in the soil at 5 cm in order to continually monitor the soil temperature. Precipitation and air temperature were monitored by weather stations operated by the University of Manitoba, Agriculture and Agri-Food Canada (Indian Head), and Agriculture and Agri-Food Canada (Lethbridge); all of which were within 1 km of the experiment sites.

2.2.1.6 Harvest and Processing

In September, mature black medic seed was collected from each plot by randomly

hand-harvesting approximately 30 plants. In order to ensure that only mature seed was collected, green or unripe seeds that were found at the tips of the plants were removed during harvest. For each plot, the plants collected were force-air dried and then threshed with a belt thresher in order to remove the pods from the plants and the seeds from the pods. In order to separate the medic seeds from the rest of the harvest material, the threshed material was passed over a series of sieves (7/64" round, 1/16" round, 0.028" x 0.028" square, 0.010" x 0.010" square) (Seedburo Equipment Company, Chicago, IL). To clean the seed sample further, it was placed in an air column (Agriculex CB-1 Column Seed Cleaner) to remove the smaller plant and soil particles. Finally, the seed samples were cleaned by hand in order to remove any dead or green seeds.

2.2.1.7 Hardseededness and Viability Tests

The initial percent hardseededness and seed viability were determined for each location (IH and WPG) and seed population ('selected George' and 'foundation George') combination. For each combination, seed from each replicate was thoroughly mixed and 50 seeds were randomly chosen. The test for hardseededness was conducted by: 1) placing each 50 seed sample in a Petri dish, 2) adding approximately 5 mL of water, and 3) placing the sample under 20°C conditions for 1 week (Taylor and Revell 1999). Unimbibed seeds (i.e., hard seeds) were counted to determine the percent hardseededness. For each sample, 10 of the remaining hard seeds were scarified using a scalpel and placed under standard conditions (20°C, moist) for 1 week in order to determine the viability and the absence of embryo dormancy (Norman et al. 2002a).

2.2.2 Seed Softening Study

2.2.2.1 Site Description

The seed softening study was conducted from the fall of 2003 to the summer of 2004 at Winnipeg, Indian Head and Lethbridge. In Winnipeg, the soil was a Riverdale silty clay, while the soil in Indian Head was an Indian Head heavy clay and the soil in Lethbridge was a sandy clay loam. At each site, a spring cereal crop was grown and harvested during the 2003 growing season (wheat at Lethbridge and Indian Head, oats at Winnipeg). Both stubble and residue were left on the soil surface in order to provide cover and improve the snow catch.

2.2.2.2 Experimental design and factors

The experimental design at each location was a randomized complete block split-split plot with four replicates. Factors included seed production location (mainplot), seed population (sub-plot) and burial depth (sub-sub plot). For seed production location, seed produced in Winnipeg and Indian Head was placed at all three sites in order to determine if there was an impact of the seed production environment on the seed softening. In this way, seeds produced in wetter environments (e.g. Winnipeg) could be tested for seed softening in drier environments (e.g. Indian Head and Lethbridge), and vice versa. For seed population, both 'selected George' and 'foundation George' seed that was produced in the two locations was used in the softening study in order to determine if there were any differences in the softening pattern due to the different origins of these populations. For seed burial depth, pouches containing seed from each production location/population combination were placed on the soil surface and at approximately 2 cm below the soil

surface. Differences in the seed softening at these two depths could have implications on how this species is best managed in a cropping system.

2.2.2.3 Preparation

Remaining seed from the seed production study from each population ('selected George' and 'foundation George') and location (Indian Head and Winnipeg) was thoroughly mixed (reps combined) and seeds were separated into lots of 100 seeds each (following generally the methods of Taylor and Revell 2002 and Norman et al. 2002a). Each lot was placed into a pouch that was made from Sefar Nitex (reference # 06-780/53) polyamide monofilament white mesh (Sefar Filtration Inc., Buffalo, NY). This mesh material had openings small enough (780 microns wide) to prevent seeds from escaping, but large enough to allow the free movement of water, air, microbes etc. The pouches were made by cutting the micromesh material into 4 cm x 4 cm squares, placing two squares together and melting three sides of the squares together using a soldering gun. A tag was also melted into each pouch in order to identify what treatment the seed belonged to. The appropriate seed was then placed into the pouch and the fourth side of the pouch was sealed. Each pouch was made large enough to allow all seeds to have contact with the soil.

In October, all the pouches were placed in the fields at the Winnipeg, Indian Head and Lethbridge locations. So as to simulate typical crop residue levels, pouches were placed between remnant straw rows. Chicken wire was placed over top of the area and nailed into the ground in order to prevent pouches from blowing away or being disturbed by animals. During the spring/summer months, the chicken wire was periodically

removed in order to hand-weed the area.

2.2.2.4 Sampling

In order to determine when stage 1 and stage 2 softening occurred during the year at each location, pouches were sampled at seven times between November 2003 and August 2004. The exact sampling dates are given in Table 2.1. Sampling was intensified during the winter and early spring since this was the time of year when the majority of softening was expected to occur.

Table 2.1 Sampling dates for seed pouches at Winnipeg, Indian Head and Lethbridge from 2003 to 2004

Sampling	Winnipeg	Indian Head	Lethbridge
1	13-Nov	12-Nov	11-Nov
2	6-Jan	4-Jan	3-Jan
3	16-Feb	17-Feb	16-Feb
4	23-Mar	21-Mar	20-Mar
5	19-Apr	18-Apr	17-Apr
6	16-Jun	15-Jun	14-Jun
7	7-Aug	6-Aug	5-Aug

The procedure for recovering seed pouches from the field was as follows. During the winter samplings, snow and ice had to be shoveled off the plot area before the nails and chicken wire could be pried up. After removing the chicken wire, the appropriate pouches were removed by chiseling them out of the frozen ground and ice. After all the

pouches had been removed the chicken wire and snow was replaced. In order to mimic "field" temperatures during the transportation of the pouches to the University of Manitoba, the pouches were placed in a cooler. In order to monitor the temperature that the pouches were exposed to between the time of sampling and the time when the pouches were processed, a temperature-sensing device (HOBO) was also placed in the cooler. The data from the HOBOs confirmed that the pouches remained within the appropriate range of cold temperatures throughout their transportation. During the spring and summer sampling times, the chicken wire was easily lifted off the plots and the appropriate pouches were removed.

2.2.2.5 Hardseededness and Viability tests

After each sampling, each pouch was opened and the seedlot in each pouch was divided in half. Since there were originally 100 seeds in each pouch, each half had approximately 50 seeds. During the dividing process, the seeds were divided in such a way that each half contained relatively similar numbers of seeds from each of the following categories: dead, germinated, imbibed and hardseed. Seed was classified as dead if it was shriveled up and no longer golden/yellow in colour. Since the viability of this species is quite high (Van Assche et al. 2003), it was assumed that a large portion of these dead seeds were seeds that had softened but were unable to successfully germinate because of external conditions (e.g., too cold, moisture stress). Seed was classified as germinated if it had already softened and was germinating in the pouch, and seed was classified as imbibed if the seed appeared to be soft and swollen. Seed that did not fit into one of the above categories was classified as hardseed. The seeds from both halves of

each pouch were placed in a Petri dish and the number of seeds in each category was recorded. Each Petri dish contained two Whatman #1 filter papers and approximately 5 mL of water.

In order to distinguish between stage 1 and stage 2 seed softening, half of the seed from each pouch was placed under a standard germination temperature (20°C), while the other half was placed under a low fluctuating germination temperature (15°C for 12 hours, 6°C for 12 hours) for a period of two weeks. This low fluctuating temperature treatment was meant to simulate stage 2 conditions. In general, stage 1 seed softening is assumed to have occurred once the "chilling" temperature requirements have been met and exposure to stage 2 conditions (low fluctuating temperatures), and only stage 2 conditions, results in germination (Figure 2.1). Stage 2 softening is assumed to have occurred when seed has already been exposed to both the required "chilling" and low fluctuating temperatures, and therefore similar germination occurs under both the standard and fluctuating germination temperatures.

Therefore, for a given sampling time, if a significant amount of softening occurred under the 15/6°C (stage 2) germination temperature but not the 20°C germination temperature, it could be concluded that: 1) a 2-stage process was required for black medic softening and 2) stage 1 requirements must have been met in the field by that time. After this initial softening, if the next sampling period showed that the 20°C treatment resulted in as much softening as the 15/6°C treatment, it could be concluded that the stage 2 conditions had been met in the field.

At each sampling time, a control treatment (seed from the same seedlot stored at room temperature (20°C)) was also subjected to these two germination temperatures.

	Germination Temperature Treatments	
	20°C	15/6°C
Stage 1 Softening ☑ "Chilling" temperature ☒ Low fluctuating temperature	No germination	
Stage 2 Softening ☑ "Chilling" temperature ☑ Low fluctuating temperature		

Figure 2.1 Assumptions of stage 1 and stage 2 softening requirements and the corresponding germination response to standard (20°C) and simulated stage 2 (15/6°C) germination temperature treatments for seed that has been exposed to either stage 1 or stage 2 conditions in the field.

Since light does not affect the seed softening of black medic (Van Assche et al. 2003), both of the germination cabinets (20°C cabinet- Model 124 S#6F9084 and 15/6°C cabinet- R414b refrigerant, Controlled Environments Ltd., Winnipeg, MB) were kept dark. During this period of time, the Petri dishes were watered every other day to ensure that there was free water available to the seeds.

After allowing the seed to germinate for two weeks, the number of remaining hardseed (unimbibed seed) was counted. This number was subtracted from the original number of hardseed per Petri dish and multiplied by two in order to determine the percentage of softened seed for each Petri dish. In order to determine if the remaining

hardseeds were viable, 10 hardseeds from each Petri dish were scarified with a scalpel and allowed to germinate under standard conditions (20°C, moist) for 1 week.

2.2.2.6 Environmental Monitoring

In order to continually monitor the soil temperature at each field location, a temperature-sensing device (HOBO) was placed alongside the pouches in each of the four replicates and at each depth (surface and 2 cm). These HOBOs were placed in the field in October and were not removed until the last sampling date (August).

Soil moisture was determined at the March, April, June and August sampling dates. Using a trowel, a random sample of the top 2 cm of soil was removed at each location. In order to prevent soil water loss during storage and transport, soil was placed in a sealed plastic bag until it was processed. These soil samples were weighed before and after they were dried at 110°C for 2 days. The difference in their weight was used to determine the gravimetric soil moisture content. Soil moisture results are shown in Appendix B.

2.2.3 Statistical Analysis

Using SAS 9.1, an analysis of variance was performed on the data (Proc GLM). The data was tested for homogeneity of variance using Bartlett's test, and if found to be non-homogeneous, was log transformed and re-analyzed. Means were separated on the basis of a protected least significant difference (PLSD) test with a 5% level of significance.

2.3 Results and Discussion

2.3.1 Seed Production Study

In terms of phenological development, 'foundation George' plants tended to flower and pod earlier than 'selected George' plants. For example, at Winnipeg 'foundation George' plants reached 50% flowering by July 8th, but 'selected George' plants did not reach 50% flowering until July 10th (data not shown). At harvest, there were noticeably more flowers and green pods on the 'selected George' plants compared to the more mature 'foundation George' plants at Winnipeg. Due to logistical problems, phenological development was not successfully recorded for the black medic plants at Indian Head.

These results provide evidence that the selection pressure exerted on the 'selected George' seed from 12 years of production on the North Dakota farm may have caused this population of black medic to exhibit a delayed phenological development compared to the 'foundation George' seed. It is possible that herbicide use over the years at this site shifted the 'selected George' population to a later developing population which would escape the herbicide applications. Also, because competition by the main crop reduces the access of the medic plants to resources, the 'selected George' population may have, over the years, shifted to later flowering and seed formation when the main crop has finished growing, and therefore more resources would be available for black medic growth and development.

Plant densities were significantly greater at Winnipeg than at Indian Head, but there was no significant difference between the 'selected George' and 'foundation George' populations or between the medic only and medic/wheat treatments (Table 2.2).

Table 2.2 The influence of the presence of a crop, production location and population on mean plant density, plant height and dry matter of black medic.

Main Factors*	Levels	Plant Density (plants per m^2)	Plant Height (cm)	Dry Matter (kg/ha)
Crop	medic	139.50	30.80	2911.6a**
	medic/wheat	131.25	33.65	1217.6b
	$LSD_{(0.05)}$	36.48	3.20	633.7
Production location	Winnipeg	137.00a	30.80	2912.5
	Indian Head	50.75b	28.28	2440.0
	$LSD_{(0.05)}$	28.89	2.59	654.5
Population	foundation George	92.50	28.58	2595.0
	selected George	95.25	30.50	2757.5
	$LSD_{(0.05)}$	28.89	2.59	654.5
Highlights of ANOVA				
Crop		NS	NS	<.0001
Production location		<.0001	NS	NS
Population		NS	NS	NS

* The crop results only include data from Winnipeg, while the production location and population results include data from both Winnipeg and Indian Head.

* *Values followed by a different letter within a main factor and variable are significantly different at $p<0.05$.

The lower plant density at Indian Head was likely due to the less favourable growing conditions (i.e., moisture stress, grasshoppers) at Indian Head compared to Winnipeg.

Medic plant height was not significantly affected by any of the factors (Table 2.2). However, the medic plants in the medic only plots were quite robust while medic plants in the medic/wheat plots were quite spindly. This was reflected in medic dry matter production, where the medic only plots produced significantly more dry matter than the medic/wheat plots (Table 2.2). These observations clearly show that overall production by a medic crop is lower when it is grown as a cover crop together with a main crop instead of as a sole crop.

The mean medic seed weight was greater for seed produced at Indian Head (1.65 mg) than for seed produced at Winnipeg (1.26 mg), but there was no significant difference between populations (Table 2.3). In comparison, Van Assche et al. (2003) found the mean seed weight for black medic to be approximately 1.39 mg. Seed mass is considered to be a plastic character that changes with environmental conditions and plant density (Wulff 1995). Since plant density may affect seed mass, the lower plant density at Indian Head may have caused the higher seed mass at this site. Also, the reason for the larger seeds at Indian Head may be due to the growing conditions. Under stressful growing conditions, the black medic plants may have allocated more of their resources to seed production in order to increase the chance of seed survival and future reproduction. Larger seeds typically mean that there are greater food reserves available, and therefore the seed may remain dormant longer and have greater longevity under stressful conditions (Bewley and Black 1982).

The initial hardseededness test showed that regardless of the treatment, black

Table 2.3 The influence of production location and population on mean seed weight and percent hardseededness of black medic.

Main Factors	Levels	Seed Weight (mg)	Hardseed (%)
Production location	Winnipeg	1.26a*	97.00
	Indian Head	1.65b	98.50
	$LSD_{(0.05)}$	0.20	2.30
Population	foundation George	1.52	97.88
	selected George	1.39	97.63
	$LSD_{(0.05)}$	0.20	2.30
Highlights of ANOVA			
Production location		0.0012	NS
Population		NS	NS

* Values followed by a different letter within a main factor and variable are significantly different at $p<0.05$

medic hardseededness was approximately 97% (Table 2.3). This high level of hardseededness is common for black medic (Moomaw 1995; Pavone and Reader 1982; Sidhu 1971). Also, there appeared to be an absence of any type of embryo dormancy, since the overall viability of the hard seed tested was 100% (see Appendix C). This high level of viability is also common for black medic (Van Assche et al. 2003). These results suggest that initial hardseededness and viability for the black medic populations tested here were similar to those reported in the literature and that different production environments, seed population and other factors had very little impact on hardseededness

and viability.

Overall, results from the seed production study suggest that: 1) selection pressures may have caused the 'selected George' population to exhibit a delayed phenological develop compared to the 'foundation George' population, 2) the presence of a main crop may influence growth and dry matter production, 3) production location may influence plant density and seed weight, and 4) initial hardseedness and viability of black medic seed is quite independent of the factors tested in this field study. This suggests that even though growth, development and production may vary for a black medic cover crop due to varying levels of competition, growing conditions and previous agronomic pressures, the initial hardseededness of mature black medic seed does not vary.

2.3.2 Seed Softening Study

In an effort to determine when during the winter/spring period black medic lose dormancy, pouches were collected on a regular basis from November to August from the field sites at Winnipeg, Lethbridge and Indian Head. The seed in each of these pouches corresponded to a particular production location (Winnipeg or Indian Head), population ('selected George' or 'foundation George') and burial depth (surface or 2 cm). Soil temperature data was also recorded during this time in order to allow for some linkage to be made between black medic softening and soil temperature.

2.3.2.1 Influence of Factors on Seed Softening

2.3.2.1.1 Winnipeg

At Winnipeg, there was no evidence of significant softening in November since

the softening that occurred at Winnipeg in November was not significantly greater than the softening that occurred for the control treatment (Figure 2.2). However, by January significant softening had occurred (Figure 2.2 and Appendix D).

Although a significant amount of softening occurred at the Winnipeg site in January and February, it was not until March that there was a significant difference between the 20°C and 15/6°C germination temperature treatments (Figure 2.3). As previously mentioned, a difference in response between the 20°C and 15/6°C treatments indicates that stage 1 softening has occurred. These results indicate that the stage 1 conditions were not met in the field until March, and therefore any softening that occurred before March must have been due to other factors. However, it is unclear what these other factors may have been.

After March, there was a significant amount of softening at the Winnipeg site (Figure 2.2), but there was no longer a difference between the germination temperature treatments, as evidenced by the lack of significant difference between the 20°C and 15/6°C treatments for the April, June and August samplings (Figure 2.3). This lack of difference indicates that the stage 2 conditions must have been met in the field by this time, since exposing the seed to simulated stage 2 conditions no longer resulted in more softening than exposing the seed to standard germination conditions (i.e. 20°C) (Appendix E).

2.3.2.1.1.1 Production Location

For all of the sampling dates, there was no significant difference in seed softening between seed production locations (Table 2.4). This suggests that even though the seed

Figure 2.2 Percent softening (15/6°C germination temperature) for black medic seed samples extracted from the Winnipeg site at intervals during 2003 and 2004 compared to the control treatment (seed stored at room temperature subjected to the same 15/6°C regime). Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results (standard errors for PLSD test derived from control and three softening site treatments).

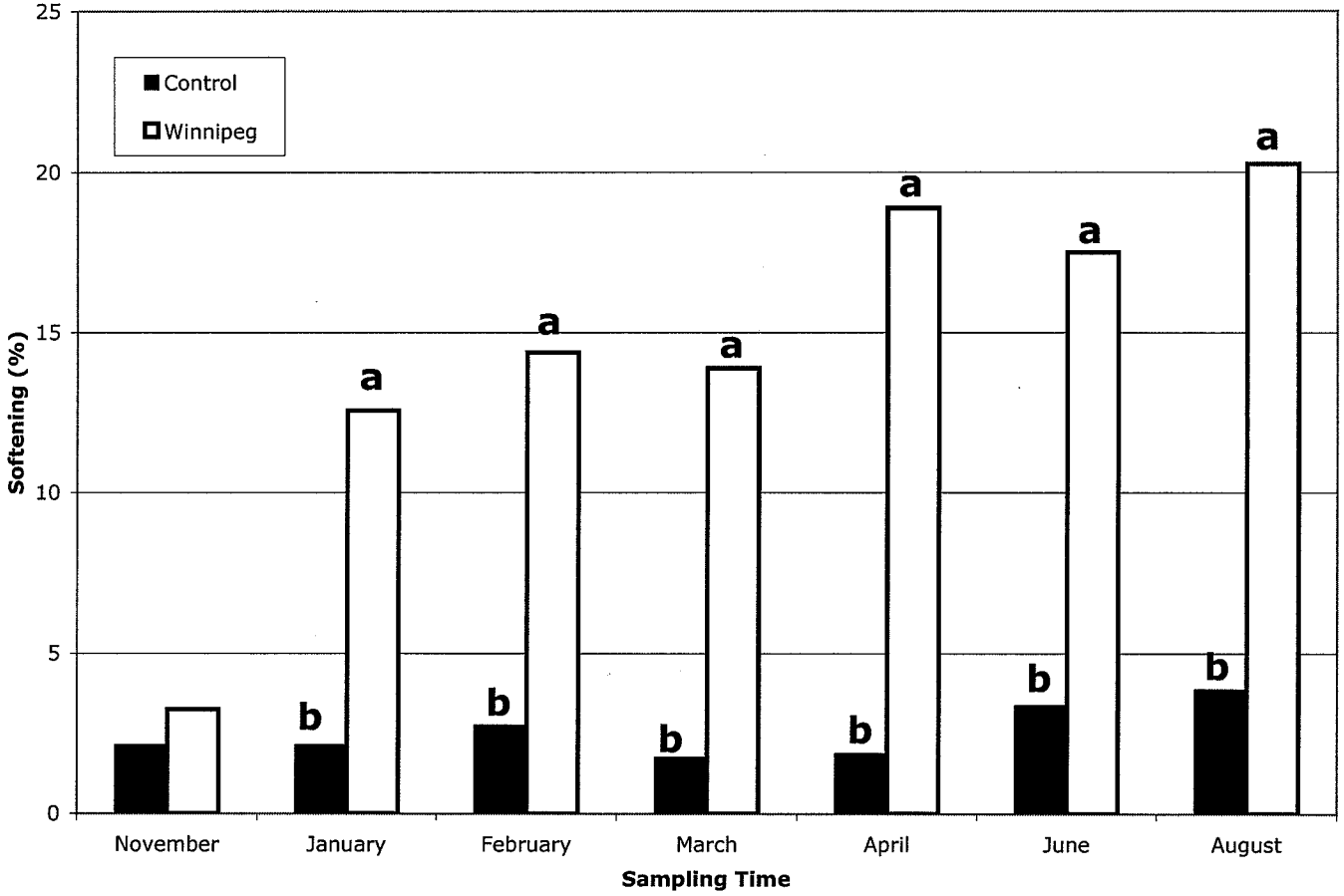


Figure 2.3 Percent softening for black medic seed samples extracted from the Winnipeg site at intervals during 2003 and 2004 and subjected to the 20°C and 15/6°C germination temperature treatments. Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results (standard errors for PLSD test derived from the 15/6°C and 20°C treatments).

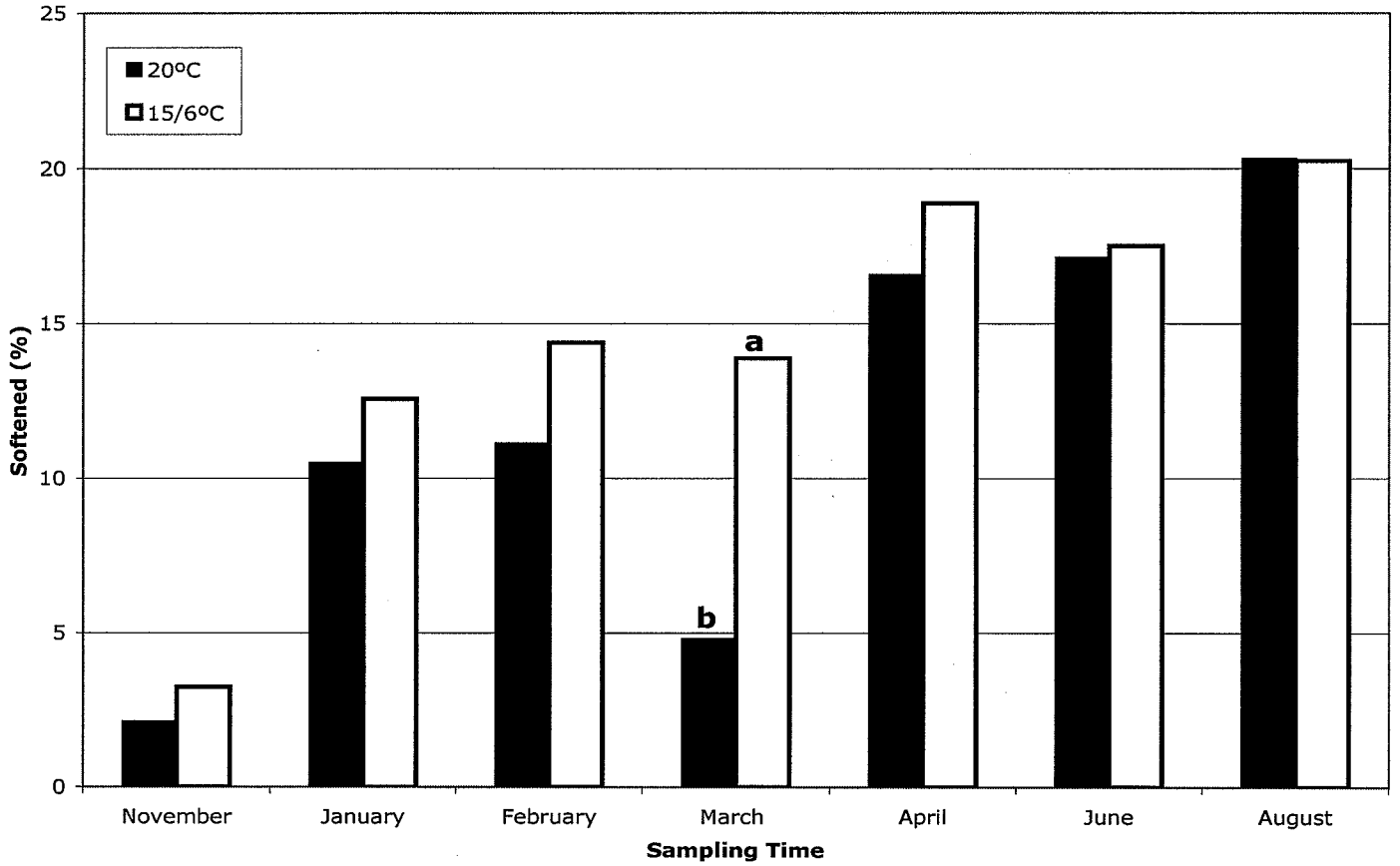


Table 2.4 Percent softening (germination at 15/6°C temperature regime) for black medic seed samples extracted from the Winnipeg site at intervals during 2003 and 2004 corresponding to the production location (Prodloc), population (Type) and burial depth (Depth) factors. Within each sampling time and main factor, values followed by different letters are significantly different at the $p<0.05$ level (PLSD).

		Sampling Time						
Main Factors	Levels	November	January	February	March	April	June	August
Prodloc	Indian Head	2.76	12.38	13.26	12.00	18.50	17.26	21.38
	Winnipeg	3.76	12.76	14.50	11.88	14.88	17.76	19.26
	$PLSD_{(0.05)}$	5.07	12.88	8.18	8.96	5.64	1.13	8.73
Type	foundation George	3.00	13.76	14.88	14.12	19.12	18.76a	22.12a
	selected George	2.50	11.38	12.88	9.76	14.26	16.26b	18.38b
	$PLSD_{(0.05)}$	2.96	10.24	9.67	7.61	6.04	2.34	3.49
Depth	buried	4.00	18.00a	21.76a	15.00a	18.76	14.26b	16.26b
	surface	2.50	7.12b	6.00b	8.88b	14.62	20.76a	24.26a
	$PLSD_{(0.05)}$	1.86	7.30	10.74	5.92	6.54	3.80	3.38

Highlights of ANOVA

	November	January	February	March	April	June	August
Prodloc	NS	NS	NS	NS	NS	NS	NS
Type	NS	NS	NS	NS	NS	*	*
Depth	NS	*	*	*	NS	*	*
Prodloc*Type	NS	NS	NS	NS	NS	NS	NS
Prodloc*Depth	NS	NS	NS	NS	NS	*	NS
Type*Depth	NS	NS	NS	NS	NS	NS	NS
Prodloc*Type*Depth	NS	NS	NS	NS	NS	NS	*

production experiment showed that there were differences in mean seed weight between the two locations, these differences did not translate into differences in seed dormancy breaking.

2.3.2.1.1.2 Population

Throughout the sampling period there was no significant effect of population on seed softening, except for the June and August samples when the 'foundation George' seed softened significantly more than the 'selected George' seed (Table 2.4). Research has shown that exposure to high temperatures (e.g. 50°C) may cause a small cohort of black medic seed to soften (Sidhu 1971). Braul (2004) found that some seed softening occurred after black medic seeds had been exposed to field conditions in late June to mid July, which he attributed to late season softening in response to a high temperature (i.e., Mediterranean) 2-stage process. Therefore, the reason for the difference in softening between the two populations may be that the 'foundation George' seed may have a sub-population of seeds that respond to these high temperatures, while the 'selected George' seeds may have a smaller proportion or none of this sub-population.

The reason for a difference in population response to environment may be as follows. Years of exposure to the different agronomic pressure may have shifted the 'selected George' population to soften mainly in spring since germination during the late summer or fall would expose the young seedlings to a number of stresses (e.g., lack of moisture, competition) and the seedlings would have a limited growing season in which to develop and produce seeds. Therefore, the sub-population of seed that would have originally softened under high temperatures would no longer exist. Also, since 'selected

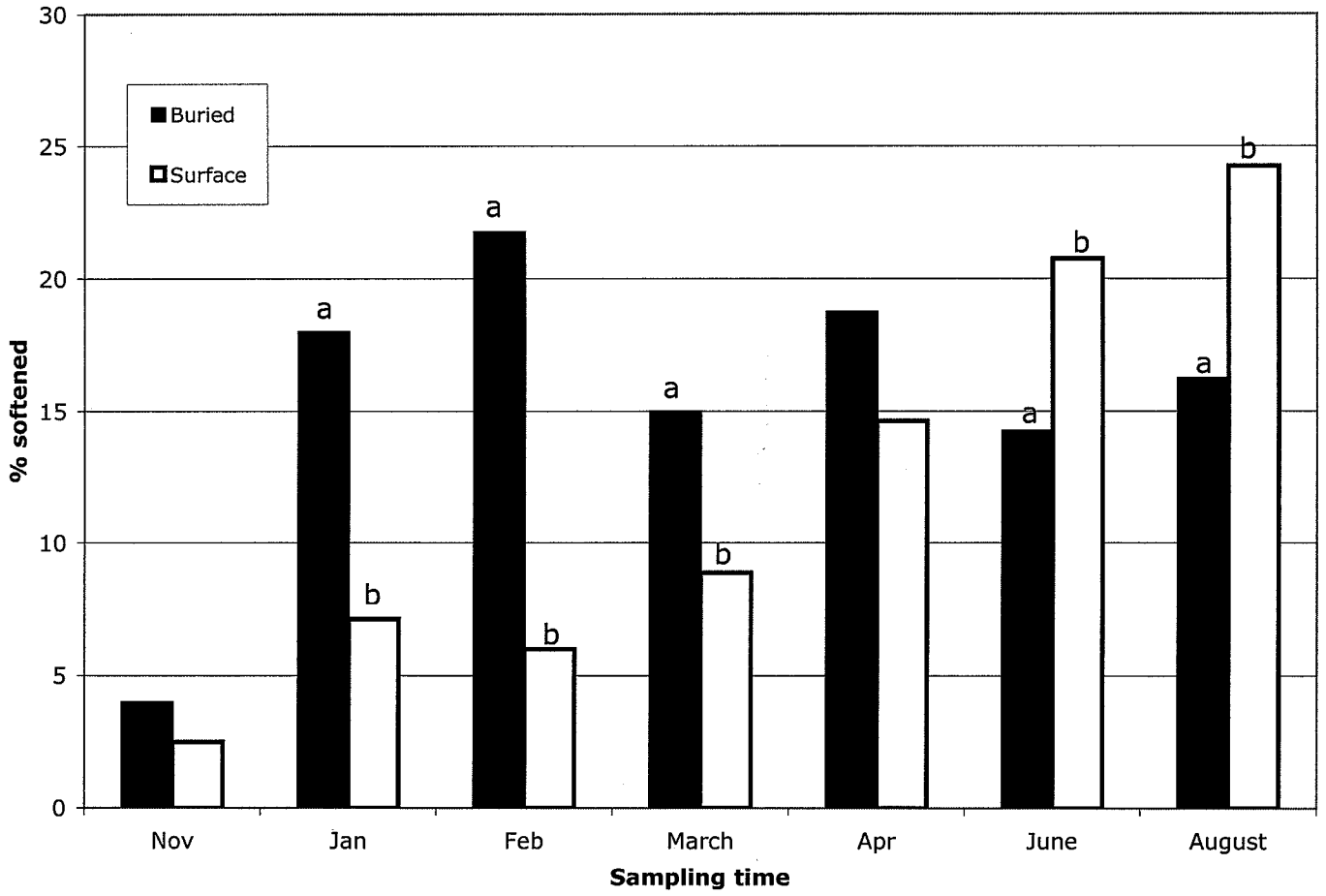
George' seed was produced as a cover crop while 'foundation George' seed was produced as a sole crop, 'selected George' seed would have evolved under shaded conditions, and therefore might have "lost" its ability to respond to high temperatures. The fact that there doesn't appear to be any softening differences between populations during the winter and spring suggests that both populations respond similarly to the temperate 2-stage seed softening process, and that it is the response of the 'selected George' population to high summer temperatures that the selection pressure from the continuous, no-till cropping system may have altered. In conclusion, it appears as though the 'selected George' population is somewhat different than the 'foundation George' population, but we do not know why.

2.3.2.1.1.3 Burial Depth

In general, there was a significant effect of burial depth on black medic seed softening at Winnipeg (Table 2.4), but there was a significant depth by sampling date interaction ($p<0.0001$) (see Figure 2.4). The January, February and March samplings showed that buried seed softened significantly more than surface seed. However, in June and August, the reverse was true, with more seeds softening at the surface than at the 2 cm soil depth.

These differences in response to burial depth may have been related to temperature differences between soil depths since temperature has been shown to be the primary reason for seed softening. For example, during the winter/spring, a more favourable soil temperature for temperate 2-stage softening may have been present at the 2 cm depth, while during the summer, a more favourable soil temperature for

Figure 2.4 Percent softening for black medic seed samples extracted from the 2 cm (buried) and surface depths at the Winnipeg site at intervals during 2003 and 2004 and subjected to the 15/6°C germination temperature treatment. Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results from the split-split plot analysis.



Mediterranean 2-stage softening may have been present at the surface. This link between soil temperature and the seed softening differences between the two depths will be investigated in the temperature section of this chapter. Other than temperature, factors such as soil moisture may have also contributed to creating a more favourable seed softening environment at the 2 cm depth during winter/spring and at the surface depth during summer, but this is only speculation.

In June, there was also a significant interaction ($p<0.008$) between production location and depth (Table 2.4). Winnipeg seed softened more than Indian Head seed at the surface, while Indian Head seed softened more than Winnipeg seed at 2 cm. It appears as though the major difference is that there is an increase in the seed softening of the Winnipeg seed at the surface compared to at 2 cm. This may also be due to more favourable softening conditions at the soil surface during the summer than at 2 cm, as was speculated above. There was also an interaction ($p<0.024$) between production location, population and depth in August, but there does not appear to be any clear biological reason for this interaction.

2.3.2.1.2 Lethbridge

There was no evidence of significant softening of black medic seed at Lethbridge in November and January, but by February the seed had softened significantly more than the control treatment seed (Figure 2.5). Also by February, there was a significant amount of softening occurring for the 15/6°C treatments, which indicated that stage 1 conditions had been met (see Figure 2.6). By March, there was significant softening for both the 20°C and 15/6°C treatments, which indicated that stage 2 conditions had been met. The

Figure 2.5 Percent softening (15/6°C germination temperature) for black medic seed samples extracted from the Lethbridge site at intervals during 2003 and 2004 compared to the control treatment (seed stored at room temperature subjected to the same 15/6°C regime). Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results (standard errors for PLSD test derived from control and three softening site treatments).

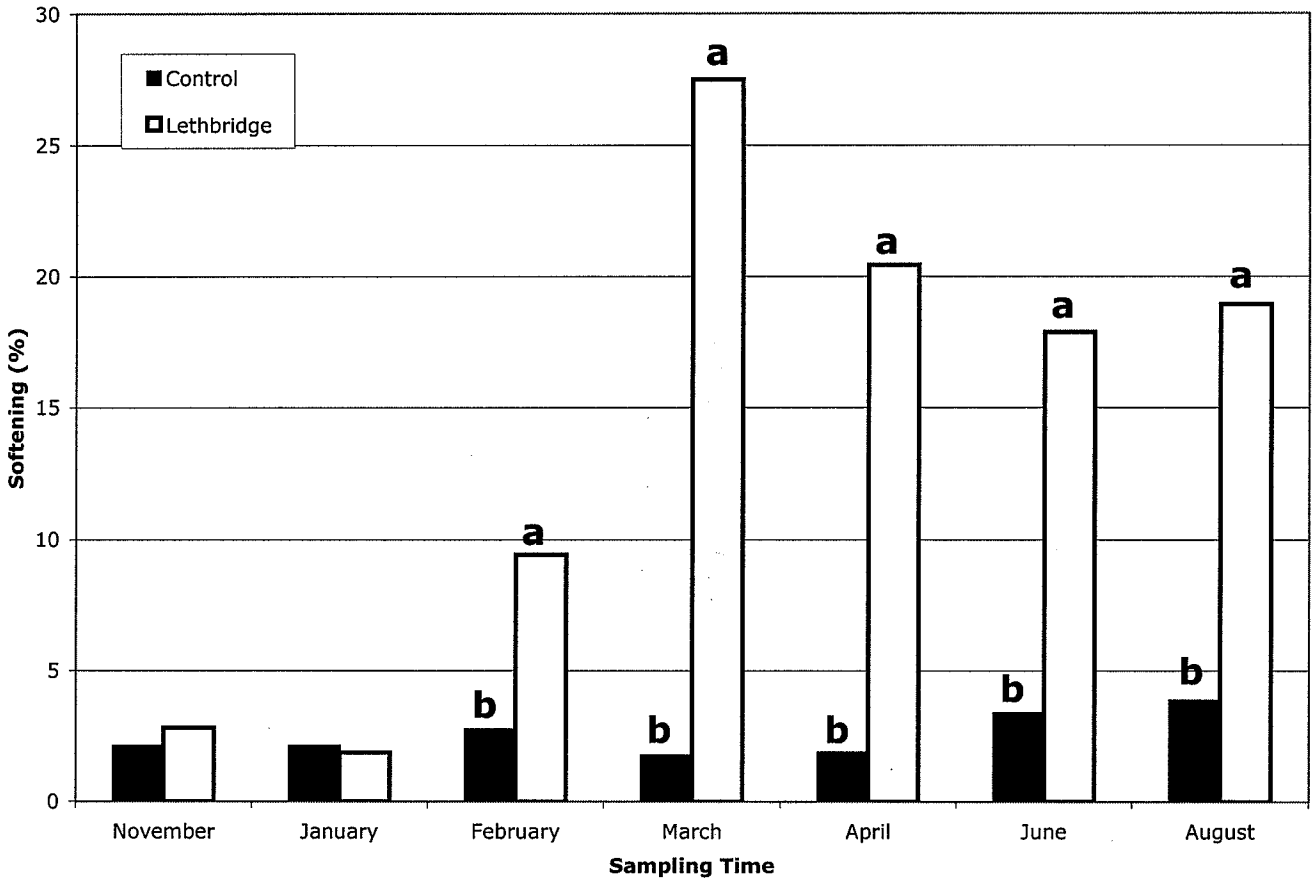
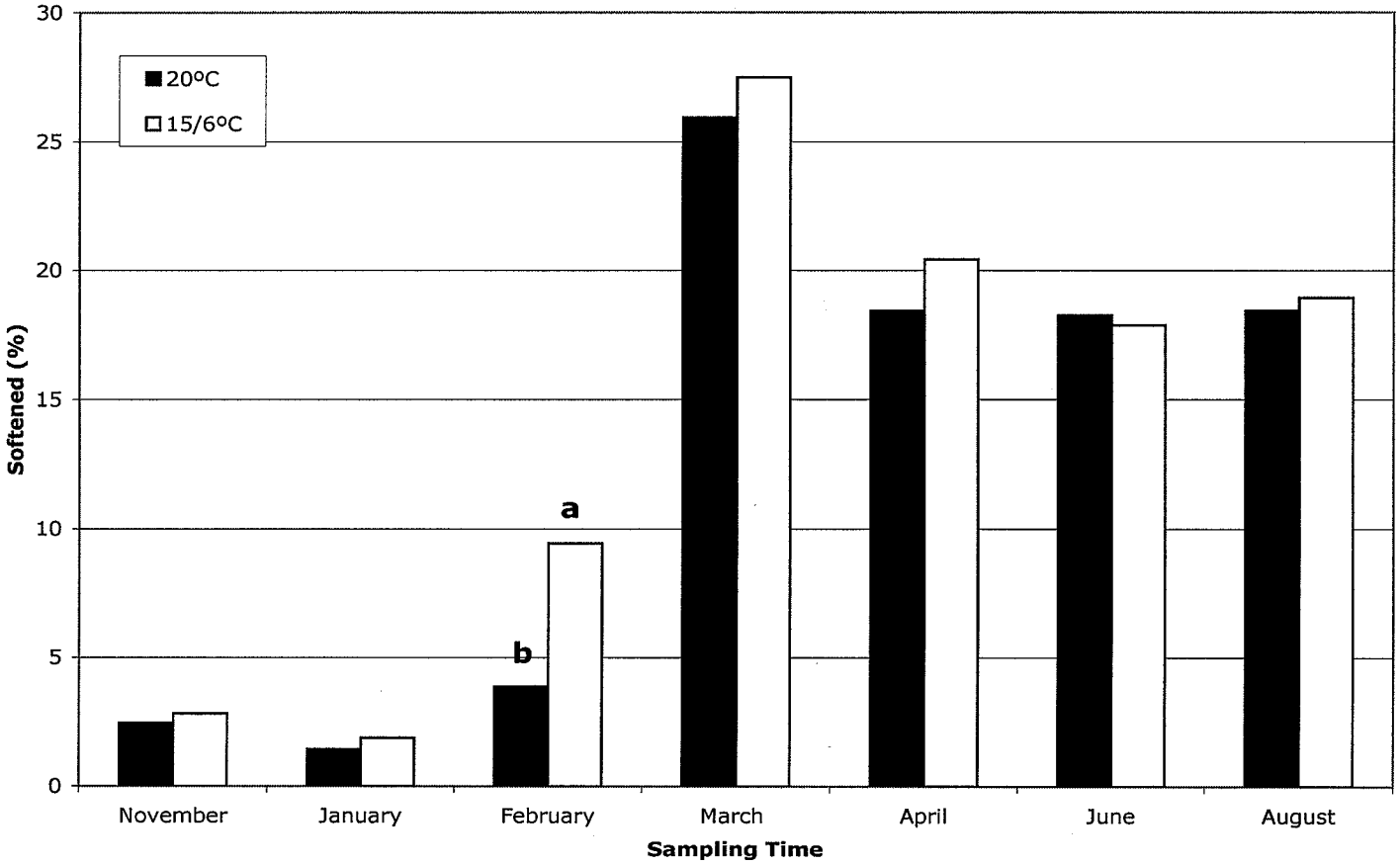


Figure 2.6 Percent softening for black medic seed samples extracted from the Lethbridge site at intervals during 2003 and 2004 and subjected to the 20°C and 15/6°C germination temperature treatments. Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results (standard errors for PLSD test derived from the 15/6°C and 20°C treatments).



stage 1 and stage 2 seed softening occurring earlier in the season at Lethbridge compared to Winnipeg is not unexpected since Lethbridge usually has milder winters, and therefore the "chilling" and fluctuating temperature stage requirements are met earlier in the season at Lethbridge than at Winnipeg (Environment Canada 2004a).

2.3.2.1.2.1 Production Location

Table 2.5 shows that the seed that was produced at Indian Head softened significantly more than the seed produced at Winnipeg during the March sampling, while the reverse trend was observed in August. The reason for these trends may be due to the differences in growing conditions at the two production locations. Perhaps due to the growing conditions, the seed produced at Indian Head had a proportionally larger sub-population that responded to the low temperature 2-stage softening process in March, while a greater proportion of the seeds produced in Winnipeg responded to the high temperature 2-stage softening process. However, the reasons that these effects were observed at Lethbridge and not at Winnipeg are unclear. Overall, the actual seed softening differences between production environments appear to be relatively small.

2.3.2.1.2.2 Population

Unlike the Winnipeg location, there was no significant effect of population on seed softening at any time during the sampling period at Lethbridge (Table 2.5). The reason for this difference between sites may be due to differences in the conditions (i.e. temperature and moisture) during the summer at these two sites. For example, since soil moisture has been shown to influence seed softening (Braul 2004), perhaps the lower soil

Table 2.5 Percent softening (germination at 15/6°C temperature regime) for black medic seed samples extracted from the Lethbridge site at intervals during 2003 and 2004 corresponding to the production location (Prodloc), population (Type) and burial depth (Depth) factors. Within each sampling time and main factor, values followed by different letters are significantly different at the $p<0.05$ level (PLSD).

		Sampling Time						
Main Factors	Levels	November	January	February	March	April	June	August
Prodloc	Indian Head	2.50	3.76	7.76	26.26a	20.00	17.38	17.24b
	Winnipeg	3.12	1.38	7.26	21.88b	15.62	18.38	20.62a
	$PLSD_{(0.05)}$	2.46	2.34	3.38	2.19	5.64	5.63	2.86
Type	foundation George	2.88	1.62	7.26	24.62	17.88	18.38	19.26
	selected George	2.76	2.12	5.76	23.50	17.76	17.38	18.62
	$PLSD_{(0.05)}$	2.05	2.10	4.66	6.23	3.82	4.35	7.08
Depth	buried	2.76	1.38	9.76a	28.62	17.00	8.76b	11.26b
	surface	2.88	2.38	5.26b	19.50	18.62	27.00a	26.62a
	$PLSD_{(0.05)}$	2.00	1.02	3.50	9.48	4.26	4.62	4.78

Highlights of ANOVA

	November	January	February	March	April	June	August
Prodloc	NS	NS	NS	*	NS	NS	*
Type	NS	NS	NS	NS	NS	NS	NS
Depth	NS	NS	*	NS	NS	*	*
Prodloc*Type	NS	NS	NS	NS	NS	NS	NS
Prodloc*Depth	NS	NS	NS	NS	NS	NS	*
Type*Depth	NS	NS	NS	NS	NS	NS	NS
Prodloc*Type*Depth	NS	NS	NS	NS	NS	NS	NS

moisture (see Appendix B) at Lethbridge compared to Winnipeg during the summer months prevented the 'foundation George' seeds from softening at Lethbridge, even though suitable temperatures may have been present. Although this may be the reason for the discrepancy between sites, it is purely theoretical.

2.3.2.1.2.3 Burial Depth

The effect of burial depth at Lethbridge was similar to that at Winnipeg. In February, seed that was buried softened significantly more than seed that was on the surface (Figure 2.7). However, in June and August, the reverse was observed, with more seeds being softened at the surface than at the 2 cm burial depth. This significant interaction ($p<0.0001$) between time and depth may be due to temperature differences between the surface and 2 cm depths, along with differences in other conditions (e.g. moisture).

In August, there was also an interaction ($p<0.009$) between production location and depth that was similar to the interaction observed at Winnipeg in June. This similar trend also may be due to a difference in temperature between the surface and the 2 cm depth, which favored the softening of Winnipeg produced black medic seed at the surface versus at 2 cm.

2.3.2.1.3 Indian Head

At Indian Head, there was no evidence that significant softening had occurred at any time during the sampling period since seed softening never significantly exceeded the seed softening of the control treatment (see Figure 2.8). Figure 2.9 shows that there was

Figure 2.7 Percent softening for black medic seed samples extracted from the 2 cm (buried) and surface depths at the Lethbridge site at intervals during 2003 and 2004 and subjected to the 15/6°C germination temperature treatment. Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results from the split-split plot analysis.

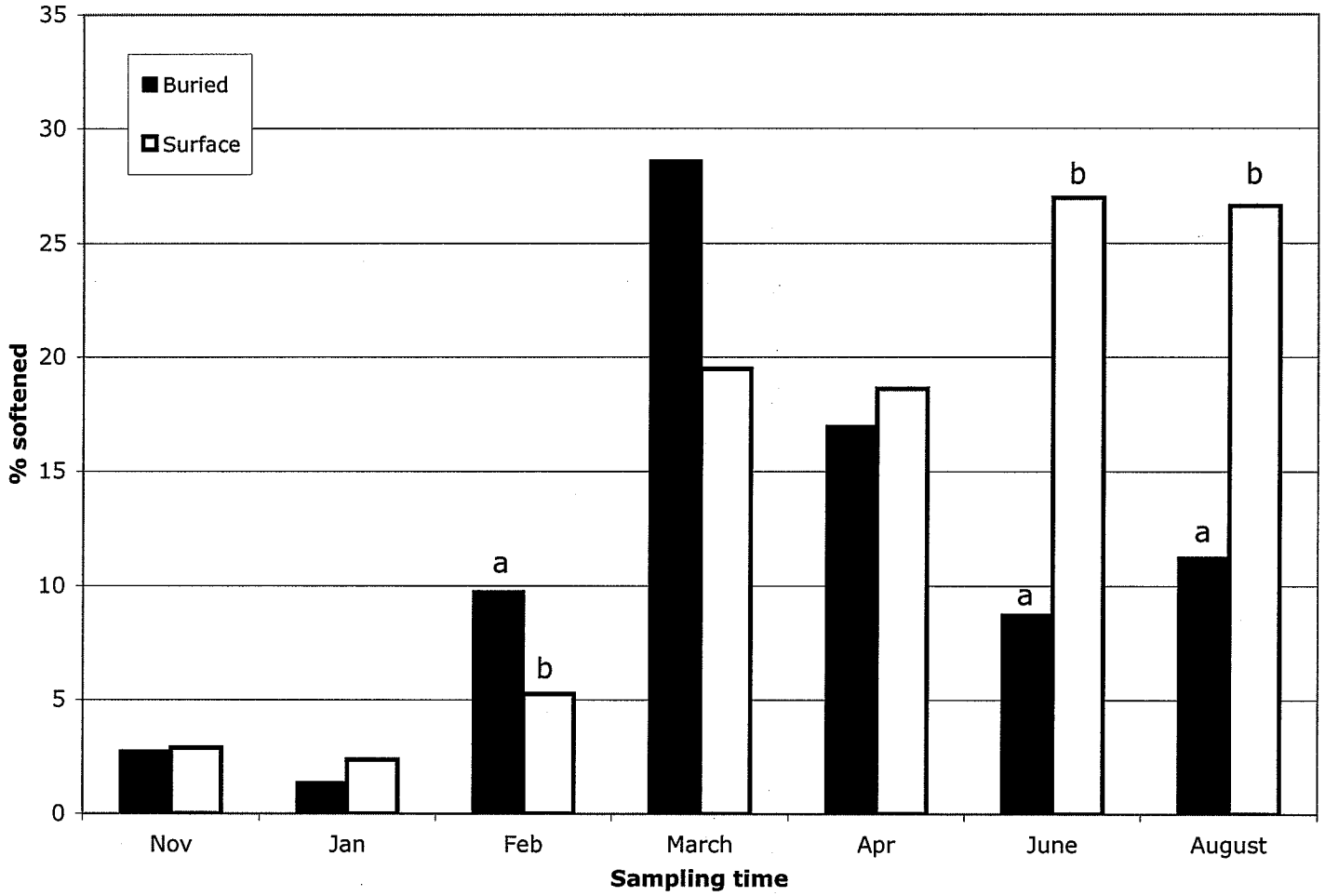


Figure 2.8 Percent softening (germination at 15/6°C temperature regime) for black medic seed samples extracted from the Indian Head site at intervals during 2003 and 2004 compared to the control treatment (seed stored at room temperature subjected to the same 15/6°C regime). Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results (standard errors for PLSD test derived from control and three softening site treatments).

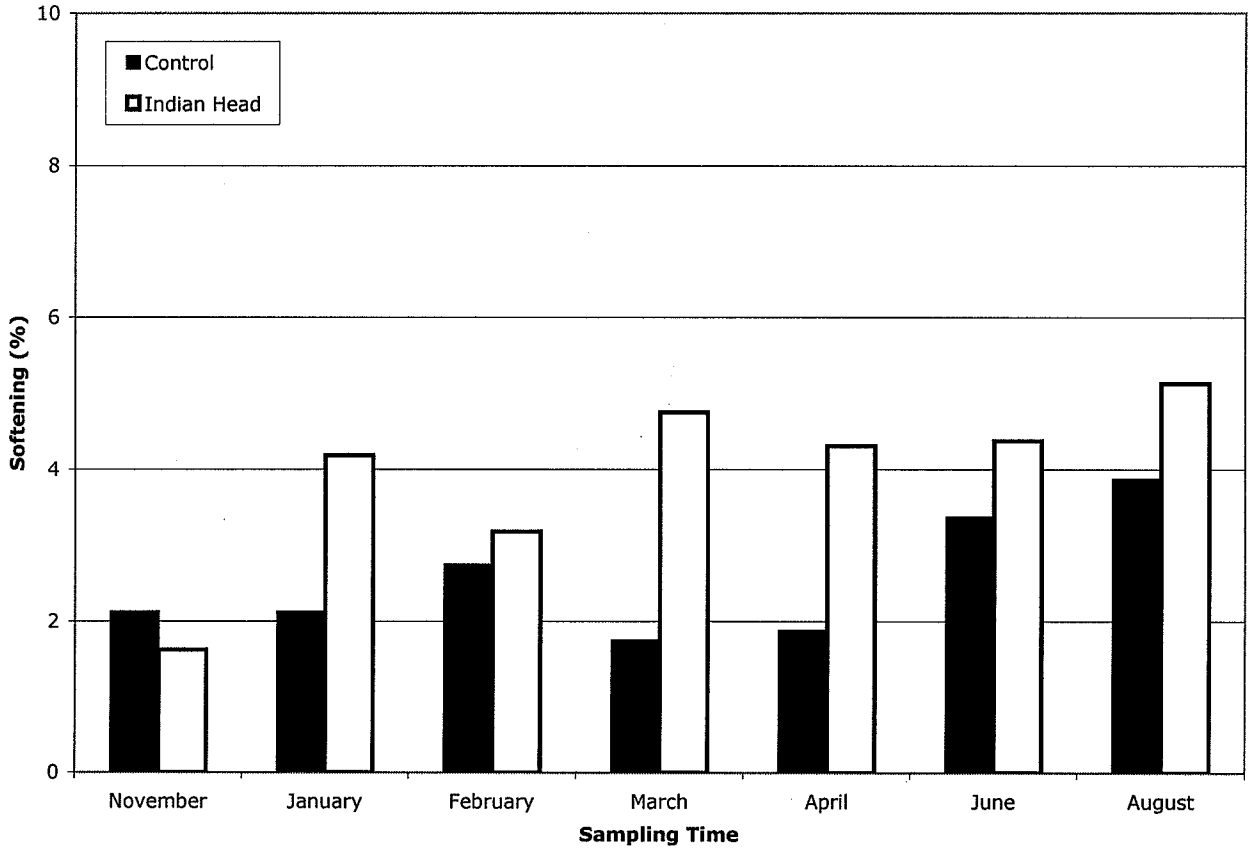
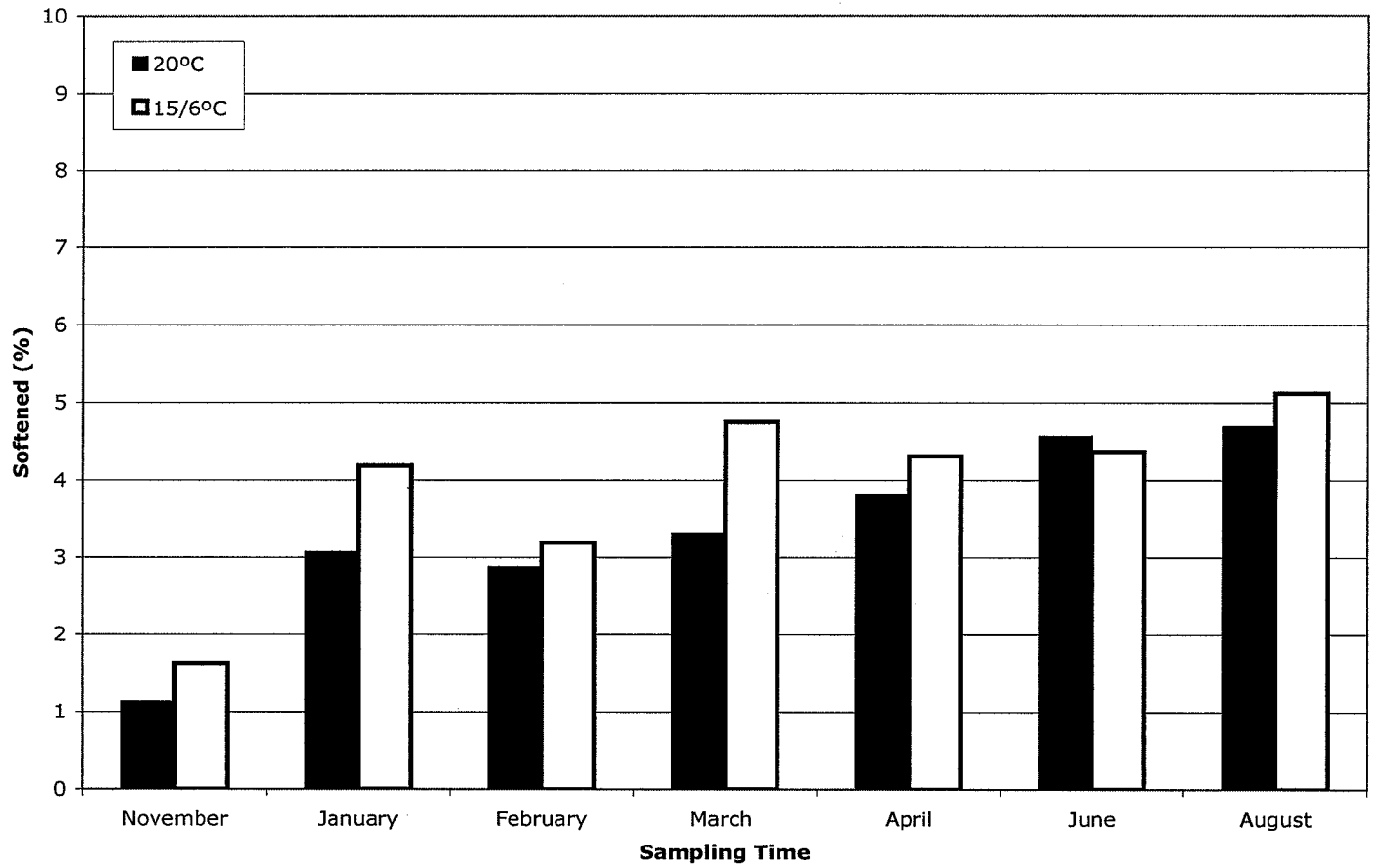


Figure 2.9 Percent softening for black medic seed samples extracted from the Indian Head site at intervals during 2003 and 2004 and subjected to the 20°C and 15/6°C germination temperature treatments. Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results (standard errors for PLSD test derived from the 15/6°C and 20°C treatments).



also no significant difference between the 20°C and 15/6°C germination temperatures for any sampling time. Had stage 1 conditions been met, a significant amount of softening would have occurred when the seed was subjected to the 15/6°C germination temperature treatment.

Although significant softening did not occur in Indian Head, there were some differences in seed softening due to population and burial depth (Table 2.6). Significantly more softening was observed for 'selected George' seed than the 'foundation George' seed in November. However, the reason for this difference between populations is unclear. Significantly more softening was also observed for buried seed than for surface seed in March and April. This may have been due to exposure to more favourable temperature and moisture conditions at the 2 cm depth than at the surface, but not favourable enough conditions to meet the stage 1 softening requirements. There was a significant interaction ($p<0.0242$) between depth and time, as shown in Figure 2.10, but unlike the interactions observed at Winnipeg and Lethbridge, this was not a cross-over interaction.

2.3.2.2 Temperature Data

For each location, hourly temperature data obtained from HOBOs at each depth was summarized into daily mean, maximum and minimum temperatures. These temperatures were plotted against time in order to observe trends (Appendix F). In general, temperatures cooled in the fall and early winter until they reached a low in January/February (<-10°C). After that, the temperatures increased and there was a period of low constant temperatures (-5°C to 5°C). This low constant temperature was followed

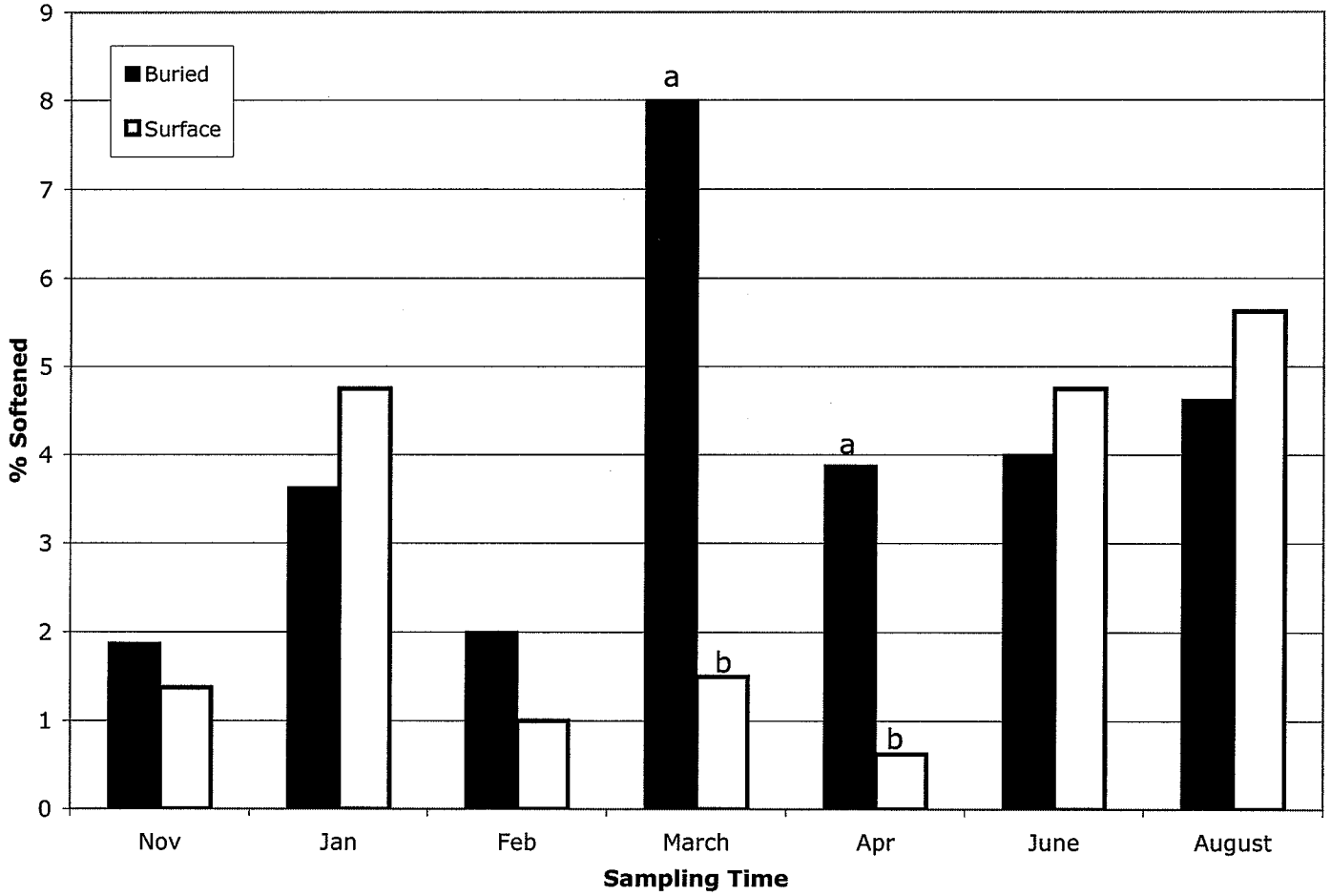
Table 2.6 Percent softening (15/6°C germination temperature) for black medic seed samples extracted from the Indian Head site at intervals during 2003 and 2004 corresponding to the production location (Prodloc), population (Type) and burial depth (Depth) factors. Within each sampling time and main factor, values followed by different letters are significantly different at the $p<0.05$ level (PLSD).

		Sampling Time						
Main Factors	Levels	November	January	February	March	April	June	August
Prodloc	Indian Head	1.50	4.50	1.00	5.50	2.88	3.62	4.00
	Winnipeg	1.75	3.88	2.00	4.00	1.62	5.12	6.26
	$PLSD_{(0.05)}$	1.52	2.86	2.15	11.38	1.38	2.83	2.30
Type	foundation George	0.88b	3.50	1.38	4.26	1.50	3.76	4.88
	selected George	2.38a	4.88	1.62	5.26	3.00	5.00	5.38
	$PLSD_{(0.05)}$	1.22	3.15	1.46	7.15	2.68	1.98	1.12
Depth	buried	1.88	3.62	2.00	8.00a	3.88a	4.00	4.62
	surface	1.36	4.76	1.00	1.50b	0.62b	4.76	5.62
	$PLSD_{(0.05)}$	1.20	2.58	1.24	6.46	1.38	2.16	1.12

Highlights of ANOVA

	November	January	February	March	April	June	August
Prodloc	NS	NS	NS	NS	NS	NS	NS
Type	*	NS	NS	NS	NS	NS	NS
Depth	NS	NS	NS	*	*	NS	NS
Prodloc*Type	NS	NS	NS	NS	NS	NS	NS
Prodloc*Depth	NS	NS	NS	NS	NS	NS	NS
Type*Depth	NS	NS	NS	NS	NS	NS	NS
Prodloc*Type*Depth	NS	NS	NS	NS	NS	NS	NS

Figure 2.10 Percent softening for black medic seed samples extracted from the 2 cm (buried) and surface depths at the Indian Head site at intervals during 2003 and 2004 and subjected to the 15/6°C germination temperature treatment. Columns with different letters within each sampling time are significantly different at the $p<0.05$ level based on the PLSD results from the split-split plot analysis.



by a dramatic shift to a period of low fluctuating temperatures during the spring, which gradually increased to higher fluctuating temperatures during the summer. These periods of "chilling" and low fluctuating temperatures correspond to the predicted stage 1 and stage 2 softening requirements.

The dramatic shift from constant to fluctuating temperatures in the spring coincides with the loss of snow cover in these areas (Table 2.7). Due to the high albedo of snow, the snow cover present at these sites would have reflected a lot of the incoming solar radiation, thereby preventing the soil from warming up (Colbeck 1988). However, once the snow melts, the soil is able to absorb this incoming solar radiation, and this leads to a dramatic increase in daytime temperatures, hence a daily fluctuation in temperature.

Table 2.7 The approximate snow loss dates for 2004 for the southern Manitoba, Saskatchewan and Alberta regions.

Location of Weather Station	Snow Loss Date
Brandon, Manitoba	3-April
Regina, Saskatchewan	5-April
Lethbridge, Alberta	14-February

Source: Environment Canada (2004b)

In order to quantify the amount of time the seed was exposed to stage 1 softening conditions before it was confirmed that the requirements had been met (i.e., February sampling date in Lethbridge and March sampling date in Winnipeg), the number of days that the mean temperature was between –5°C to 5°C was recorded for each location. Temperature data from all locations showed a period of low constant temperatures between –5°C and 5°C in the late winter and/or early spring (Appendix F). This data is supported by the average soil temperatures cited by Environment Canada (2004b) for this

region, and therefore this suggests that a range between –5°C to 5°C is a reasonable range for the required "chilling" temperature. Since stage 1 conditions were not met until February in Lethbridge and March in Winnipeg, the temperatures present in the fall and early winter (October to December) likely did not play a role in stage 1 softening. Therefore, it appears logical that only temperatures following the initial extreme freezing period (temperatures <-10°C) would be worthy of investigation for seed softening.

2.3.2.2.1 Winnipeg

Starting in mid-January, there were approximately 49 days (buried seed) and approximately 46 days (surface seed) that the seed was exposed to temperatures between –5°C and 5°C before the March sampling (Figures 2.11 and 2.12). The presence of these "chilling" temperatures for this extended period of time suggests that conditions may have been suitable for stage 1 softening. This corresponds with the seed softening data, which showed that stage 1 softening had occurred by March. By the April sampling date (Figure 2.11 and 2.12), the temperature had shifted from a low constant temperature between –5°C and 0°C to a low fluctuating temperature, which suggests that conditions may have been suitable for stage 2 softening. This corresponds with the seed softening data, which showed that stage 2 softening had occurred by April. The presence of these two temperature stages, and the corresponding softening results, provide evidence that seed softening occurred in Winnipeg due to a 2-stage process.

In order to help explain the differences in seed softening between the two depths, the mean, maximum and minimum temperatures at each of these depths were investigated (Appendix F). From November to April, there appeared to be no difference

Figure 2.11 Mean maximum, average, and minimum daily soil temperatures (2 cm) at Winnipeg illustrating the conditions for possible stage 1 and stage 2 softening. Arrows represent the different sampling times within the time period and are accompanied by a description of the softening results.

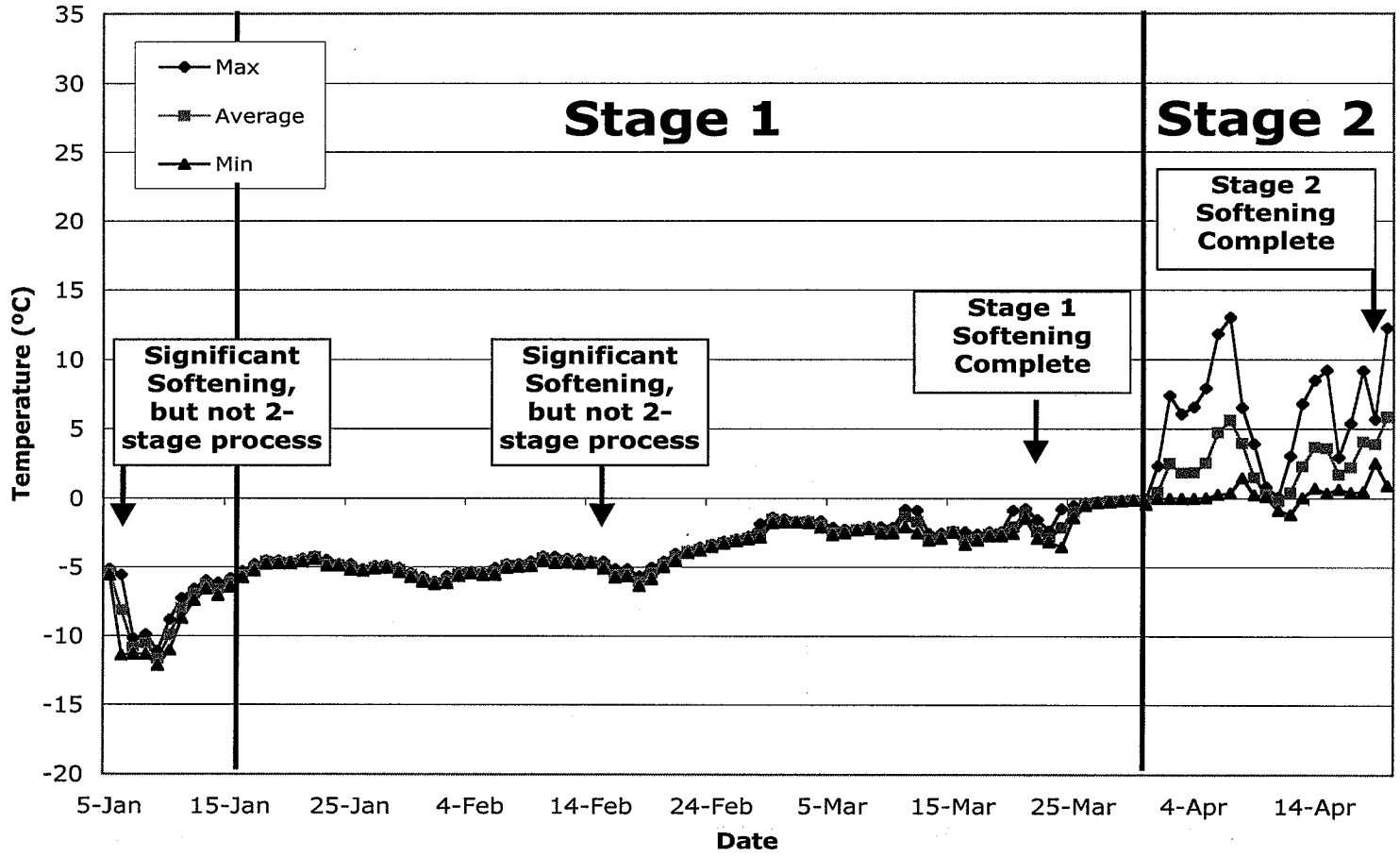
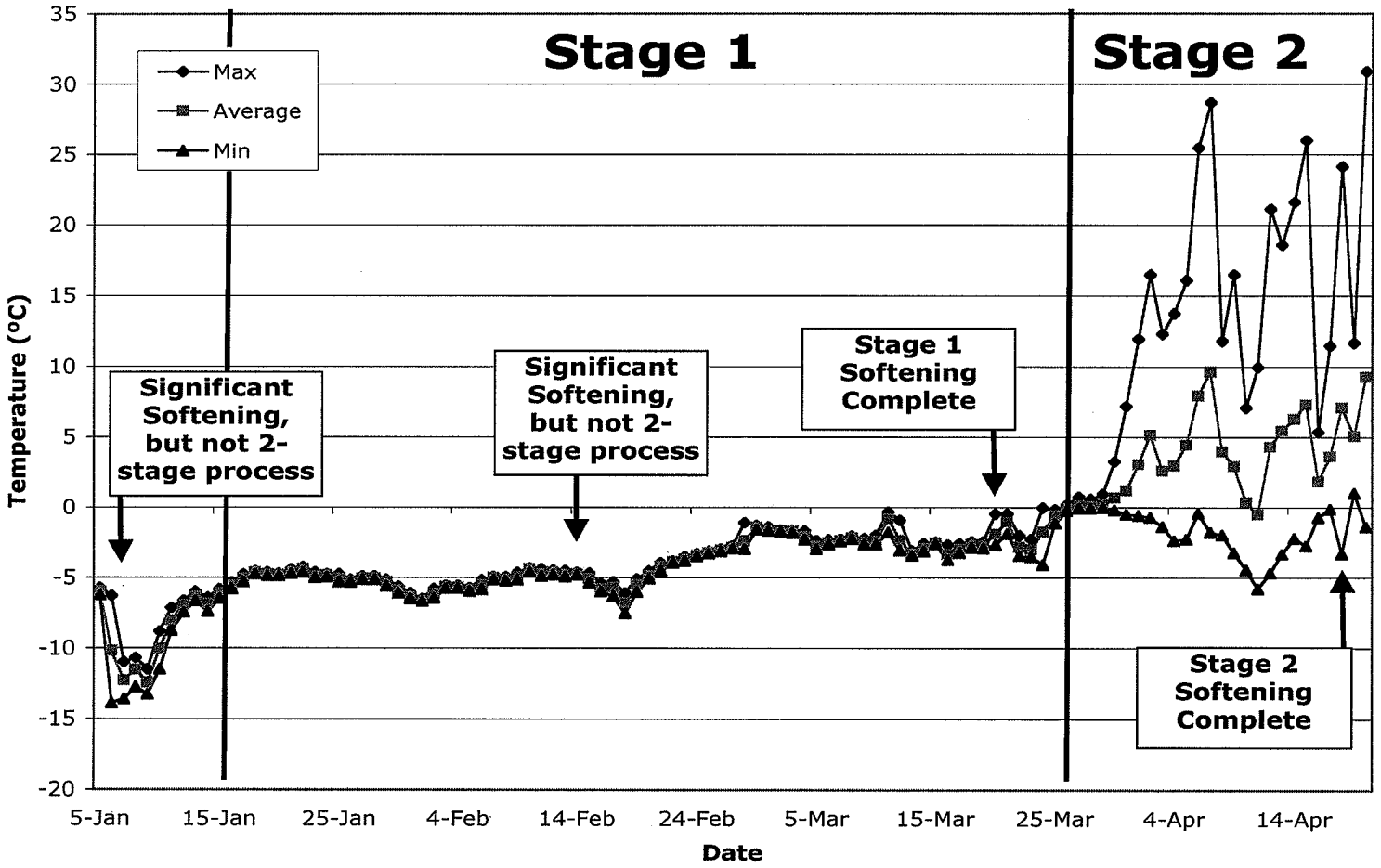


Figure 2.12 Mean maximum, average, and minimum daily soil temperatures (surface) at Winnipeg illustrating the conditions for possible stage 1 and stage 2 softening. Arrows represent the different sampling times within the time period and are accompanied by a description of the softening results.



in maximum and mean soil temperatures between the two depths. However, there did appear to be a slightly warmer minimum temperature for the 2 cm depth compared to the surface depth during the early part of this period (November to January). This temperature data suggests that during the winter and early spring, differences in seed softening between the depths were not due to differences in maximum and mean temperatures, but may have been due to differences in minimum temperatures. The slightly higher minimum temperatures at the 2 cm depth may have provided a more favourable softening environment than at the surface, and therefore allowed for more softening to occur. However, it is unlikely that this slight difference in minimum temperature is solely responsible for the softening differences observed between soil depths during January, February and March.

From April to August, the temperatures on the soil surface were more extreme (i.e. higher maximum temperatures and lower minimum temperatures) and higher (i.e. higher mean temperature) than at the 2 cm depth (Appendix F), and this may account for the greater softening at the surface in these months (Table 2.4). For instance, the temperatures required for the high temperature 2-stage softening process proposed by Braul (2004) may have only been satisfied by the more extreme surface temperatures and not by the temperatures at the 2 cm depth. In conclusion, although temperature appears to explain some of the softening differences between the two depths, the overall reasons for these differences remains unknown.

2.3.2.2.2 Lethbridge

During January and February, there were approximately 37 days (buried seed) and

approximately 38 days (surface seeds) that were between –5°C and 5°C (Figures 2.13 and 2.14). This suggests that conditions may have been suitable for stage 1 softening. This corresponds with the seed softening data, which showed that stage 1 softening had occurred by February. By the March sampling, the temperature had changed to a period of low fluctuating temperatures, which suggests that conditions may have been suitable for stage 2 softening. This corresponds with the softening data, which showed that stage 2 softening had occurred by March. This linkage between the presence of the stage 1 and stage 2 softening requirements and the corresponding softening results confirms the importance of the 2-stage process for black medic seed softening in Western Canada.

There appeared to be no difference between the two depths for the mean, maximum and minimum temperatures between October and February (Appendix F). This suggests that the significant difference in softening that was observed in February between the two depths is likely not due to temperature and is instead due to some unknown factor. From mid-February to August, the soil temperatures were more extreme and higher at the soil surface than at the 2 cm depth. As with the Winnipeg results, this difference in temperature between the two depths is likely one of the reasons for the greater amount of softening observed at the soil surface compared to at the 2 cm depth during June and August. However, temperature is likely not the only reason for this observed softening difference.

2.3.2.2.3 Indian Head

During January and early February, there was a period of low temperature (<-5°C) at both the 2 cm and surface depths (see Figures 2.15 and 2.16). By late February

Figure 2.13 Mean maximum, average, and minimum daily soil temperatures (2 cm) at Lethbridge illustrating the conditions for possible stage 1 and stage 2 softening. Arrows represent the different sampling times within the time period and are accompanied by a description of the softening results.

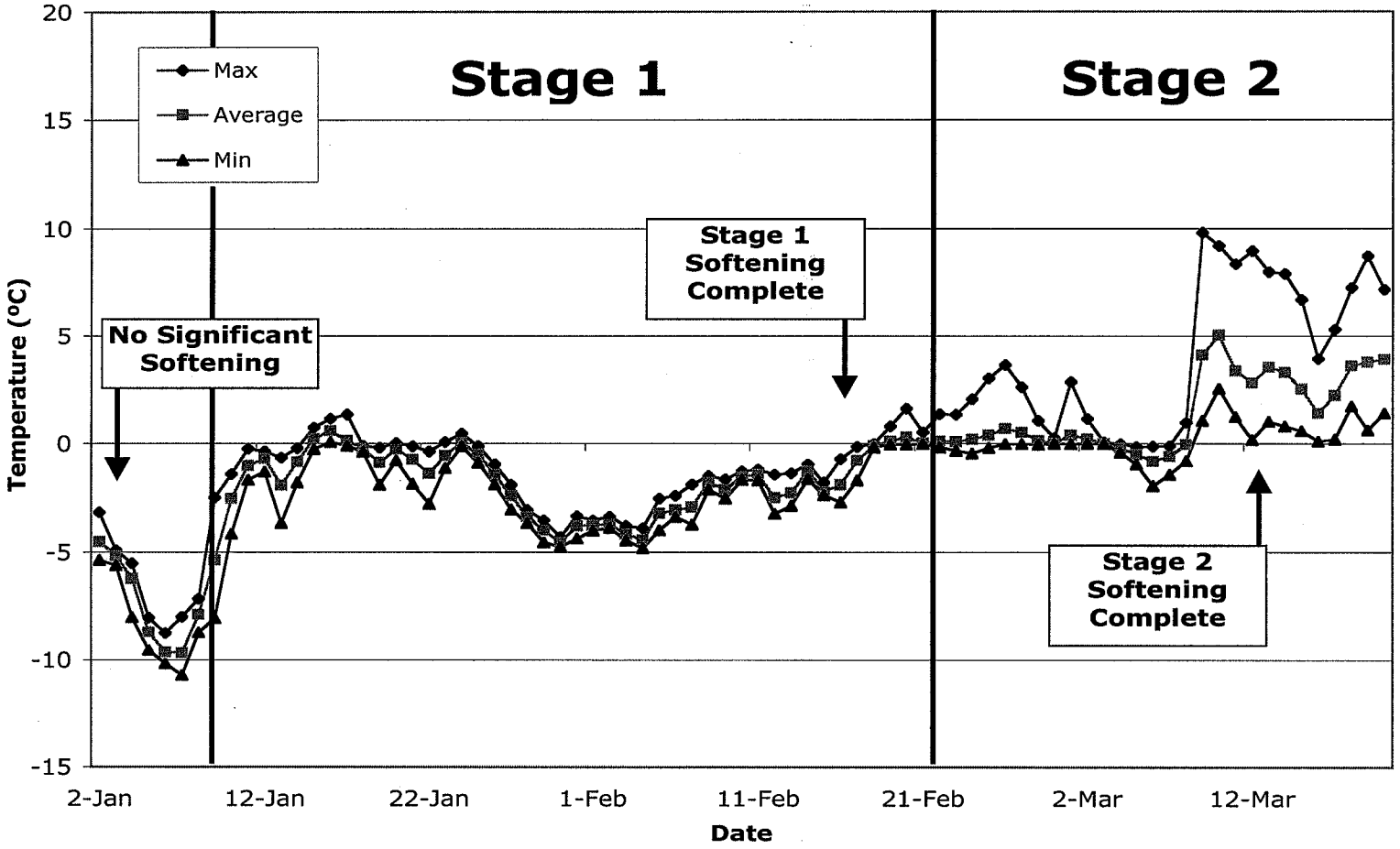


Figure 2.14 Mean maximum, average, and minimum daily soil temperatures (surface) at Lethbridge illustrating the conditions for possible stage 1 and stage 2 softening. Arrows represent the different sampling times within the time period and are accompanied by a description of the softening results.

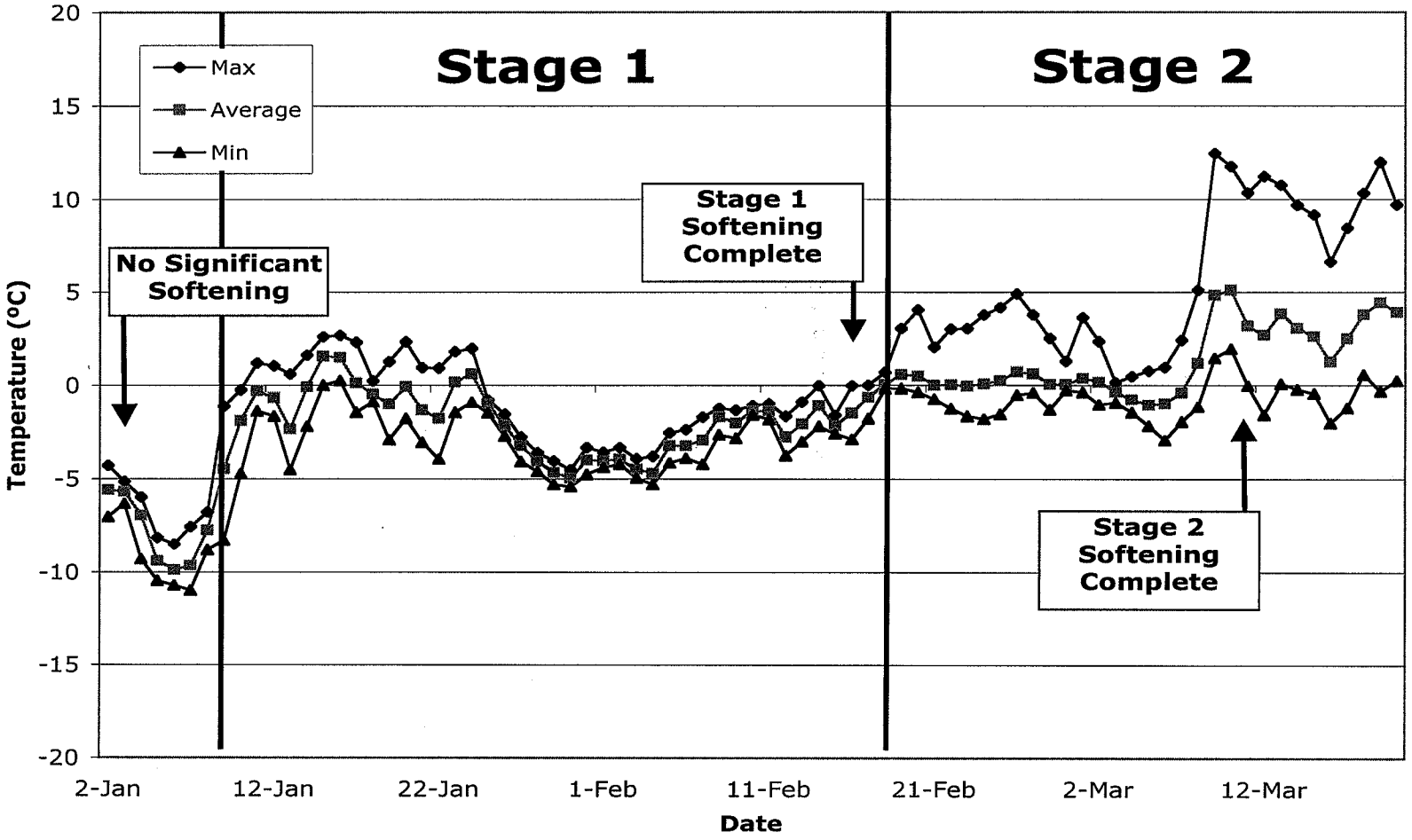


Figure 2.15 Mean maximum, average, and minimum daily soil temperatures (2 cm) at Indian Head illustrating the conditions for possible stage 1 and stage 2 softening. Arrows represent the different sampling times within the time period and are accompanied by a description of the softening results.

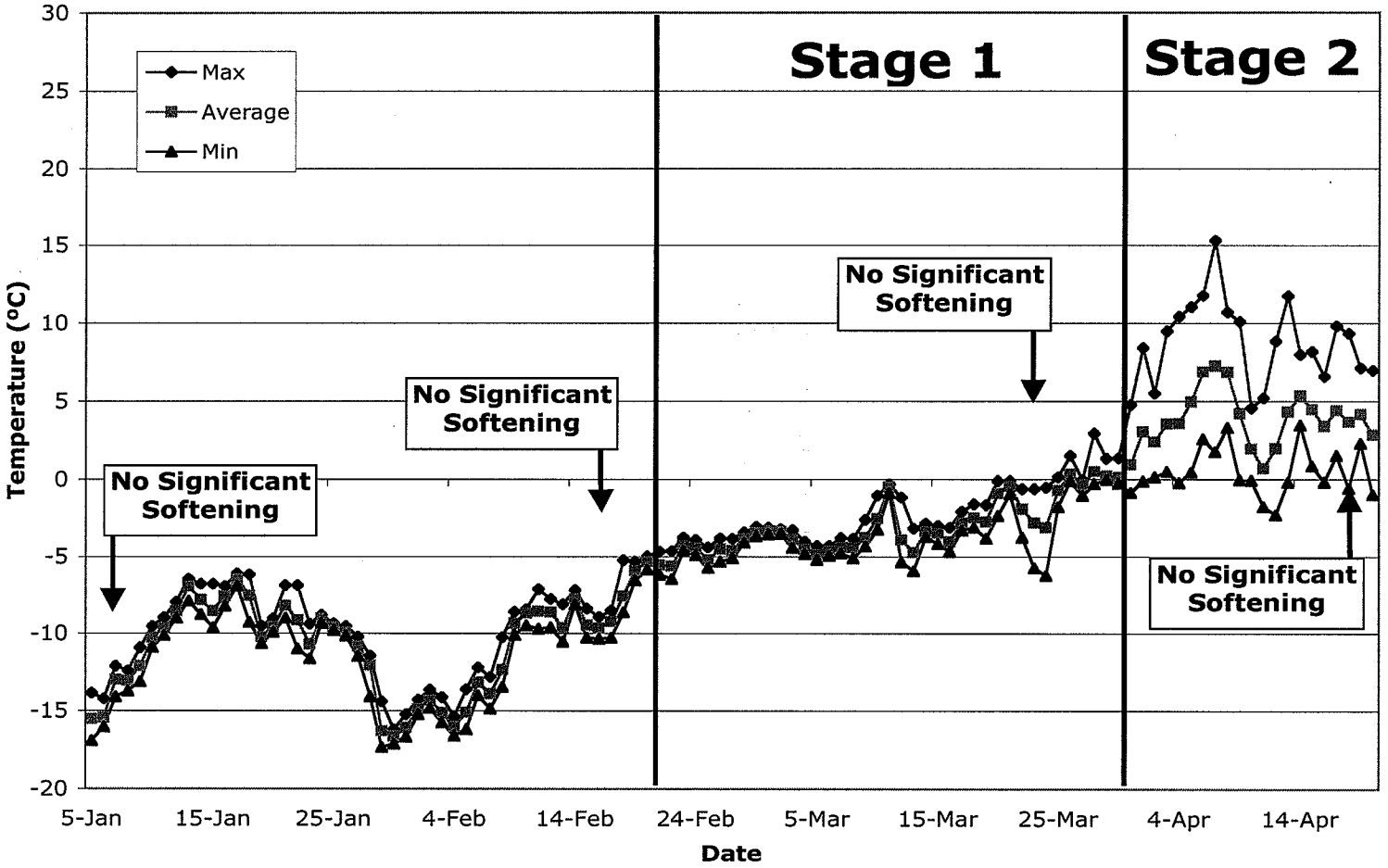
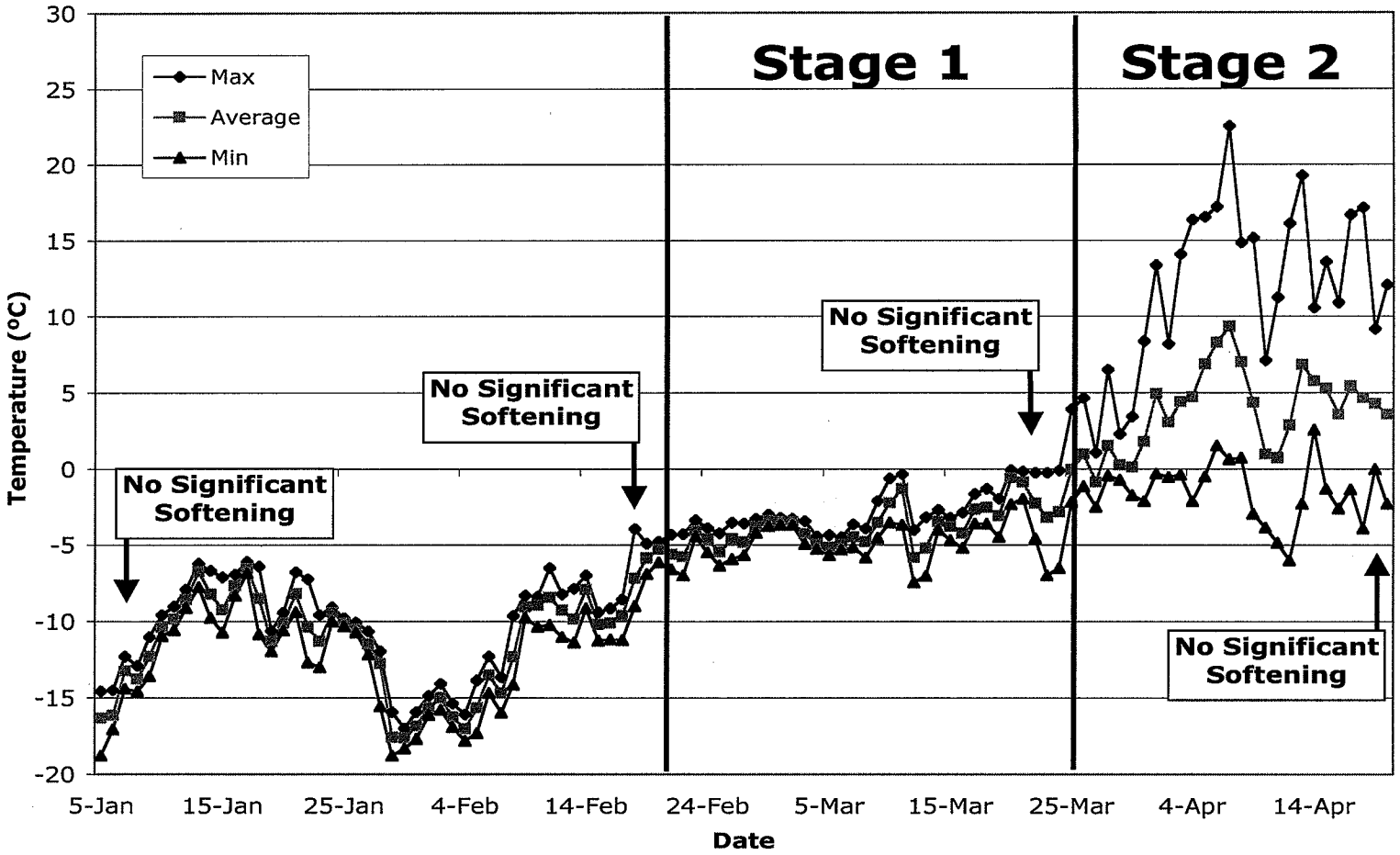


Figure 2.16 Mean maximum, average, and minimum daily soil temperatures (surface) at Indian Head illustrating the conditions for possible stage 1 and stage 2 softening. Arrows represent the different sampling times within the time period and are accompanied by a description of the softening results.



the temperature had warmed up to between –5°C and 0°C, and this lasted for approximately 26 days for the buried seed and approximately 23 days for the seed at the surface. The presence of these "chilling" temperatures suggests that conditions may have been suitable for stage 1 softening. By April, the temperature had shifted from these "chilling" temperatures to a period of low fluctuating temperatures, which suggests that conditions may have been suitable for stage 2 softening.

While soil temperature conditions at Indian Head suggest that conditions were suitable for stage 1 softening, no significant amount of softening occurred at Indian Head. The "chilling" temperatures present at Indian Head were the same "chilling" temperatures present at Winnipeg and Lethbridge, and therefore since softening occurred at these other locations, it must not have been the absence of the appropriate "chilling" temperatures that prevented seed softening from occurring at Indian Head. One difference between the conditions at Indian Head compared to the other two locations was that Indian Head experienced low temperatures (<-5°C) for an extended period during the winter. The low temperature at Indian Head may have prevented stage 1 softening from occurring for two reasons. Due to the extended period of low temperatures, the seed was exposed to the "chilling" temperatures for a shorter period of time than at the other two locations, and therefore the exposure time may not have been long enough to meet the stage 1 softening requirements. The other reason may be that the low temperatures may have caused something to occur in the seed that prevented it from softening. Based on observations, it is unclear why stage 1 softening did not occur at Indian Head.

The temperature data may also help explain the difference in softening between the surface and 2 cm buried seed in March and April (see Appendix F). Beginning in

mid-February, the minimum soil temperature was less extreme (i.e. warmer) at the 2 cm depth than at the surface. As speculated, this warmer minimum temperature at the 2 cm depth may have been the reason why there was significantly more softening at the 2 cm depth than at the soil surface.

2.4 Conclusion

Although production location, population and the presence of a main crop may influence different aspects of growth and development, overall, seed production location and population had very little influence on the initial hardseededness and seed softening of black medic. In contrast, there appears to be an influence of burial depth on seed softening, with the buried seeds softening more during winter and early spring, and the surface seeds softening more during summer. Although the overall reasons for these trends are unknown, they appear to be somewhat linked with soil temperature, with more favourable softening temperatures occurring during the winter/spring at the 2 cm depth and during the summer at the surface.

Observation of seed softening at Winnipeg and Lethbridge during the late winter and early spring suggest that black medic does appear to respond to a 2-stage softening process. These results are supported by the temperature data, which suggests that the stage 1 ("chilling" temperature: -5°C to 5°C) and stage 2 (low fluctuating temperature) softening conditions did indeed occur at Winnipeg and Lethbridge. At Winnipeg, the softening data suggested that stage 1 softening had occurred by the March sampling and that stage 2 softening had occurred by the April sampling, and the temperature data confirms this. At Lethbridge, the softening data suggested that stage 1 softening had

occurred by the February sampling and that stage 2 softening had occurred by the March sampling, and the temperature data confirms this. Therefore, this confirms that a 2-stage process is involved in the seed softening of black medic in Western Canada.

At Indian Head, even though the temperature data suggested that the stage 1 and stage 2 conditions were present, significant softening did not occur, and therefore the stage 1 requirements must not have been met. Although the temperatures within the stage 1 period were similar between locations, the length of time spent at these "chilling" temperatures was shorter at Indian Head than at the other two sites, and this may be the reason why softening did not occur. However, before we can make a definitive conclusion on why significant softening did not occur at Indian Head, we need to determine what the actual temperature and exposure time requirements are for the stage 1 softening of black medic.

Chapter 3: Temperature and Exposure Time Requirements for Stage 1 Softening of Black Medic (*Medicago lupulina*)

3.1 Introduction

Results from the field experiment showed that even though stage 1 "chilling" conditions appeared to be present at Indian Head, Winnipeg and Lethbridge, significant softening only occurred at Winnipeg and Lethbridge. Since significant softening did not occur at Indian Head, it can be assumed that the stage 1 requirements were not met. In order to better understand the stage 1 requirements for black medic seed softening, a controlled environment study was established. Although it has been suggested that the stage 1 requirements for seed softening in black medic involve the seed being exposed to a "chilling" or low constant temperature for a period of time, we do not know what constitutes a "chilling" temperature and how long the seed must be exposed to this temperature before softening can occur.

Van Assche et al. (2003) have shown that a "chilling" temperature of 5°C was sufficient at satisfy stage 1 requirements of black medic in Belgium. However, on the Canadian Prairies, soil temperatures during the winter are usually below 0°C (Environment Canada 2004a), yet softening still occurs (Braul 2004). Therefore, it is important to determine the difference that exposure to a cool temperature (5°C) versus a freezing temperature (–5°C or colder (–23°C)) has on black medic softening. Also, while Van Assche et al. (2003) documented that an 8 week exposure to "chilling" temperatures resulted in stage 1 softening they did not establish a minimum time period requirement.

The objective of this controlled environment experiment was to determine the influence that different stage 1 temperatures and exposure times have on the seed

softening of *Medicago lupulina*. The specific questions addressed were:

1) Are there differences in stage 1 softening for the temperatures 20°C, 5°C, -5°C and –23°C?
2) What is the minimum exposure time required for stage 1 softening?

3.2 Methods and Materials

3.2.1 Experimental Design and Approach

Since the field experiment results showed that there was no significant difference in stage 1 softening between the 'selected George' and 'foundation George' populations or between the Winnipeg and Indian Head production locations, only one black medic population/production location needed to be used in this study. Therefore, only the 'Selected George' seed produced in Winnipeg was used.

The seed was pre-incubated for 2 days at 20°C on wet filter paper (general approaches of Van Assche et al. 2003) and seeds that germinated or imbibed were discarded and the remaining hardseed was used in this experiment. Hardseed was divided into 80 lots of 50 seeds each and each lot was placed into a Petri dish lined with 2 layers of Whatman #1 filter paper. These Petri dishes were divided into 20 groups (4 replicates per group), which corresponded to different stage 1 temperature (20°C, 5°C, -5°C, -23°C) and exposure time (2, 4, 6, 8, and 10 weeks) combinations. Each group was placed into individual plastic bags, in order to keep moisture out, and placed into the appropriate cold room or growth cabinet for the appropriate period of time. The experimental design of this study was a split-plot design with the mainplot being stage 1 temperature and the sub-plot being exposure time.

For the stage 1 temperatures, the 5°C treatment was meant to simulate the conditions used in the Van Assche et al. (2003) experiment. Due to the cold room that was used, the actual temperature that the seed was exposed to ranged from 3.3°C to 4°C, with a mean of 3.6°C (measured using a HOBO). The –5°C treatment used in the present study was meant to simulate typical winter soil temperatures in Western Canada. The actual temperatures in the cold room used ranged from –2°C to –5°C, with a mean of -4.1°C (measured using a HOBO). This range in temperature more accurately portrays the soil temperatures experienced during a normal winter on the Canadian Prairies than a constant –5°C temperature does (Environment Canada 2004a). The –23°C treatment was established in order to determine the effect of extremely cold temperatures, such as those experienced in the soil during a winter with no snow cover, on seed softening (Fowler 1983). A control treatment (20°C) was also established as one of the stage 1 temperature treatments.

3.2.2 Hardseededness and Viability Tests

At 2 week intervals, Petri dishes were removed from each of the stage 1 temperatures, at which time approximately 5 mL of distilled water was added. Subsequently, these Petri dishes where placed in a 15/6°C germinator (i.e., 15°C temperature for 12 hours and a 6°C temperature for 12 hours). This 15/6°C germination temperature was used to simulate stage 2 conditions. If significant softening occurred when seed from a particular treatment (i.e. temperature/time combination) was exposed to the 15/6°C germination temperature, it could be concluded that the stage 1 requirements were met by that particular temperature/time combination. These Petri

dishes were inspected every other day, at which time softened seeds were counted and removed, and water was added to ensure that there was some free water available to the seeds. At the end of two weeks, the Petri dishes were removed from the germinator and the viability of the remaining hardseeds was tested. The viability tests confirmed that the remaining hardseeds for all the treatments were viable.

3.2.3 Statistical Analysis

Using SAS 9.1, an analysis of variance was performed on the data (Proc GLM). The data were tested for homogeneity of variance using Bartlett's test and were log transformed if found to be non-homogeneous. Means were separated on the basis of a protected least significant difference (PLSD) test with a 5% level of significance. Data were analyzed for main effects using a split-plot ANOVA and were also analyzed separately for each exposure time and temperature treatment in order to better resolve the individual effect of each of these factors.

3.3 Results and Discussion

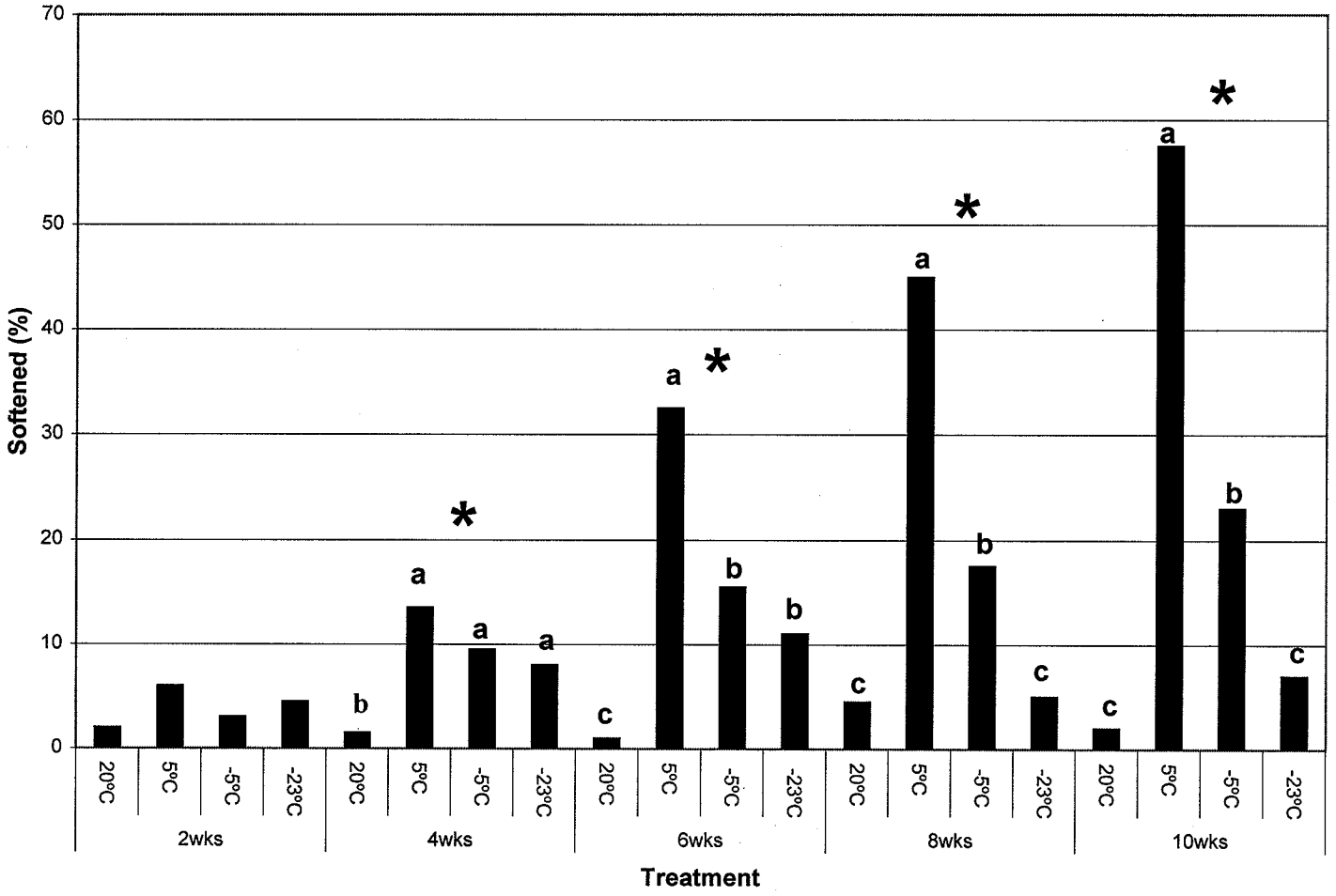
Results from the split-plot ANOVA indicated that there was a significant effect of temperature and exposure time on stage 1 softening (<0.0001), as well as a significant interaction between temperature and time (<0.0001). In order to more clearly explain what effect each temperature and time treatment had on stage 1 softening, the results from the separate exposure time and temperature analyses will be discussed in the following sections.

3.3.1 Temperature Effect on Stage 1 Softening

The mean percent seed softening for each stage 1 temperature treatment at each exposure time is shown in Figure 3.1 (see Appendix G for ANOVA results). For the –23°C temperature treatment, although some significant softening occurred (i.e. significantly more softening than the control temperature treatment (20°C)), the results varied with time (see Figure 3.1). Exposure of the seeds to this cold temperature for 4 to 6 weeks resulted in significant softening, but prolonged exposure to this temperature (8 weeks or more) resulted in the seeds no longer softening more than the control treatment. This result was not due to a decrease in seed viability because the seed viability results showed that the seed remained viable throughout the 10 weeks. Perhaps the significant seed softening observed for this temperature treatment is due to a temperature process other than the 2-stage process, and this is why we see a decrease in seed softening by 8 weeks. However, since the –23°C temperature treatment did not consistently result in significantly more softening than the control treatment, it is unlikely that this extreme temperature constitutes a "chilling" temperature.

In contrast, both the 5°C and –5°C temperature treatments consistently resulted in significantly more softening than the 20°C treatment (Figure 3.1), and therefore both of these temperatures appear to meet the stage 1 temperature requirements. When comparing softening between these two "chilling" temperatures, it appears that the 5°C treatment resulted in significantly more softening than the –5°C treatment. This indicates that although both of these temperatures can be considered "chilling" temperatures, exposure of medic seed to the warmer of these "chilling" temperatures may result in more softening. This finding helps support the field experiment results, which provided

Figure 3.1 Percentage of black medic softened under a 15/6°C germination temperature for the different temperature and exposure time treatments. Within each exposure time, significant differences ($p<0.05$) between treatments are noted by an asterisk (*) and columns with different letters are significantly different at the $p<0.05$ level according to the PLSD results.



evidence that the higher minimum soil temperature (i.e. closer to 5°C) at the 2 cm depth versus the soil surface may have contributed to the greater amount of softening that was observed at the 2 cm depth during the winter/spring at the Indian Head and Winnipeg sites. Overall, although it is uncommon to see soil temperatures greater than 0°C during the winter and early spring in Western Canada (Environment Canada 2004a), these results suggest that the warmer the soil temperature is during this period, the greater the softening will be.

For this study, the maximum softening that occurred was approximately 58% and this corresponded to the seed that was exposed to the 5°C temperature treatment for 10 weeks (see Figure 3.1). As for the –5°C treatment, the maximum amount of seed softening that occurred was approximately 23%, which is quite similar to the maximum amount of softening that occurred in the field experiment (27%). Research has shown that the percentage of black medic seeds that soften within a given period of time tends to vary. For example, Pavone and Reader (1982) found that approximately 40% of black medic seed softened in the first year, while Van Assche et al. (2003) found that after 2.5 years in the soil, only 8% of black medic seed had softened. However, the similarity between the maximum softening for the –5°C treatment and the field experiment suggests that as long as the stage 1 and stage 2 requirements are met, black medic seed softening in Western Canada could be as high as approximately 25% during the first year after establishment.

Since it appears as though the –5°C temperature treatment corresponds relatively well with the "chilling" temperatures experienced in the field, it would be interesting to determine whether the results from the –5°C treatment could be used to predict stage 1

softening in the field. Table 3.1 illustrates what the predicted stage 1 softening would be, based on the –5°C softening results, compared to what the actual softening was at each of the field experiment sites. For the actual softening results, the amount of softening that occurred at the 2 cm depth during the sampling period following stage 1 softening conditions (i.e. March for Winnipeg and Indian Head; February for Lethbridge) was used. The predicted values were calculated by extrapolating the –5°C softening results for the appropriate exposure time for each field site (i.e., 49 days for Winnipeg, 37 days for Lethbridge and 26 days for Indian Head). Results show that the –5°C temperature treatment from this study quite accurately predicted the stage 1 softening at each of the field sites (Table 3.1). This suggests: 1) the method used to calculate the amount of time the seeds were exposed to the "chilling" temperatures in the field appears accurate, and 2) temperature is clearly the primary factor involved in stage 1 softening since environmental differences between the controlled environment study and the field environment, such as moisture, do not appear to have significantly altered stage 1 seed softening.

Table 3.1 Actual and predicted stage 1 softening for the Winnipeg, Lethbridge and Indian Head sites corresponding to the number of days exposed to stage 1 conditions

		Stage 1 Softening	
Location	Days	Actual (%)	Predicted (%)
Winnipeg	49	15	17
Lethbridge	37	10	13
Indian Head	26	8	9

3.3.2 Time Effect on Stage 1 Softening

The results showed that after 2 weeks, there was no significant effect of any temperature on seed softening (see Figure 3.1). However by 4 weeks, seed exposed to either of the two "chilling" temperatures (-5°C, 5°C) softened significantly more than the control (20°C) treatment. After 6 weeks, the 5°C treatment resulted in significantly more softening than any of the other treatments, while the –5°C treatment still resulted in more softening than the control treatment. After 8 and 10 weeks, there was significantly more softening for the 5°C treatment than any of the other treatments and significantly more softening for the –5°C treatment than the –23°C and 20°C treatments.

These results suggest that seed must be exposed to "chilling" temperatures for at least 4 weeks before stage 1 softening can occur. This conclusion also appears to be supported by the field study, which showed that where black medic seed was exposed to "chilling" temperatures for greater than 5 weeks (Winnipeg and Lethbridge), significant softening occurred. On the other hand, where seed was exposed to "chilling" temperatures for less than 4 weeks (Indian Head), no significant softening occurred. Since it appears that at least 4 weeks is needed to meet stage 1 requirements, the low level of softening observed at Indian Head is likely due to insufficient exposure of the seed to stage 1 softening conditions.

It also appears that as seed is exposed to the "chilling" temperature for longer periods, more of the seed is softened (see Table 3.2). For the 5°C treatment, the amount of softening steadily increased with time after the minimum 4 week period. There also appeared to be a slight increase in softening for the seed exposed to the –5°C temperature treatment, especially between the 4 and 6 week exposure periods. These results help

Table 3.2 Percentage of black medic seed softened under a 15/6°C germination temperature for seeds exposed to various temperatures for varying time periods. Within each temperature, values with different letters are significantly different at the $p<0.05$ level according to the PLSD results.

	Temperature			
Exposure Time	5°C	-5°C	-23°C	20°C
2	6.00d	3.00c	4.50bc	2.00
4	13.50d	9.50bc	8.00ab	1.50
6	32.50c	15.50ab	11.00a	1.00
8	45.00b	17.50ab	5.00b	4.50
10	57.50a	23.00a	7.00b	2.00
$PLSD_{(0.05)}$	9.08	8.60	2.96	2.96

Highlights of ANOVA

	5°C	-5°C	-23°C	20°C
Temperature	*	*	*	NS

explain the difference in stage 1 softening between the three field sites. As shown in Table 3.1, the seed at Winnipeg was exposed to the "chilling" temperatures for 12 days longer than the seed at Lethbridge, and this resulted in a 5% difference in seed softening. This suggests that not only does prolonged exposure to the suitable "chilling" temperatures appear to have no detrimental effect on seed softening, it actually appears to increase the amount of softening that occurs.

3.4 Conclusion

The results from the controlled environment study showed that while –23°C does not appear to constitute a stage 1 or "chilling" temperature, both –5°C and 5°C do. However, the seed must be exposed to these temperatures for at least 4 weeks before softening can occur. The results also suggested that prolonged exposure to the "chilling" temperatures past the 4 week minimum may result in increased seed softening. Overall, this study helped clarify what the stage 1 requirements are for black medic seed softening, which in turn helped explain the field experiment results.

Chapter 4: General Discussion

4.1 Black Medic Seed Softening in Western Canada

The results from the seed softening study and the controlled environment experiment confirm that a 2-stage process is required for the softening of black medic in Western Canada (see Figure 4.1).

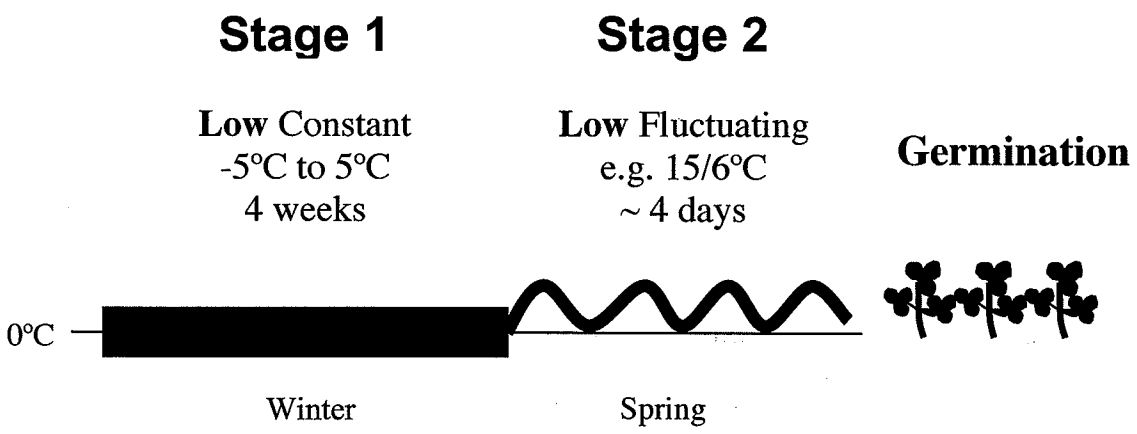


Figure 4.1 Model of the 2-stage process responsible for the majority of black medic softening in Western Canada.

In order for stage 1 softening to occur, it appears that temperatures ranging from –5°C to 5°C are required. The significant response of black medic to the colder "chilling" temperatures (i.e., -5°C) in both the field and controlled environment studies confirms that these black medic populations are well suited to the Canadian prairie environment. However, there also appears to be some softening response that is unrelated to these "chilling" temperatures, such as the cold temperature softening response observed for the –23°C treatment in the controlled environment experiment, as well as the high

temperature softening response that was first noted by Braul (2004) and may have occurred in the field experiment at the Winnipeg and Lethbridge sites. The presence of these alternative softening methods reiterates the phenotypic and genotypic diversity of this species, which allows it to adapt to different environmental conditions (Rumbaugh and Johnson 1986; Sidhu 1971). However, although these alternative softening methods may be present, the 2-stage softening process suggested in Figure 4.1 appears to be responsible for the majority of black medic softening in Western Canada

As for the time requirement for stage 1 softening, it appears as though seed needs to be exposed to the "chilling" temperatures for at least 4 weeks before softening can occur. This minimum time requirement may present a problem in some areas of Western Canada where there is often only a short time period between the freezing temperatures (i.e., less than -5°C) and the fluctuating temperatures (stage 2 conditions). An insufficient time period for "chilling" may in fact be why so little seed softened at Indian Head.

For the stage 2 requirements, the results of the study confirm that a range of fluctuating temperatures, including 15/6°C, are suitable. Also, as with most hardseeded legumes (Taylor 1981), the seed only needs to be exposed to these fluctuating temperatures for a few days (i.e., approximately 4 days) before stage 2 softening can occur. For example, stage 2 requirements were met at the 2 cm depth at Lethbridge even when the maximum daily fluctuation was only 10°C/0°C and the length of exposure to these fluctuating temperatures was only 3 days. In Western Canada, such fluctuating temperatures typically begin immediately after the snow has melted off the soil surface. Hence, it is likely that it is this short period of intense fluctuation that is important and not the actual temperatures and times involved.

A 2-stage softening process driven by temperature appears to be responsible for the dormancy breaking of a number of different legume species under different environmental conditions. In Mediterranean climates (i.e., Australia), a high temperature 2-stage softening process is required for the softening of many hardseeded legumes (e.g. subterranean clover and burr medic) (Taylor 1981; Quinlivan 1961). In contrast, in Western Canada, a low temperature 2-stage softening process appears to be required for black medic softening. Since black medic originated from a Mediterranean climate, it is interesting to see how it has been able to shift its seed softening strategy over the years in order to adapt to the temperate environment of the Northern Great Plains. Although the actual temperatures involved in the 2-stage seed softening process vary between environments, the main purpose of this 2-stage process is the same. The legumes in both of these environments have adopted a dormancy breaking strategy that ensures that the majority of the seed will respond to the appropriate temperature cues and germinate during the time of year when the environmental conditions are most favourable.

4.2 Black Medic in a Western Canadian Cropping System

Before black medic can be widely used in Western Canadian cropping systems, we need to understand how to best manage this cover crop species. This research has shown that a 2-stage process is key to the softening of black medic, and therefore key to the yearly recruitment of this species. This research has also shown that meeting the requirements of this 2-stage process, particularly the stage 1 requirements, may sometimes be a problem. However, a number of management practices can be used to help mitigate this problem. For example, research has shown that snow cover provides

the soil with excellent insulation against the cold, and that the soil temperature (5 cm depth) difference between a fallow field with no snow cover compared to an adjacent stubble field with a 10 cm snow cover can be as large as 10°C (Fowler 1983). This indicates that in colder areas of the prairies where sufficient snow cover may be a problem, such as southern Saskatchewan or colder areas of southern Alberta, farmers should adopt different management strategies, such as the use of no-till or shelterbelts, in order to increase the amount of snow catch on their fields. Trapping more snow will not only help to increase the soil temperature, thereby potentially increasing levels of seed softening, but will also help to increase the amount of water stored in the soil, which in turn will promote the recruitment of the black medic in the spring (Ries and Power 1981).

The results from the field experiment also suggest that when selection pressures are applied, the black medic population responds and evolves with respect to seed dormancy pattern, which allows the population to soften earlier or later in the season. Since a population shift may affect the competitiveness of black medic and its overall performance as a cover crop (e.g., its yearly capacity to regenerate), farmers should be aware of how different selection pressures (e.g., herbicide use) may shift black medic populations, and they should manage their systems accordingly.

Also, the field experiment results suggest that seed burial depth may affect the seed softening, and therefore potential recruitment, of black medic seed in Western Canada. It appears that buried seed softens more in early spring, while seed on the soil surface softens more in the summer. Since different tillage practices can affect the placement of seed in the soil, it is likely that using a certain tillage practice may affect when the black medic seed will soften in a season. For example, since a no-till system

tends to result in seeds remaining at or near the soil surface, a no-till system may promote more summer recruitment than a conventional tillage system would. Since the timing of seed softening may affect the ability of black medic to produce a suitable stand, as well as its competitiveness with the main crop, it is important that the impact that tillage may have on the softening of black medic be considered when managing a cropping system that contains this cover crop species.

4.3 Recommendations for Future Research

Although this research has answered a number of questions regarding black medic seed dormancy, there are still a number of questions remaining that should be investigated.

1) Long-Term Black Medic Seed Softening in Western Canada

At present, we only know what the short-term (1 season) seed softening pattern of black medic is in Western Canada and it would be useful to know what happens to the seed over a number of seasons (long-term seed softening). Results from this research could provide important information on the longevity of black medic seed in a Western Canadian cropping system, the yearly recruitment possibilities, and the sustainability of this cover crop system.

2) Plasticity of Black Medic Seed Dormancy

The response of black medic to high summer temperatures has been noted by Braul

(2004) and supported by the field results in the present study. Although a temperate 2-stage process appears to be the major cause of softening of black medic in Western Canada, it appears that the black medic populations have the ability to respond to a high temperature 2-stage process, and therefore may be able to successfully soften in Mediterranean climates, such as Australia. It would be interesting to investigate if a temperate black medic cultivar, such as 'George', could indeed successfully soften under a Mediterranean climate. Research could include whether or not a sub-population of seeds would soften, grow successfully and reproduce in this type of environment, and if so, could the black medic fully adapt to this environment overtime. This research could provide important information on the plasticity of black medic seed dormancy, which in turn could lead to a better understanding of black medic's ability to adapt to different environments.

3) Management of Black Medic

In order for the best management practices to be used for cropping systems that include black medic, more research is needed on the impact of different management practices (e.g. tillage, repeated herbicide use, etc.) on black medic seed softening. Research should also be conducted on how to manage the black medic in order to maximize its benefits (e.g. soil erosion reduction), while minimizing the problems with its use (e.g. competition with the main crop).

4) Benefits of Black medic as a Cover Crop

Before most farmers will use black medic as a cover crop in their cropping

system, the benefits of its inclusion must be known. Therefore, the actual benefits of having black medic as a cover crop in a cropping system need to be quantified. These benefits should include both the agronomic/environmental benefits (e.g. reduced soil erosion, weed suppression) and the economic benefits (e.g. reduced fertilizer and pesticide costs) of including black medic in a cropping system.

Chapter 5: Conclusions

The results from the field experiment and the controlled environment experiment confirm that a 2-stage process is required for the successful softening of black medic in Western Canada. For stage 1, it appears that seed needs to be exposed to temperatures ranging from –5°C to 5°C for at least 4 weeks, and that prolonged exposure to these "chilling" temperatures may result in greater seed softening (Figure 4.1). Overall, it appears that for black medic seed softening in Western Canada, if stage 1 requirements are not met, significant softening will not occur, but if they are met, and seeds are exposed to some type of fluctuating temperature for a short period of time (i.e., stage 2 conditions), significant softening will occur.

There also appears to be an effect of several factors on black medic production and seed softening. The results from the field experiment suggest that although the presence of a companion crop (i.e., wheat) and the differences between seed production environments and populations may affect the growth and development of black medic, initial seed dormancy and seed viability are unaffected. However, for the two populations, there does appear to be a shift away from late season recruitment (i.e. high temperature softening) for the 'selected George' population compared to the 'foundation George' population, which suggests that the selection pressures may have indeed altered the seed dormancy of the 'selected George' population. There also appears to be an influence of seed burial depth on seed softening, with more seed softening (low temperature 2-stage process) occurring during the winter/spring at the 2 cm depth, and more seed softening (high temperature softening process) occurring at the surface during the summer.

In summary, this research has provided us with valuable information about black medic seed dormancy loss under Canadian prairie conditions, which will hopefully lead to an improved understanding of how this species should be managed and utilized in Western Canadian cropping systems.

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Appendix A

Soil test results for the levels of the N, P, K and S nutrients (E- excessive level, O- optimum level, M- marginal level, D- Deficient level) and pH at 0-6 cm and 6-24 cm depths for the Winnipeg (WPG), Indian Head (IH) and Lethbridge (LETH) sites.

		N		P		K		S		
Site	Depth (cm)	ppm	lbs/acre	ppm	lbs/acre	ppm	lbs/acre	ppm	lbs/acre	pH
WPG	0-6	50	172	>60	>410	594	3468	16	62	7.1
	6-24	12		48		380		5	0	7.5
		M-O		O-E		O-E		O		
IH	0-6	10	69	18	69	>600	>3090	20	84	7.8
	6-24	8		5		315		7		8.1
		D-M		D-M		O-E		O-E		
LETH	0-6	17	106	32	112	495	2135	4	25	7.9
	6-24	12		8		191		3		8.1
		M		M-O		O-E		M		

Appendix B

Gravimetric soil moisture for the March, April, June and August samplings for Winnipeg, Indian Head and Lethbridge.

	Sampling Date			
Site	March	April	June	August
Winnipeg (%)	60	38	29	22
Indian Head (%)	40	14	20	6
Lethbridge (%)	14	7	18	4

Appendix C

Viability results for 'selected George' and 'foundation George' seed produced at Winnipeg and Indian Head.

Site	Population	Rep	Viability (%)
Winnipeg	'selected George'	1	100
		2	100
		3	100
		4	100
	'foundation George'	1	100
		2	100
		3	100
		4	100
Indian Head	'selected George'	1	100
		2	100
		3	100
		4	100
	'foundation George	1	100
		2	100
		3	100
		4	100

Appendix D

Percent softening for black medic seeds (15/6°C germination temperature) samples extracted from the Winnipeg, Lethbridge and Indian Head fields at intervals during 2003 and 2004 compared to the control treatment (seed stored at room temperature). Within each sampling time, values followed by different letters are significantly different at the $p<0.05$ level (PLSD). Due to differences in sample size, the PLSDcontrol was used to compare each site to the control and the PLSDsites was used to compare values between sites.

	Sampling Time						
Sites	November	January	February	March	April	June	August
Winnipeg	3.25a	12.56a	14.38a	13.88b	18.88a	17.50a	20.25a
Lethbridge	2.81a	1.88b	9.43b	27.50a	20.44a	17.88a	18.94a
Indian Head	1.62b	4.19b	3.19c	4.75c	4.31b	4.38b	5.12b
Control	2.12ab	2.12b	2.75c	1.75c	1.88b	3.38b	3.88b
$PLSDcontrol_{(0.05)}$	1.40	3.72	4.91	5.13	3.39	4.21	4.20
$PLSDsites_{(0.05)}$	1.14	3.04	4.01	4.19	2.77	3.44	3.43

Appendix E

Percent softening for black medic seeds samples extracted from the Winnipeg, Lethbridge and Indian Head sites at intervals during 2003 and 2004 and subjected to the germination temperature treatments (20°C and 15/6°C). Within each sampling time and site, values followed by different letters are significantly different at the $p<0.05$ level (PLSD).

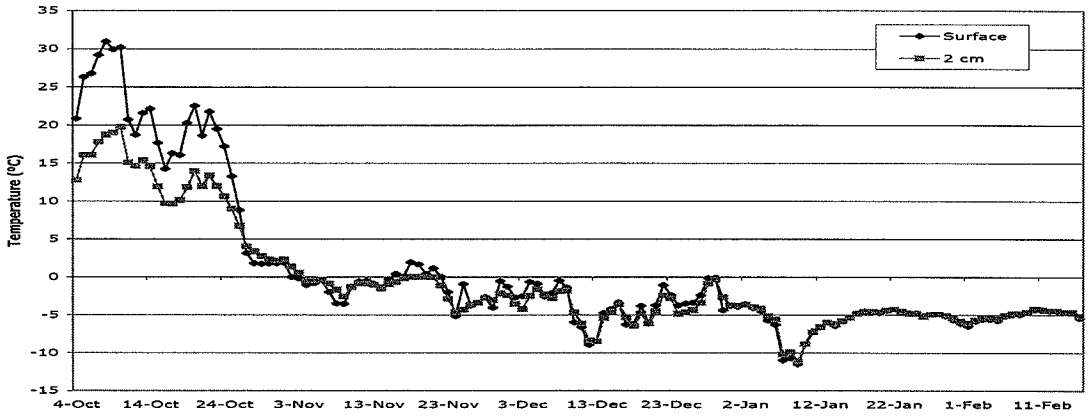
Sites	Germination Temperature	Sampling Time November	January	February	March	April	June	August
Winnipeg	20°C	2.12	10.50	11.12	4.81b	16.56	17.12	20.31
	15/6°C	3.25	12.56	14.38	13.88a	18.88	17.50	20.25
	$PLSD_{(0.05)}$	1.19	6.04	6.32	3.15	3.61	3.21	3.49
Lethbridge	20°C	2.44	1.44	3.88b	25.94	18.44	18.25	18.43
	15/6°C	2.81	1.88	9.44a	27.50	20.44	17.88	18.94
	$PLSD_{(0.05)}$	1.04	0.88	2.19	5.41	4.50	5.33	5.40
Indian Head	20°C	1.12	3.06	2.88	3.31	3.81	4.56	4.69
	15/6°C	1.62	4.19	3.19	4.75	4.31	4.38	5.12
	$PLSD_{(0.05)}$	0.79	1.59	1.25	3.61	1.30	1.21	1.32

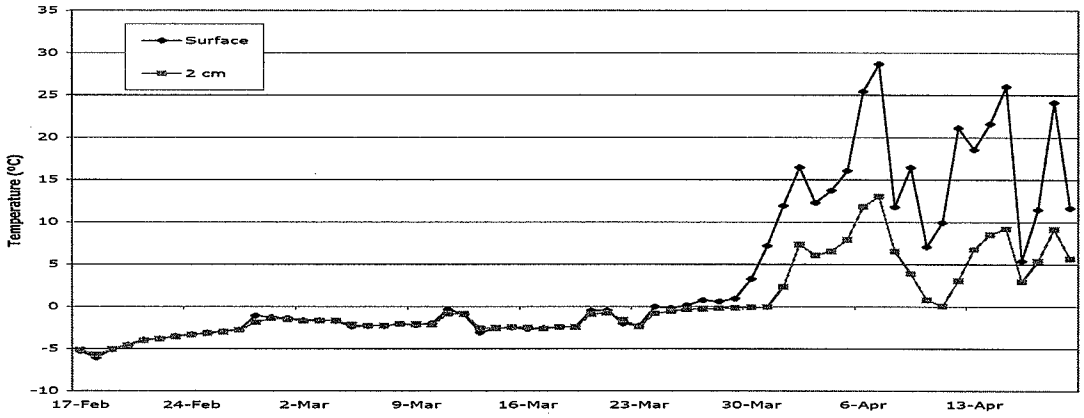
Highlights of ANOVA

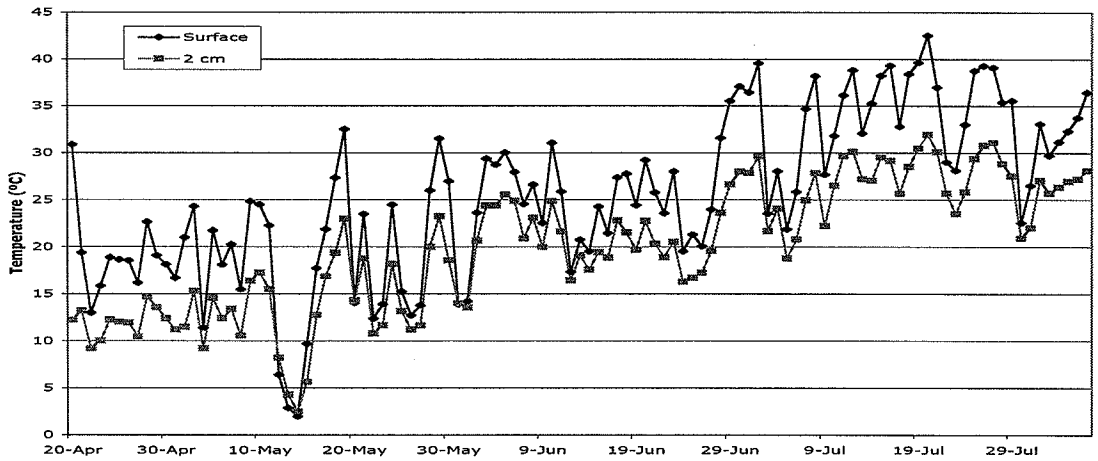
	November	January	February	March	April	June	August
Winnipeg	NS	NS	NS	*	NS	NS	NS
Lethbridge	NS	NS	*	NS	NS	NS	NS
Indian Head	NS	NS	NS	NS	NS	NS	NS

Appendix F

Maximum daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Winnipeg

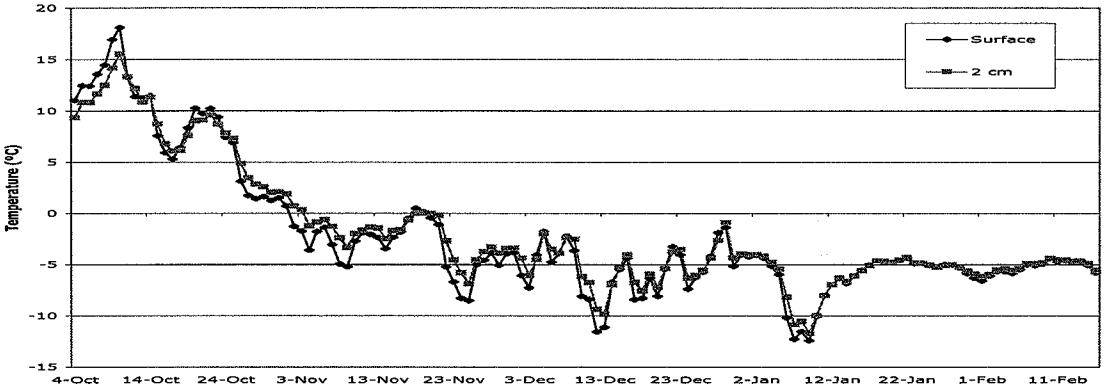


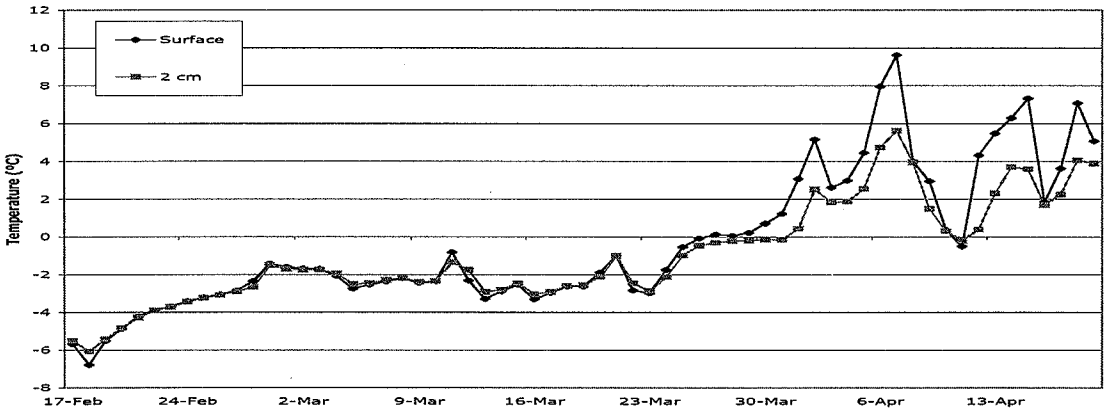


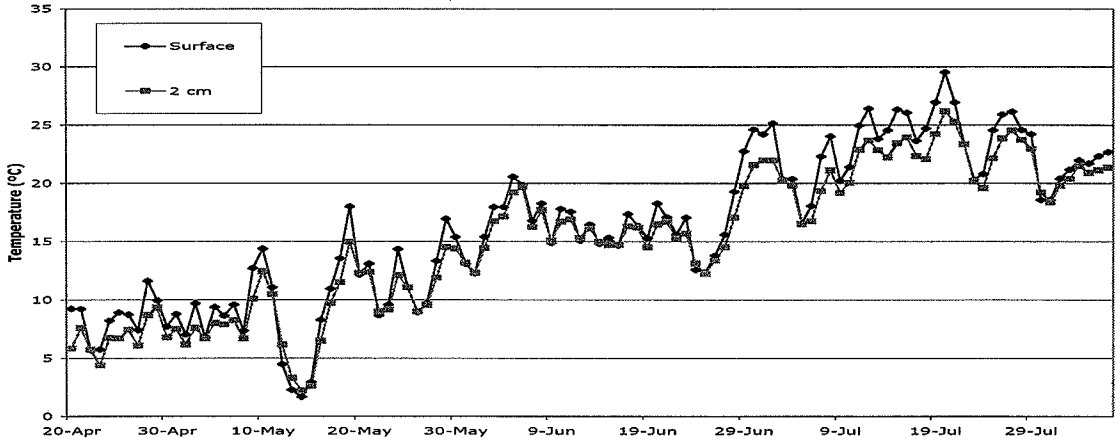


Appendix F con't

Mean daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Winnipeg

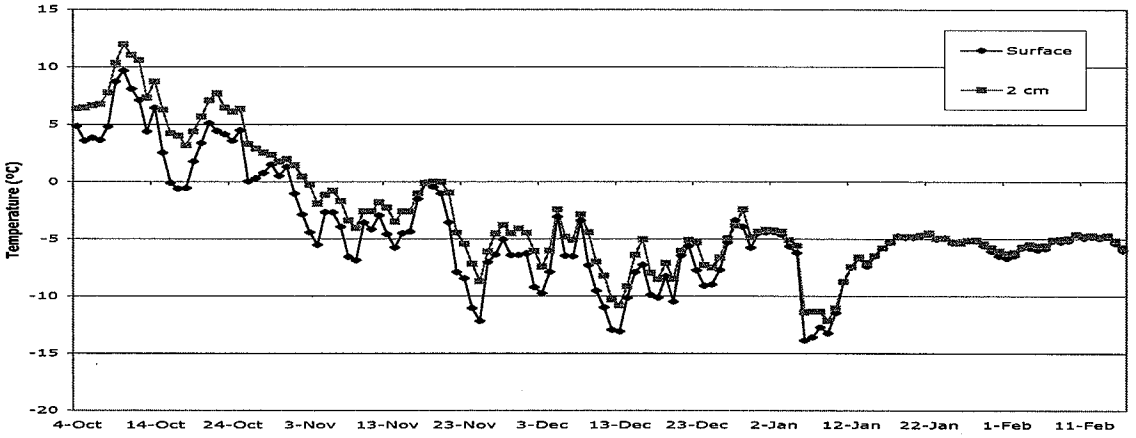


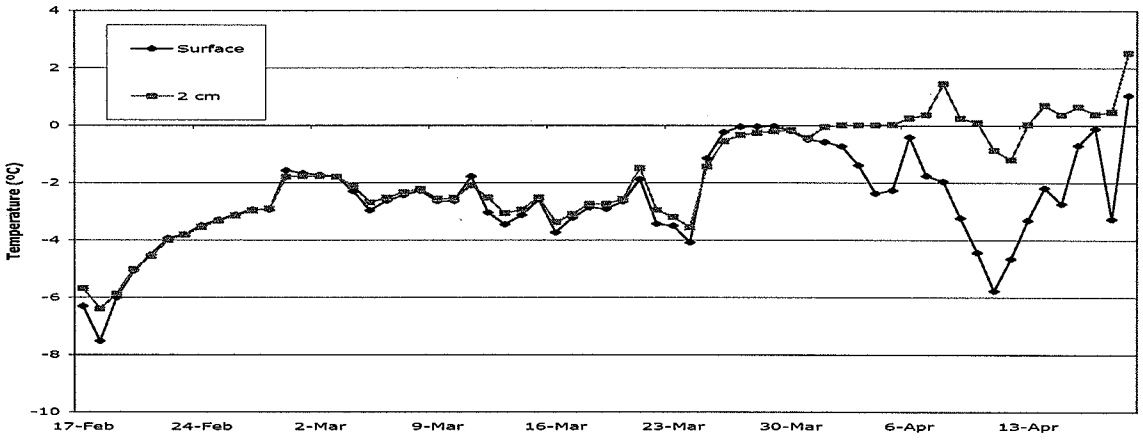


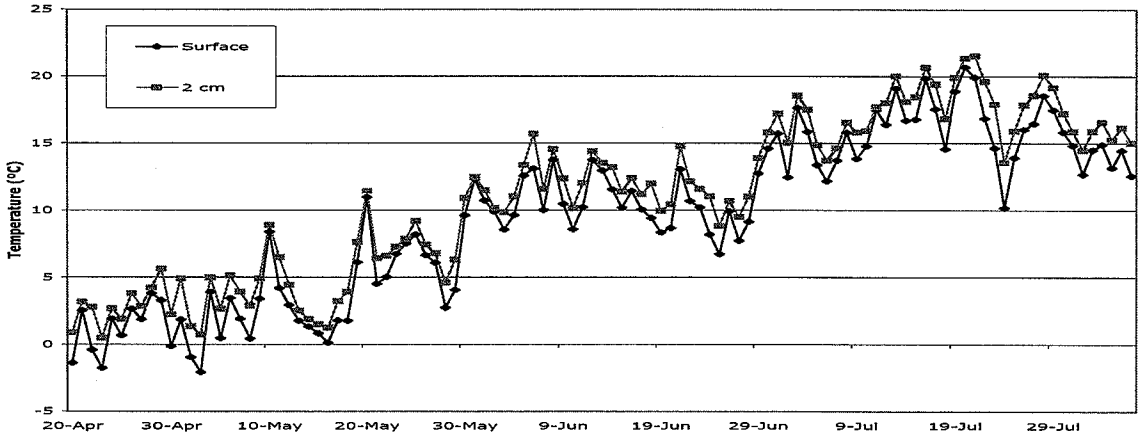


Appendix F con't

Minimum daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Winnipeg

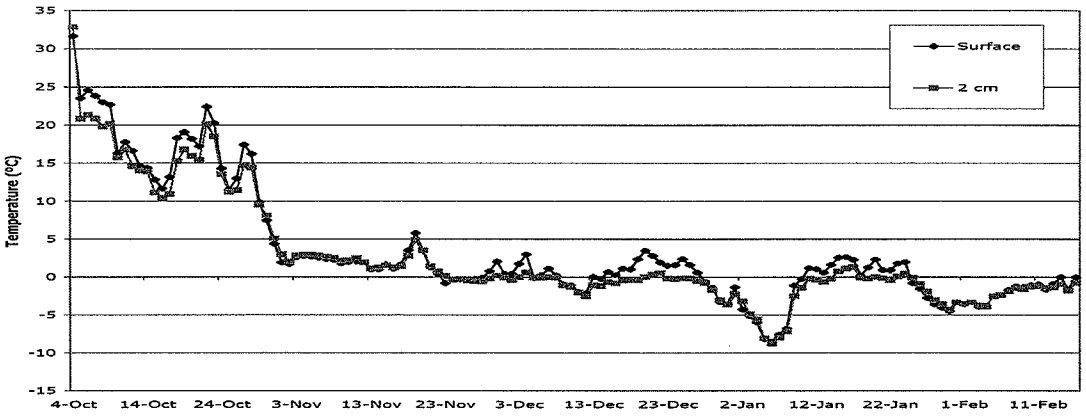


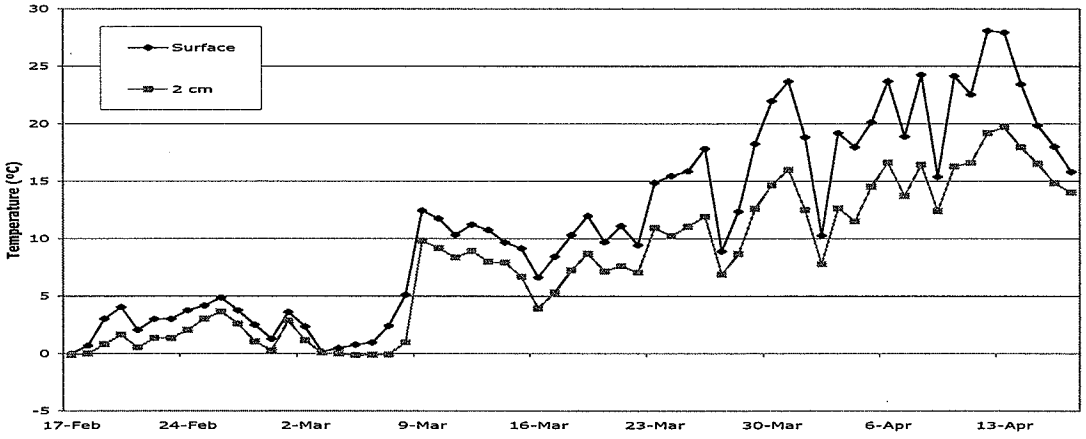


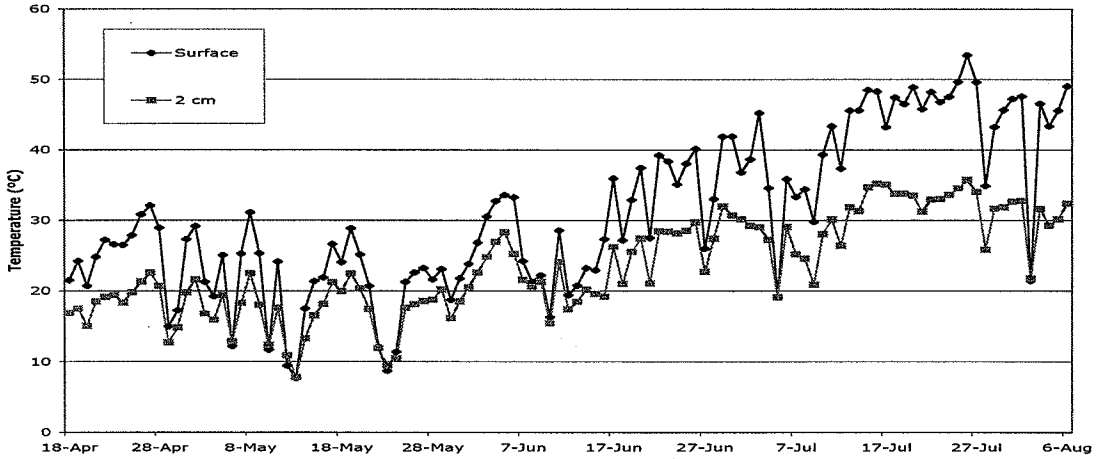


Appendix F con't

Maximum daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Lethbridge

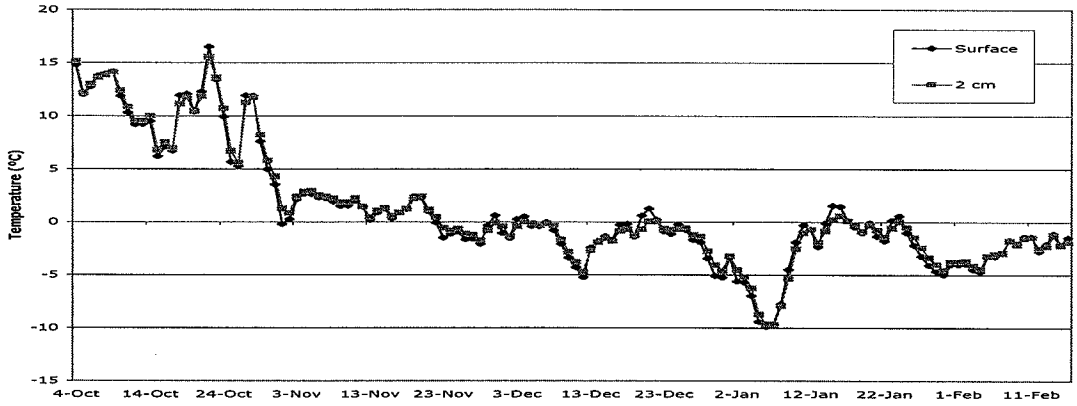


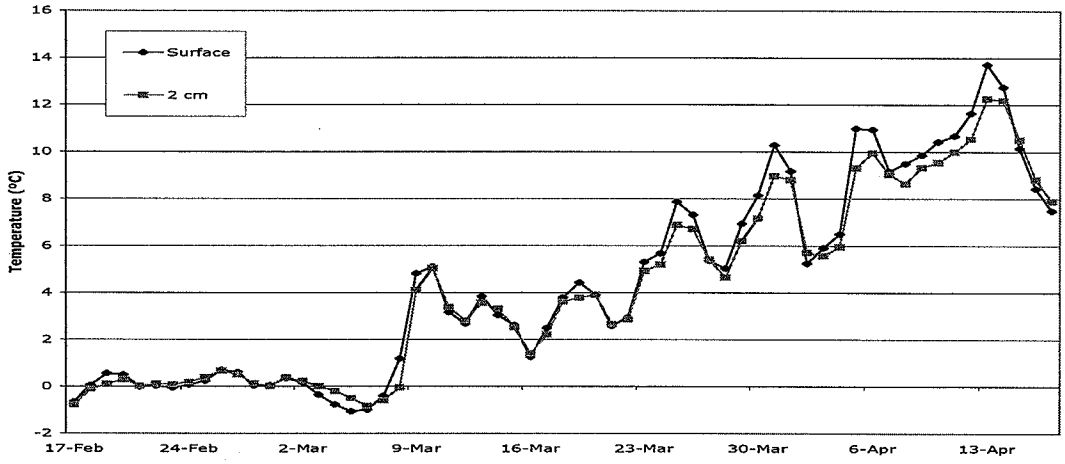


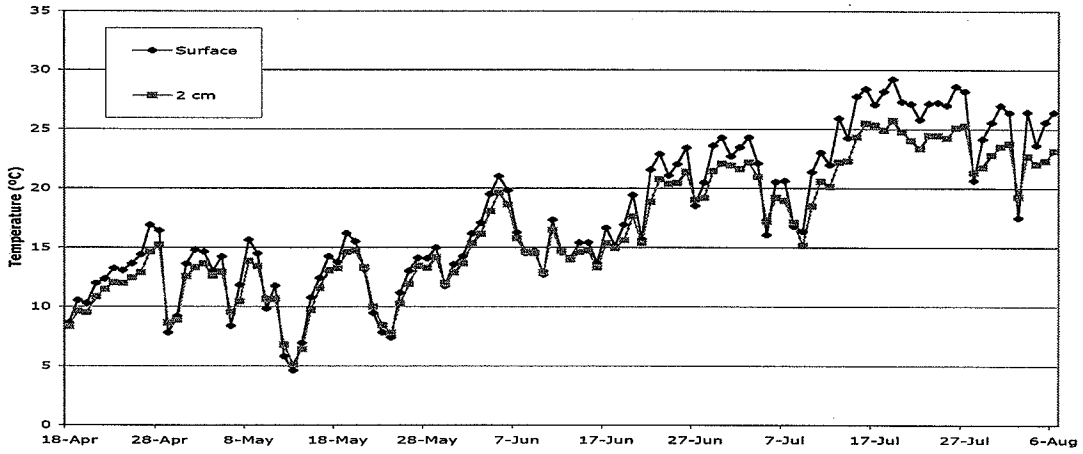


Appendix F con't

Mean daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Lethbridge

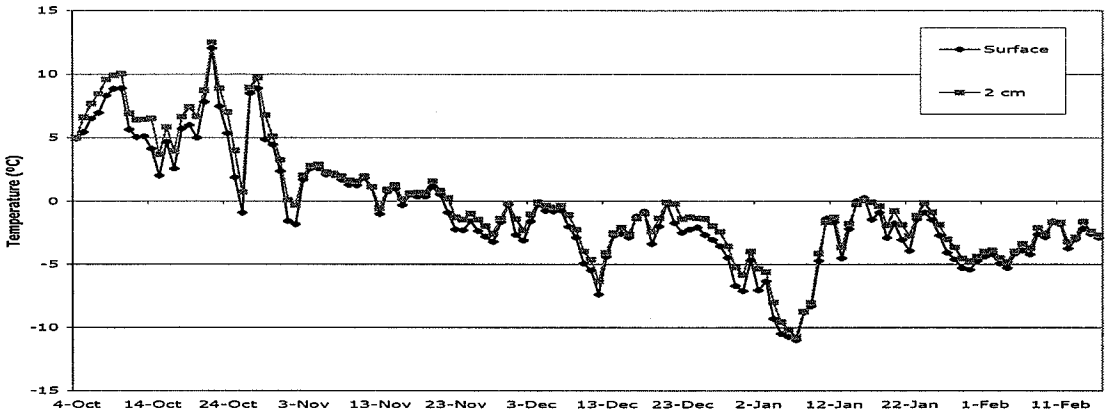


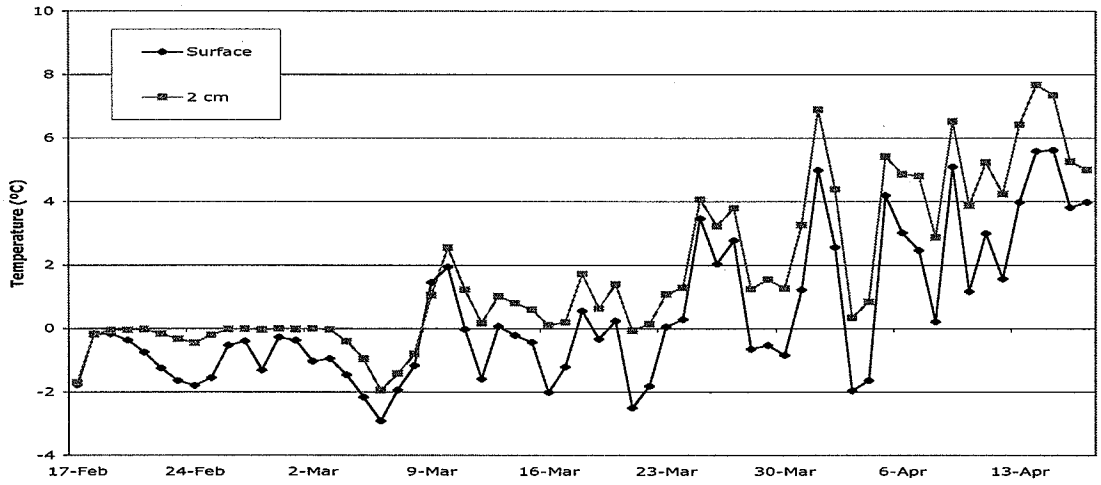


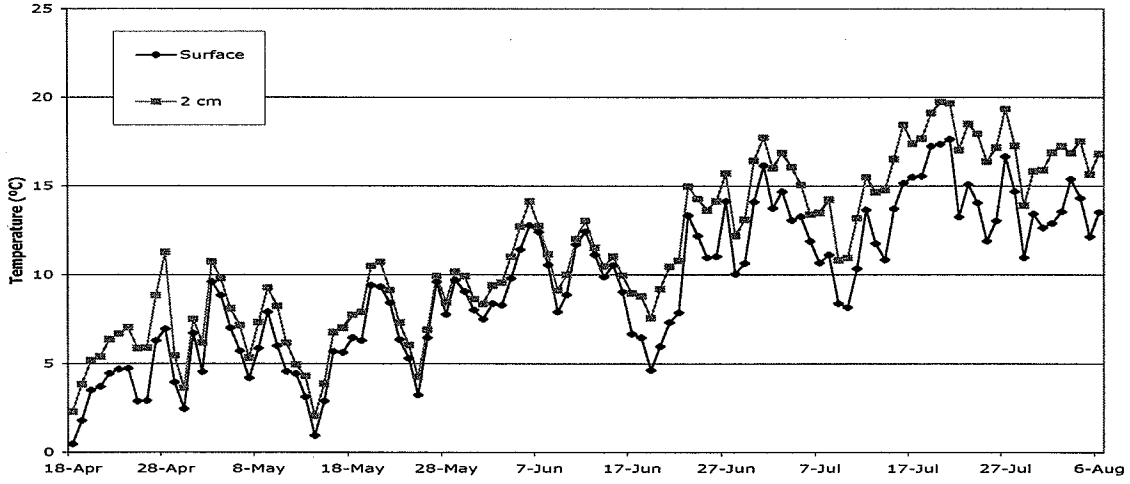


Appendix F con't

Minimum daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Lethbridge

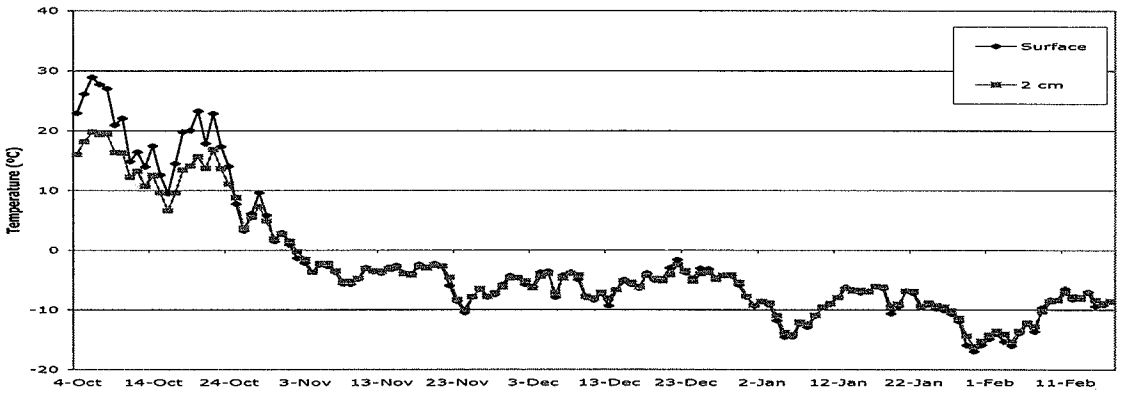


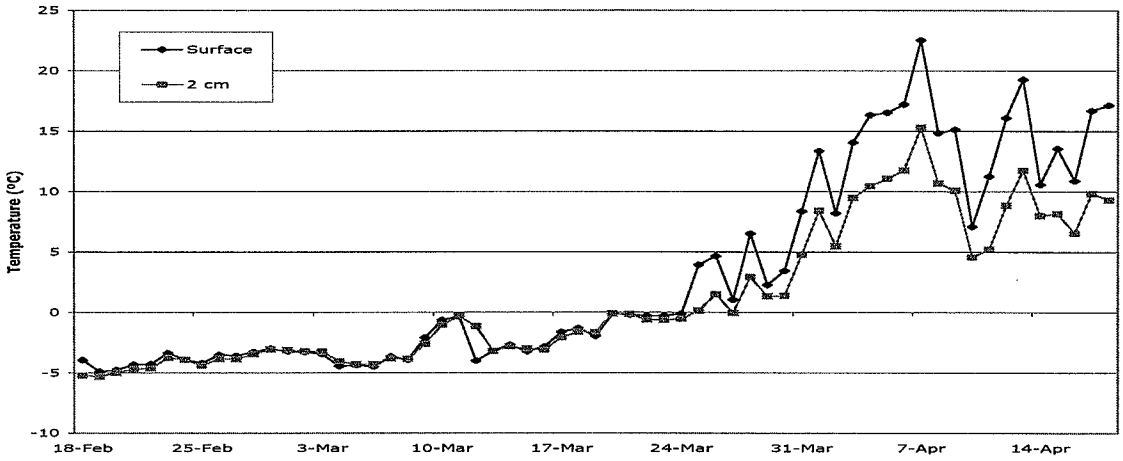


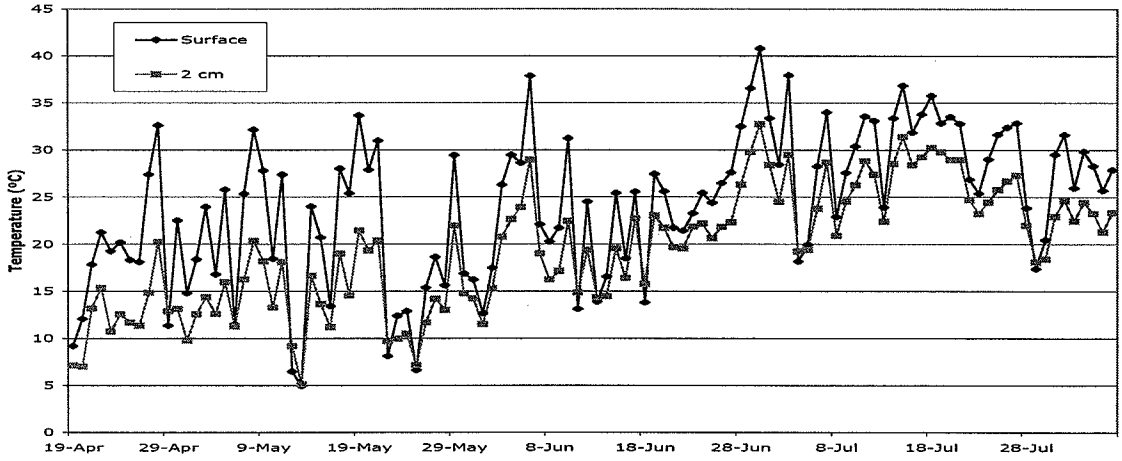


Appendix F con't

Maximum daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Indian Head

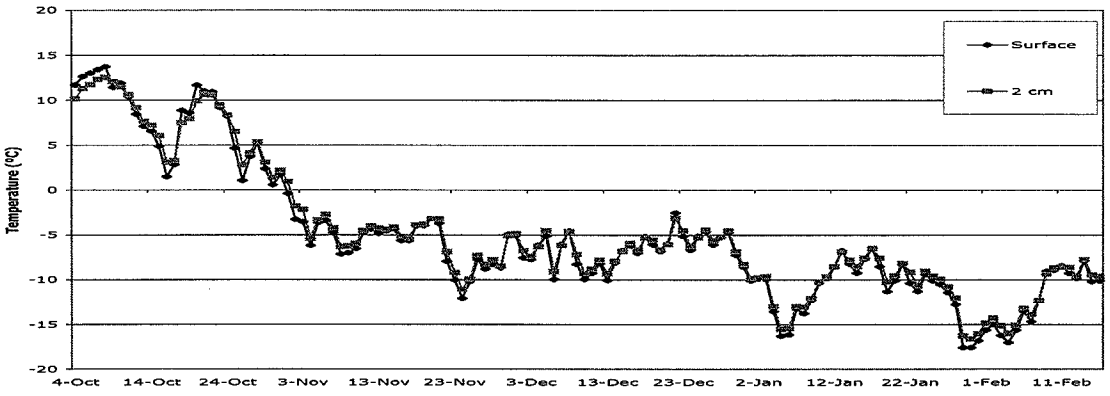


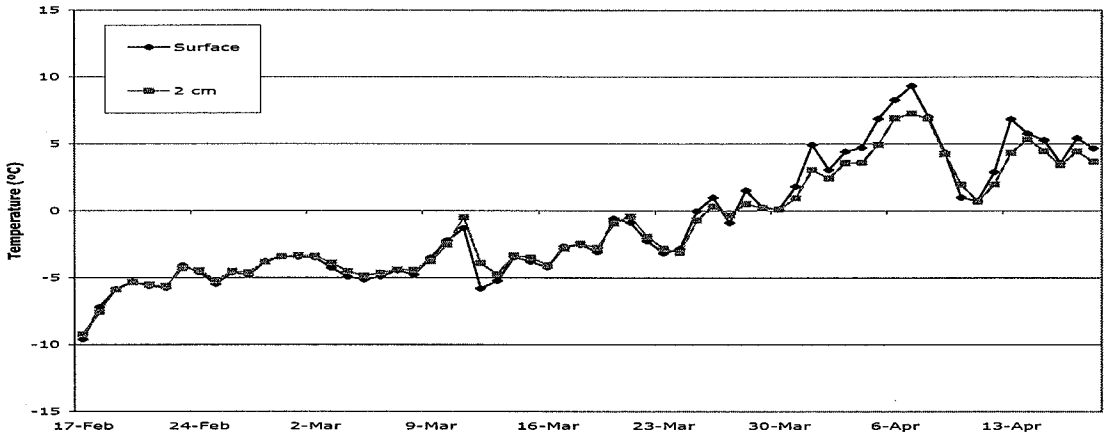


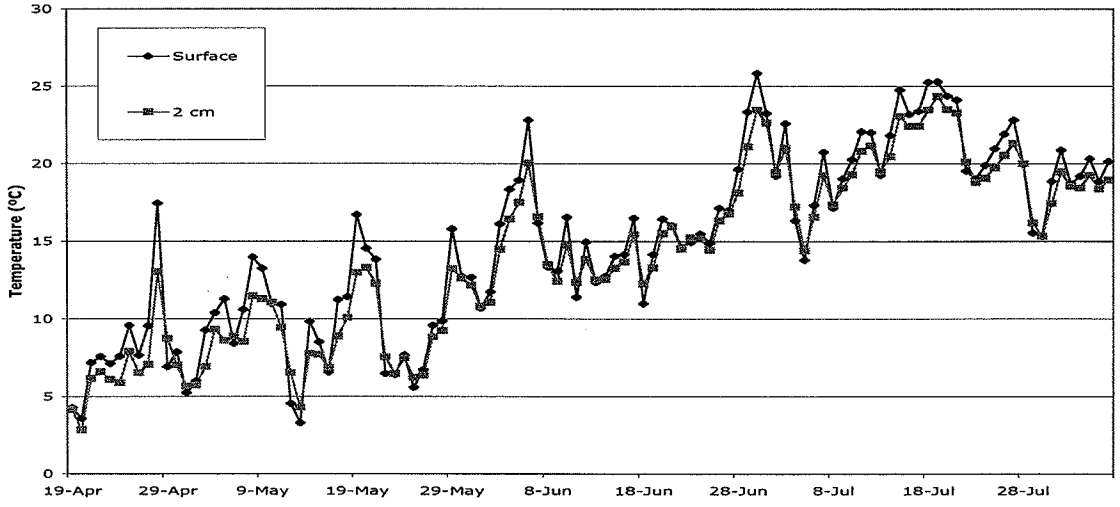


Appendix F con't

Mean daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Indian Head

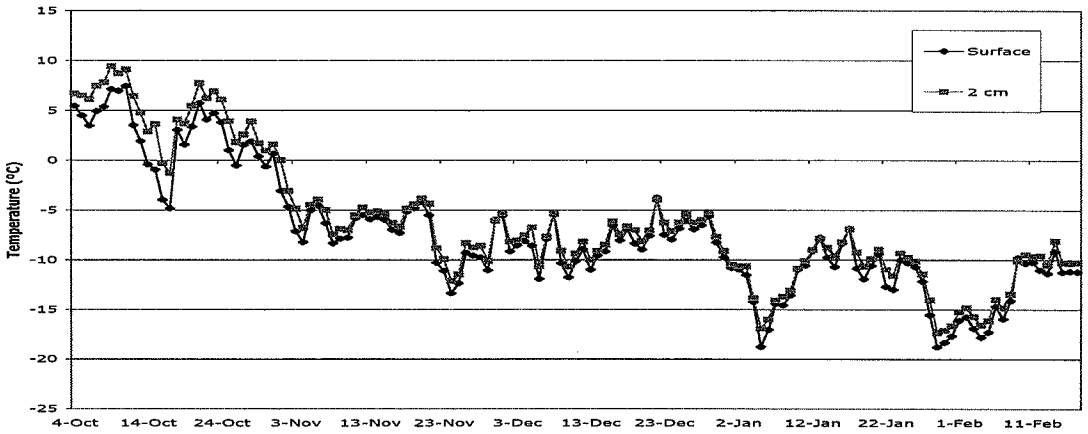


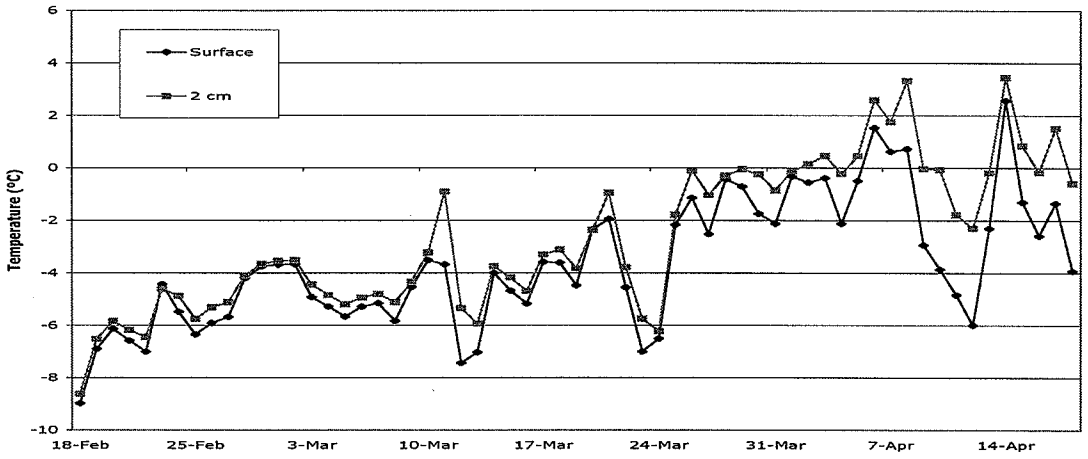


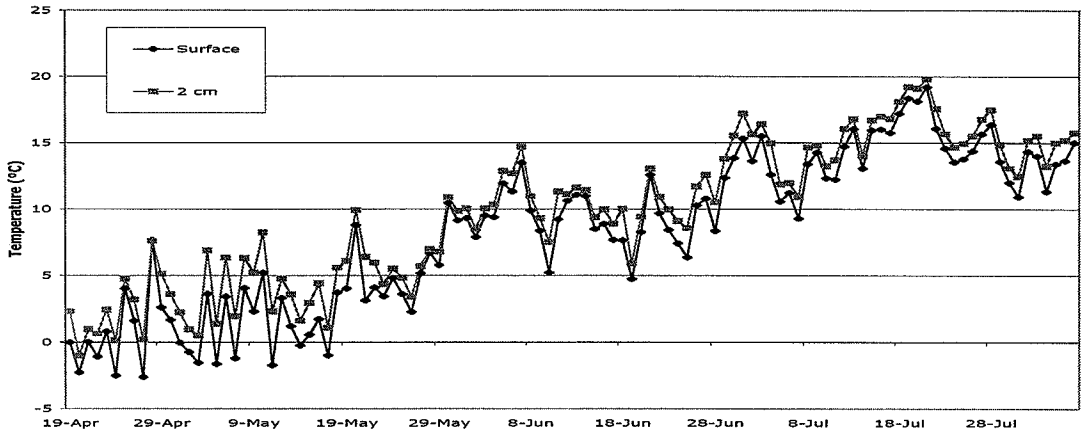


Appendix F con't

Minimum daily soil temperatures for the surface and 2 cm depths from October 2003 to early August 2004 at Indian Head







Appendix G

Percentage of black medic softened under a 15/6°C germination temperature for the different temperature and exposure time treatments. Within each exposure time, columns with different letters are significantly different at the $p<0.05$ level (PLSD).

	Exposure Time				
Temperature	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
20°C	2.00	1.50b	1.00c	4.50c	2.00c
5°C	6.00	13.50a	32.50a	45.00a	57.50a
-5°C	3.00	9.50a	15.50b	17.50b	23.00b
-23°C	4.50	8.00a	11.00b	5.00c	7.00c
$PLSD_{(0.05)}$	4.22	6.04	8.04	9.48	5.54

Highlights of ANOVA

	2 weeks	4 weeks	6 weeks	8 weeks	10 weeks
Temperature	NS	*	*	*	*