

**Recruitment Characteristics of Black Medic (*Medicago lupulina* L.)  
as a Self-Regenerating Cover Crop  
in a Continuous Grain Cropping System.**

by

Alden J. Braul

A thesis  
submitted in partial fulfillment  
of the requirements for the degree of

MASTER OF SCIENCE

Department of Plant Science  
University of Manitoba  
Winnipeg, MB

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## ABSTRACT

### **Recruitment Characteristics of Black Medic (*Medicago lupulina* L.) as a Self-Regenerating Cover Crop in a Continuous Grain Cropping System.**

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Although cover crops improve the sustainability of cropping systems in the Canadian prairies, their use is limited because of annual reseeding costs. These costs may be eliminated through the development of self-regenerating legume cover crops. Black medic (*Medicago lupulina* cv. 'George') was selected as a potential candidate for use as a self-regenerating cover crop in a continuous grain cropping system. The process of black medic recruitment consists of dormancy loss, germination, and seedling emergence. Seed microsite conditions, specifically soil temperature and soil moisture, control black medic recruitment and are affected by crop type, tillage system, and nitrogen (N) fertilizer rate.

In 2002 and 2003, field experiments were conducted to investigate the impact of crop type, tillage system, and nitrogen (N) fertilizer rate on black medic recruitment. Black medic recruitment was significantly greater in flax compared to oats during 2 of the 3 site-years. These differences were the result of (a) higher soil temperatures in flax which may have enhanced dormancy loss and germination and (b) higher soil volumetric water content in flax resulting from additional surface residue from the crop grown the previous year. In general, black medic recruitment was significantly greater for zero tillage (ZT) compared to conventional tillage (CT). This difference was attributed to higher soil volumetric water content (VWC) at 0-2 cm in ZT compared to CT. Distinct seed placement resulting from tillage also influenced black medic recruitment. Zero

tillage left the majority of the seed in the recruitment zone (0-2 cm depth) compared to CT. In addition, these seeds, positioned at or near the soil surface, were exposed to temperature fluctuations that enhanced dormancy removal. Nitrogen fertilizer rate did not significantly affect black medic recruitment. However, a trend towards greater black medic recruitment under low N rates was observed.

Laboratory studies indicated that temperature and moisture controlled black medic germination. Non-dormant (scarified) black medic seed germinated over a range of constant temperatures (10 to 30 °C) and at a diurnal fluctuating temperature of 5/40 °C. However, physically dormant black medic seed only germinated when exposed to a fluctuating 5/40 °C moist treatment. Appropriate temperature fluctuations in the presence of moisture were required for dormancy removal.

Black medic recruitment fluctuations varied between site years. Maximum black medic recruitment at Carman in 2002, Carman in 2003, and Winnipeg in 2003 was 26, 298, and 1214 plants m<sup>-2</sup>, respectively. These differences were attributed to variable environment conditions for dormancy loss and germination, genetic characteristics and size of the seed bank, soil texture differences, number of years established, and the management history of the plots. Broadleaf herbicide applications had a large impact on black medic recruitment and black medic tolerance varied according to the growth stage and active ingredient of the herbicide.

Black medic recruitment generally occurred early in the growing season. This flush was explained on the basis of the two-stage softening process where a chilling treatment (stage 1) was followed by fluctuating temperatures in the range of 5/15 °C (stage 2). This sequence of temperature combinations may rupture the seed coat or act

as a signal to cause changes in the seed coat to allow water uptake and germination.

Black medic recruitment also occurred late in the growing season during one site year.

Seed dormancy loss during this time period may be due to another two-stage softening process involving a different sequence and combination of temperatures including a high temperature preconditioning treatment (stage 1).

Cropping systems with low N inputs and limited broad spectrum broadleaf herbicide applications are best suited for a self-regenerating black medic cover crop. Further studies are warranted to quantify the benefits of black medic as self-regenerating cover crop in a continuous grain cropping system.

## 1. INTRODUCTION

Cover crops are low-growing legume plants grown in association with a main crop. Cover crops improve the sustainability of cropping systems in Canada by suppressing weeds, reducing fertilizer inputs, increasing organic matter, reducing nitrate ( $\text{NO}_3$ ) contamination of surface and ground water, increasing yields, and improving disease control. In addition, yields of the main crop are minimally affected and heat and water resources are more efficiently used (Thiessen - Martens and Entz 2001). Despite these numerous benefits, the use of cover crops is limited, primarily due to high seeding costs. Several researchers have highlighted the need for a cover crop capable of reseeding itself to reduce input costs (Moomaw 1995; Frye et al. 1988). A self-regenerating cover crop would increase the adoption of cover crops.

Annual *Medicago* species, commonly referred to as medics, are used as self-regenerating cover crops throughout the world. Australia has been a world leader in using annual medics and clovers to replace fallow in an integrated dryland cropping and livestock production system (Goose 1998). In South America, small farmers recognize the value of self-regenerating medics in corn as a valuable cover crop and forage source for livestock (Lazarte et al. 1997). Self-regenerating annual winter medics are also used as cover crops in Norway (Brandsaeter and Netland 1999). In the United States, annual medics were promoted to replace the fallow period in Montana (Koala and Sims 1982 in Rumbaugh and Johnson 1986) and grow in association with soybeans in Nebraska (Moomaw 1995). For the Canadian prairies, more information is required on the

suitability of annual medics and clovers as self-regenerating cover crops that are adapted to local environmental conditions and cropping systems.

Black medic (*Medicago lupulina* L.) was selected as a potential candidate species for use as a self-regenerating cover crop in continuous grain production systems in the Canadian prairies. Previous studies highlight the benefits of black medic as a cover crop. However, no information was available on the self-regeneration characteristics of black medic within a continuous grain cropping system. Therefore, the purpose of this thesis was to understand the ecology of black medic self-regeneration within a continuous grain cropping system environment. Laboratory germination experiments and field recruitment studies were designed to address the following objectives.

1. To understand the effect of temperature on the germination of scarified black medic seed.
2. To understand the effect of temperature and moisture on dormancy loss and germination of black medic seed.
3. To determine the effect of crop type, tillage system, and N fertilizer rate on the recruitment of black medic in a continuous grain cropping system.
4. To link the two-stage softening theory of medics in Australia to the softening process of black medic in the temperate climate of the Canadian prairies.



## 2. LITERATURE REVIEW

### 2.1 Introduction

Annual medics are used as self-regenerating cover crops in many parts of the world. The advantage of a self-regenerating crop is the elimination of annual re-seeding costs. Understanding and managing the self-regeneration characteristic of annual medics is necessary to guarantee timely recruitment within years and between years.

Recruitment timing is controlled by the breakdown of the hard seed coat which is influenced by moisture and temperature conditions immediately surrounding the seed, commonly referred to as the seed microsite. These conditions are influenced by the management practices in the cropping system which indirectly modify the environmental conditions at the microsite.

### 2.2 Annual Medic Species

#### 2.2.1 Origin and Distribution

The genus *Medicago* consists of 1 shrub, 21 perennials, and 34 annual species. The annual *Medicago* species are commonly referred to as “medics”. Medics evolved in North Africa and the Middle-East into very adaptable species capable of growing in a wide range of soils, temperature regimes, and growing season lengths (Rumbaugh and Johnson 1986) and later spread world wide as contaminants in seed exports. In Australia, medics are central to the ley farming system as a forage crop. In the United States, selected species are cultivated and others have become naturalized (Bauchan and Scheaffer 2002). In many other parts of the world, medics appear in cropping systems

both as weeds and favourable species (Pavone and Reader 1982; Frick and Thomas 1992; Peters and Schmidt 2001).

Medics are adapted to a Mediterranean type climate where they are winter annuals, germinating with the onset of the first fall rains in September to November (Northern Hemisphere) and producing up to 12 tons hectare<sup>-1</sup> of forage during the winter (Goose 1998). Seed produced during the winter period falls on the ground and lays on the soil surface during the hot and dry summer months. During this time, a combination of soil surface temperatures near 60 °C during the summer period and cooler fluctuating temperatures in fall fracture the hard seed coat, the primary physical seed dormancy mechanism preventing germination.

Medics have also adapted to more northerly temperate climates where they are spring annuals (Rumbaugh and Johnson 1986). Chilling temperatures during the winter period and fluctuating spring soil temperatures fracture the seed coat leading to dormancy loss and allowing germination to proceed.

### **2.2.2 Agricultural Application**

Although medics are common throughout the world, their extensive adaptation to farming systems is limited to Australia where they replaced fallow by integrating dryland cropping and livestock production (Goose 1998). This “ley” or “meadow” farming system implies growing crops in rotation with regenerating annual legume pastures that are grazed by sheep. Generally, the medic pasture alternates with wheat on a two year cycle. Regeneration from the soil seed bank occurs every year, and in the pasture phase, the medic provides forage for livestock. In the cereal phase, regenerating medics may

briefly furnish forage before seeding of the cereal crop (Goose 1998). The strengths of the ley farming system include improved soil fertility, enhanced crop and livestock production, and production diversification (Reeves and Ewing 1995).

There are two reasons why annual medics are so successful in Australia. First, medics maintain their longevity in the soil seed bank. This characteristic is important because the seeds must remain dormant in the soil seed bank for at least 2 years during the cereal phase. Delayed germination is caused by the hard seed coat which prevents medic seeds from exhausting their total reproductive capacity in any single attempt to establish themselves. The hard seed coat makes the seed impermeable to water until specific temperature conditioning requirements occur at the seed microsite and cause the seed coat to fracture. This environmental cueing mechanism regulates the germination of seed in the soil seed bank, spreading seedling recruitment over several seasons (Tadmor et al. 1971) or within a season (Quinlivan 1971), and guaranteeing long term survival. Sometimes called a drought avoidance mechanism, the hard seed coat is especially valuable in Mediterranean climates to avoid false breaks in germination and maintain the soil seed bank. Plant breeders in Australia select for softening patterns adapted to local climatic conditions that improve the recruitment survivorship of the medic in the pasture phase of the rotation.

The second factor contributing to the success of medics in Australia is the economic benefit provided by the integration of livestock and cereal production through the use of medics. During the pasture phase, sheep wool is produced while the soil undergoes a soil rebuilding phase through the addition of organic matter and N by the medic crop. The benefits of this cycle are further realized in the cereal phase with

reduced weed pressure and increased soil fertility. The ecological and economic benefits of this system are particularly important, especially when wool prices are strong (Mohamed Saleem and Fisher 1993; Latta 2001).

Given the relative success of ley farming in Australia, technology transfer of the medic system to developing countries has been the focus of many development organizations. Mohamed Saleem and Fisher (1993) analyzed a case study of ley farming in West Africa where development agencies promoted the system to intensify crop-livestock production and to improve human welfare while protecting the environment. High opportunity costs of growing forages, inadequate technical knowledge, and the attempts by development workers to apply technologies outside of their ecozonal limits were cited as the major challenges to overcome.

At the International Center for Agricultural Research in the Dry Areas (ICARDA) in Aleppo, Syria, replacement of weedy fallows with medics was examined under four tillage treatments (Cocks 1994). By improving the productivity of the fallow year with medics, high quality forage was produced and soil N levels increased for the next planting. In order for sufficient medic recruitment during the fallow year, traditional deep ploughing operations needed to be replaced by more shallow cultivation. Recommendations to improve the medic system included the selection of indigenous species, adaptation of locally available tillage equipment, and improved grazing management to restore the medic soil seed bank each year.

These lessons in adapting medics to cropping systems around the world are applicable to adapting medics to Canadian prairie cropping systems. Selecting

appropriate species and understanding the effects of management on recruitment are key research priorities to aid adaptation.

## 2.3 Black Medic

### 2.3.1 Origin and Distribution

Black medic (*Medicago lupulina* L.) is a member of the legume family and grows as an annual, biennial, or short-lived perennial (Turkington and Cavers 1979; Bauchan and Scheaffer 2002). Black medic is taxonomically organized as an annual species as follows:

Family:	Leguminosae
Subfamily:	Papilionoidae
Tribe:	Trifolae
Genus:	<i>Medicago</i> L.
Section:	Lupularia
Species:	<i>lupulina</i> L.

The specific origin of black medic is still in question based on conflicting research that links its origin to the Mediterranean region, Europe, and Asia. Currently, black medic is widely distributed over temperate and subtropical regions of the world including Great Britain, Ireland, S. Europe, N. Africa, America, Asia, and the Atlantic Islands (Sidhu 1971). Since its introduction to North America, likely in the 1600's with contaminated alfalfa seed shipments from Europe, black medic has naturalized from Northern Mexico to as far north as Edmonton, Canada (Sidhu 1971). It grows best in moist soils and under cooler temperatures than other annual *Medicago* species

(Rumbaugh and Johnson 1986). In the Great Basin area of Utah, black medic is commonly found at elevations higher than 1,400 m (Rumbaugh and Johnson 1986). Ecologists study black medic to learn about its ability to colonize a wide range of habitats and maintain a diverse genetic pool comprised of numerous genotypes.

### **2.3.2 Morphological Characteristics**

Black medic has a shallow tap root up to 60 cm in length that is resistant to cold and drought. Depending on the genotype, the stem may be erect or decumbent with few to many basal branches that develop from the crown of the root. Leaflets are stipulate, petiolate, trifoliate (first leaf simple), elliptic to obovate, 1-2 cm long. The inflorescence consists of a raceme 3-10 mm that is globose to cylindrical with a variable number of flowers (11-47) and fruits per head (3-47). The flower is 2-3 mm long, bright yellow with 4 alternate stamens mostly self-pollinated, although heterozygosity is not uncommon. The tripping mechanism is present but non-functional. Pods are one-seeded, black, up to 3 mm long, slightly curved with seeds up to 2 mm long, hard, shiny, and yellow to olive green. In southern Ontario, flowering occurs from April to October and fruit production from May to October. The chromosome number is  $2n=16$  or  $2n=32$  (Turkington and Cavers 1979; Sims et al. 1985).

### **2.3.3 Population diversity**

Black medic populations are a mosaic of distinct genotypes (Sidhu 1971). These genotypes are maintained since black medic is an obligate self-pollinating species, with a non-functional tripping mechanism (Sidhu 1971). As a result, interbreeding among

individuals of the same or different populations is not expected (Sidhu 1971). Sidhu (1971) observed that variations in habitat or climate shifted the proportions of the different genotypes within a population.

#### **2.3.4 Seed Bank Characteristics**

A seedbank includes all the seeds in the soil profile (Thompson and Grime 1979; Cavers 1995). Often only a small percentage of the seed bank will germinate in any given year (Harper 1977). Some researchers suggest that only about 6% of viable seed in the top 10 cm are likely to produce seedlings for some species (Roberts 1984).

In the case of black medic, understanding seedbank characteristics are useful for understanding why this species is persistent. Roberts and Feast (1972) found that black medic seed was still viable after 6 years of storage in cultivated or uncultivated soil. Pavone and Reader (1982) observed that over 60% of the black medic seed bank did not germinate in spring. These observations indicate that black medic relies on a persistent seed bank for regeneration. Thompson and Grime (1979) suggested the strategy of a persistent seed bank is commonly used by short-lived species found in unpredictable environments. A persistent seed bank stabilizes population dynamics to spread risk and diminish large fluctuations in response to short term environmental changes (drought or biotic disturbance) (Cavers 1983). Persistent seed banks are also recognized for conserving genetic diversity, buffering populations against changes due to genetic drift, selection or immigration (Levin 1986). These characteristics describe the black medic seed bank.

Black medic recruitment is usually not limited by the seed bank, but rather the availability of appropriate temperature and moisture conditions at the microsite. These conditions determine the degree and timing of recruitment. The usefulness of seed banks for predicting populations is currently debated. Some researchers claim that seed banks accurately describe and predict weed populations, while others argue that the best indicator for future weed populations is not the seedbank, but rather the previous year's weed population (Derksen et al. 1998; Ball and Miller 1989). No studies are available which trace black medic recruitment and seed bank size over the long term.

### **2.3.5 Agricultural Application**

Black medic has many agricultural applications. It can be used as a cover crop and green manure. In the United Kingdom, the dry matter and nitrogen accumulation of black medic was compared to that of red clover (*Trifolium pratense* L.) and white clover (*Trifolium repens* L.) (Stopes et al. 1996). Legume dry matter accumulation after 6 months during winter for red clover, white clover, and black medic was 800, 600, and 600 kg ha<sup>-1</sup>, respectively and N accumulation was 21, 17, and 14 kg ha<sup>-1</sup>, respectively. After 25 months and 10 mulching treatments, dry matter accumulation for red clover, white clover, and black medic was 25.4, 25.0, and 20.4 t ha<sup>-1</sup>, respectively, and N accumulation was 741, 592, and 459 kg ha<sup>-1</sup>, respectively. Although black medic dry matter and N production was less than for red and white clover, black medic is still used extensively in organic farming systems in other European countries because it is less susceptible to diseases affecting red and white clover.



Power and Koerner (1994) investigated black medic as a cover crop in eastern Nebraska. Based on a July 1 to November 12 production period, the highest-yielding species were arrowleaf clover (*Trifolium vesiculosum* Savi), rose clover (*Trifolium hirtum*), and black medic producing 3.7, 3.6, and 3.4 t ha<sup>-1</sup> dry matter, respectively. This research indicates the ability of black medic to improve the sustainability of cropping systems by building up organic matter.

In Montana, Sims (2001) recognized and promoted the application of Australian ley farming systems in northerly climates using black medic as the self-regenerating legume species. He selected the black medic cultivar 'George' in 1979 by collecting adapted ecotypes from 4 counties around Bozeman, MT (Sims 2001). This seed was bulked and planted into a field in May 1979. Due to the high portion of the hard seed they used, the stand was weak in 1979. In 1980 the residual seed from the first planting germinated and formed a strong stand which was incorporated into the top 10 cm of the soil profile after seed maturation. In 1981 the field was seeded to wheat and in 1982, black medic regenerated from the soil seedbank forming a vigorous stand. Seed harvested in August 1982 was designated 'George'. This cultivar was intended for use as a green manure, summer fallow replacement crop in dryland cropping rotations (400 mm or more annual precipitation) prior to wheat or barley (*Hordeum vulgare* L.). After the grain production year, black medic regenerated from the soil seed bank, serving both as a cover crop and forage. Black medic used in this manner increased wheat yields by 92% and water use efficiency by 81% in Montana field experiments (Rumbaugh and Johnson 1986).

Also in Montana, black medic was compared to five commercial, annual medics from Australia: 'Gaertn' (*M. truncatula*); 'Ghor', 'Jemalong'; and 'Cyprus' (*M. littoralis*) (Sims and Slinkard 1991). After the first year of production, black medic had lower stand densities, lower dry matter production, and lower seed yields than other species. However, after the second year (spring wheat crop) and the third year (green manure crop), black medic stand density, dry matter production, and seed yields were substantially greater than the Australian medics. Superior performance over the longer-term was attributed to greater seed bank persistence of black medic. Grain and protein yields, total N uptake, and water use efficiency were greater for spring wheat following black medic than the Australian medics. According to Sims (2001) and Oien (1998), the ley system design with black medic was not adopted because (a) producers preferred to maintain a "weed free" and bare field during the fallow period, (b) government subsidies did not recognize non-traditional cropping systems, and (c) crop insurance programs reduced coverage on continuous cropping systems like ley systems.

Black medic as a winter annual legume cover crop was examined in Norway by Brandsaeter and Netland (1999). Winter hardiness and growth characteristics for black medic were compared with hairy vetch (*Vicia villosa* Roth.), crimson clover (*Trifolium incarnatum* L.), and subclover (*Trifolium subterraneum* L.). The results showed the following ranking of spring biomass as an expression of winter hardiness: hairy vetch>black medic>crimson clover>subclover. Black medic winter hardiness was an important characteristic in northern climates to capture unused heat and moisture resources in spring before seeding operations. In addition, black medic was recognized

for its low canopy, and therefore mowing was not required when transplanting crop plants such as tomatoes (Brandsaeter and Netland 1999).

The use of medics as a reseeding annual legume crop in arid rangelands was investigated by Rumbaugh and Johnson (1986). In their study, 70 accessions of black medic were compared to 514 accessions of 33 other annual *Medicago* species. Black medic showed superior reseeding and groundcover characteristics, greater ability to improve pastures, and more prolific seed production that rapidly built up the soil seedbank. The 70 accessions of black medic were collected from regions around the world. They varied in ground cover characteristics, plant size, date of first bloom, and regeneration characteristics. Noted was a phenotypically heterogeneous population capable of adapting to a variety of environmental circumstances.

Black medic was also used in an intercropping system as a cover crop growing underneath the main crop. The earliest record of this practice being recommended was in 1935 in a book by A. Petersen entitled Klee und Kleeartige als Futterpflanzen auf Acker und Weide (Hartl 1989). Hartl (1989) studied the influence of black medic and *Trifolium resupinatum* on weeds and the yield of organic winter wheat when grown as a cover crop. Black medic and *T. resupinatum* reduced winter wheat dry matter by 12 and 14 %, respectively, compared to winter wheat grown alone. No significant differences between wheat seed yields in the different treatments were observed. The dry matter of live weeds at harvest was reduced by 44 and 49% for the black medic and *T.resupinatum* cover crops, respectively, grown with winter wheat, compared to winter wheat sown alone. These results highlight the usefulness of black medic in controlling weeds and maintaining production of the main crop.

In Michigan, black medic (cultivar 'George') and two other annual *Medicago* species were intercropped with barley (*Hordeum vulgare*) (Moynihan et al. 1997). Depending on location, barley seed yields were reduced or increased with the medic intercrop compared to monoculture barley fertilized with N. Fall weed biomass was reduced in medic intercrops by 65% across environments compared to the barley monoculture. Black medic was the least competitive of the annual medics tested.

Researchers in Nebraska examined the intercropping cover crop system by seeding black medic, hairy vetch, and turf-type tall fescue into a young stand of soybeans (Moomaw 1995). No reduction in soybean yield was observed suggesting that competition between the main crop and cover crop was minimal. Black medic ground cover at the end of the season was greater than hairy vetch. Only black medic produced seed in the soybean canopy. Based on field observations, the researchers suggested that the rapid seed production characteristic of black medic could be exploited as a self-regenerating cover crop at different times in the crop rotation.

Goldstein and Young (1987) studied the economic contribution of a black medic self-regenerating cover crop in a cropping system. A conventional cropping system using a wheat/barley/wheat/pea rotation was compared to a low-input system using a three year pea-black medic/black medic/wheat rotation. The low-input system was termed a "perpetuating-alternative legume system" (PALS) since the black medic was allowed to produce seed and regenerate in subsequent stands of spring peas. In both systems, wheat yields were assumed to be equal. However, pea yields under PALS were assumed to be lower due to competition with the black medic. Final analysis indicated that PALS was

more profitable when commodity prices were based on actual world prices, rather than current government target prices.

A North Dakota farm case study collected information on the use of black medic as a self-regenerating cover crop in a flax-wheat rotation (Hagen 2001). In 1993, black medic was seeded as a green manure, allowed to set seed, and disked into the soil. Since 1993, black medic has successfully regenerated every year from the soil seed bank and produced seed under the main crop. The producers estimate that black medic adds 25 kg N ha<sup>-1</sup> annually and provides some weed control. Herbicides are used to suppress the black medic in spring to reduce competition with the main crop. Black medic also provides excellent fall grazing.

In summary, the agricultural applications and benefits of black medic as a cover crop, inter-crop, green manure, and self-regenerating legume have been researched. Despite the benefits associated with black medic in a variety of cropping systems, the practice of using black medic is not common. Factors limiting the adoption of black medic as a self-regenerating cover crop may be (a) low levels of self-regeneration from the seed bank, (b) concern regarding the potential of black medic to become a weed, or (c) the failure to identify a cropping system best suited to capture the benefits of a self-regenerating cover crop of black medic.

## **2.4 Components of Seedling Recruitment**

Seedling recruitment has often been thought of as seedling emergence. However, seedling recruitment is much more than this. Seedling recruitment is the culmination of seed formation, dormancy development, dormancy loss, germination, and shoot

elongation (Forcella et al. 2000). These biological processes operate on different time scales and often require different environmental conditions to move the seed to the next developmental stage (Benech-Arnold et al. 2000).

#### **2.4.1 Formation of viable and nondormant seed**

The first step in seedling recruitment is the formation of viable and nondormant seed. After fertilization of the zygote, the embryo undergoes differentiation and maturation, but remains nonviable. A viable and nondormant embryo is formed as a result of the development of the testa which is permeable to water and fully differentiated with well developed thickenings in its palisade and hypodermal cells (Bewley and Black 1985).

This nondormant phase prior to the onset of dormancy was studied in attached and detached black medic seeds by Sidhu and Cavers (1977). Attached seed describes seed where the inflorescence is still connected to the parent when the seeds are buried in the soil. Detached seed describes seed where the inflorescence is detached from the parent but the seeds are still attached to the peduncle. The average duration of the nondormant phase for attached or detached seed was 20 days in the greenhouse and 8 days in the field. Therefore, a window of 1 to 3 weeks prior to the onset of dormancy exists when black medic can germinate given the right environmental conditions.

The implication of this non-dormant phase is important to consider when black medic is used as a self-regenerating cover crop in dense crop canopies where relative humidity is high. Under these environmental conditions, non-dormant black medic may

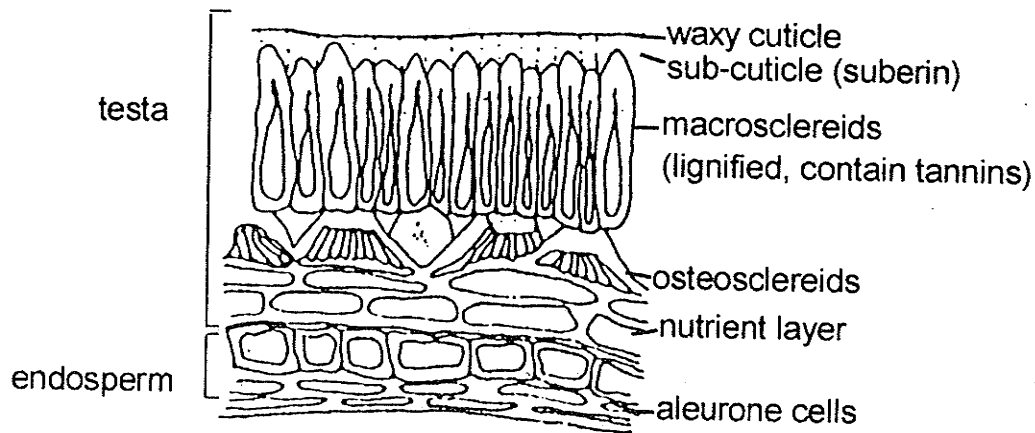
pre-maturely germinate while attached to the plant, and subsequently die. The result would be reduced seed rain reduced seedbank populations.

#### **2.4.2 Dormancy Development**

Benech-Arnold et al. (2000) define dormancy as an internal seed condition that impedes germination in the presence of favourable moisture, thermal, and gaseous conditions. It is controlled by a complex interaction of environmental, edaphic, physiological, and genetic factors (Dyer 1995; Buhler et al. 1997; Andersson and Milberg 1998). Since the dormancy release of a seed population strongly affects the periodicity and degree of recruitment (Benech-Arnold et al. 2000), it is essential to understand the factors that regulate seed dormancy. It is also important to recognize that dormancy characteristics are unique to each plant species. Based on this understanding of dormancy, the following discussion will summarize the current scientific understanding of dormancy development and release as it relates to black medic and similar species.

Dormancy is often divided into two separate categories - primary and secondary dormancy (Taylorson 1987). Primary dormancy or innate dormancy prevents precocious germination and is present in newly formed seeds. A characteristic hard and water impermeable seed coat is considered as the primary dormancy mechanism preventing seed germination in legumes (Probert 2002). This form of primary dormancy, known as physical or seed coat imposed dormancy, is the result of unknown chemical changes in the testa that render it impermeable to water (Sidhu and Cavers 1977). The testa is composed of a waxy cuticle, suberin, and thick-walled palisade and osteosclereid layers

(Figure 2.1). According to Bewley and Black (1985), each of these layers contribute to waterproofing and in some legume species, the waxy cuticle plays a major role. Other research (Probert 2002) indicates that the thickened outer walls of the palisade cells, rather than the cuticle (outer seed coat), constitute the moisture barrier.



**Figure 2.1:** A section of the seed coat of *Melilotus alba* (Bewley and Black 1985).

The level or intensity of primary dormancy is variable and depends on the genetic variability between and within legume species. In Australia, numerous studies highlight these differences in physical dormancy. Latta and Quigley (1993) compared the persistence of *Medicago truncatula* with other annual medics in the Victorian Mallee region of Australia finding significant differences in dormancy intensity between species and cultivars within the same species. Cocks (1992) seeded several medic species into a sward and found significant differences in sward composition over 4 years, attributing the change to variable patterns of seed softening and species adaptability to the local environmental conditions. In Canada, the genetic diversity between alfalfa cultivars results in varying levels of physical dormancy (Stout et al. 1999; Acharya et al. 1999). A



study of various populations of black medic by Sidhu (1971) showed significant differences in levels of physical dormancy between and within populations which were attributed to genetic diversity.

Environmental conditions during seed development and maturation also affect the intensity of primary dormancy of many species (Andersson and Milberg 1998). In Australia, cool and moist conditions during the seed production phase enhanced the level of hard seed for subterranean clovers (Quinlivan 1965) and annual medics (Lodge et al. 1990). In North America, alfalfa seed produced in the warm growing season of southern California usually has a hard-seed content of less than 20%, while that grown in the cooler regions of the Pacific northwest has a hard seed content between 40 and 50% (Bass et al. 1988). Sidhu (1971) also attributed differences in dormancy levels in black medic to the environment conditions during the seed production phase.

Secondary dormancy follows primary dormancy and represents a dormant period that is re-induced in seed that was once dormant and whose dormancy had been reduced or eliminated (Benech-Arnold et al. 2000). The ability to cycle from primary to secondary dormancy provides seeds the ability to move in and out of dormancy over time. In this way, the seed is prepared for environmental conditions which are most suitable for germination. Black medic dormancy re-induction will be discussed in the following section.

### **2.4.3 Dormancy Loss**

Dormancy loss in many plant species is attributed to a number of variables that act simultaneously or separately to alter the environmental conditions at the seed

microsite level. These factors include changes in light, moisture, temperature, and gaseous environment (Taylorson 1970). Dormancy changes have been successfully modeled using soil temperature as the primary factor (Bouwmeester and Karssen 1992). Soil temperature and soil moisture conditions and their interactions are generally considered to be the most important factors that influence dormancy, whereas other factors such as light and nitrate concentrations often terminate dormancy (Benech-Arnold et al. 2000). The intensity of dormancy varies (Baskin and Baskin 1998) and is measured on the basis of the temperature range required to break dormancy (Benech-Arnold et al. 2000).

#### **2.4.3.1 Physical**

In legume species, loss of physical dormancy is the result of structural changes in the palisade layer of the seed coat which seeds to imbibe moisture. To date, these structural changes are not well understood. The seed coat is considered to have specialized regions for water loss and uptake such as the hilum (McDonald et al. 1988), micropyl, and the structurally weak lens region (Taylor 1981; Gopinathan and Babu 1985; Baskin 2003). However, water penetration is also observed at random sites on the seed coat, suggesting that the specialized regions are not absolutely important for water uptake (Quinlivan 1968; Zeng 2001). Most scientists agree that water is absorbed over the entire seed coat through the seed coat fractures.

Temperature effects are considered to be the main factor driving the structural changes in the seed coat of legumes that lead to dormancy loss. Based on studies in a Mediterranean climate, Taylor (1981) divided the process of medic dormancy loss into

two temperature regulated stages. For dormancy loss to occur, the seed needed to be exposed to two distinct temperature conditions. In the first preconditioning stage, a constant temperature greater than 20 °C or fluctuating diurnal temperatures typical of field conditions (15/60 °C) produced latent soft seeds. These temperature conditions were typical during the hot and dry summer period. The amplitude of diurnal temperature fluctuations was found to be optimum at a maximum temperature of 50 °C for some medics, and for sub clover species, the seed softening increased to a maximum temperature of 70 °C (Taylor and Ewing 1988). Several researchers found that the maximum temperature of the diurnal cycle rather than the fluctuation was the most important factor in determining the breakdown rate of hard seed (Quinlivan 1966; Fairbrother and Pederson 1993). The second stage produced a seed that was permeable to water and was referred to as the final softening phase. As in the preconditioning phase, temperature was the driving factor. The second phase occurred very quickly and required a few appropriate diurnal temperature cycles, unique to individual genotypes. Taylor (1996a) found that the temperature requirements of the final softening stage were similar for a particular population during the first year of softening, even though the environmental conditions (moisture and temperature) during seed production and maturation differed. However, temperature requirements of seeds that soften after the first year were different if the original seed production environment differed between the seeds. Smith et al. (1996) found that seeds of a genotype which soften in the first year may do so in response to a different stimulus than those which soften in subsequent years. This two-stage process set the framework for subsequent hard seed coat softening studies.

Two important questions about the softening process still remained after the discovery of the two stage softening process. The first was how temperature causes the seed coat to fracture and the second, why this softening process depends on two separate temperature regimes. A theory proposed by Zeng (2001) answers these questions based on physical and chemical changes in seed coat lipids. According to the theory, the surface of the seed is covered by a hydrophobic layer made of a complex combination of lipids, primarily cutin, suberin, and waxes. These lipids respond to temperature changes like those common in Western Australia during the summer. The relative proportion of these different lipids influences the rate of seed softening. According to Zeng (2001), the first stage of the softening process proposed by Taylor (1981) results from a physical weakening of the hydrophobic bonds of the lipids caused by high temperatures. The final softening process is a chemical process that occurs when lipids are hydrolyzed by the reaction between fat and water. Therefore free water must be available in the microsite and likely becomes available when humidity rises with decreasing temperatures. For this reason, low temperatures of 15 or 10 °C are required for the second softening process. The combination of physical and chemical degradation ruptures the seed coat and makes the seed permeable to water.

In Canada, primary dormancy loss in alfalfa, red, alsike, white and sweet clover seed (*Melilotus officinalis*) was also linked to temperature. Early work by Leggatt (1927) showed the interaction between legume hard seed coats and their environment. High soil temperatures promoted germination of dormant alfalfa seed in the field. Freezing conditions during the winter resulted in only 4% softening of impermeable alfalfa seed, while 74% softening of impermeable alfalfa seed occurred during one growing season.

As well, low temperatures followed by a period of alternating temperatures was favourable for the germination of red, alsike, white and sweet clover seed. The low temperatures occurred during the winter period. Alternating temperatures occurred in spring when the soil warmed during the day and cooled at night. This apparent timing mechanism resulted in early spring germination. It was also observed that dormancy loss increased under moisture limiting conditions and high temperature.

Stout et al. (1999) designed several laboratory experiments to gain an understanding of dormancy loss in alfalfa seed under field conditions in British Columbia. When the range in diurnal temperatures was large and the temperature was high (ie: 5/35 °C), hard seed percentages were much lower than at a constant 20 °C treatment. Immediately after the 5/35 °C treatment, many hard seeds became 'fresh seeds', meaning that they imbibed water but did not germinate.

Acharya et al. (1999) also studied the effect of temperature on dormancy levels in alfalfa seed. No significant decline in dormancy resulted when several alfalfa cultivars were held under controlled environmental conditions in a storage room at 20 °C for 64 months. However, at 35 °C, the proportion of hard seeds in seed samples decreased continuously for all cultivars.

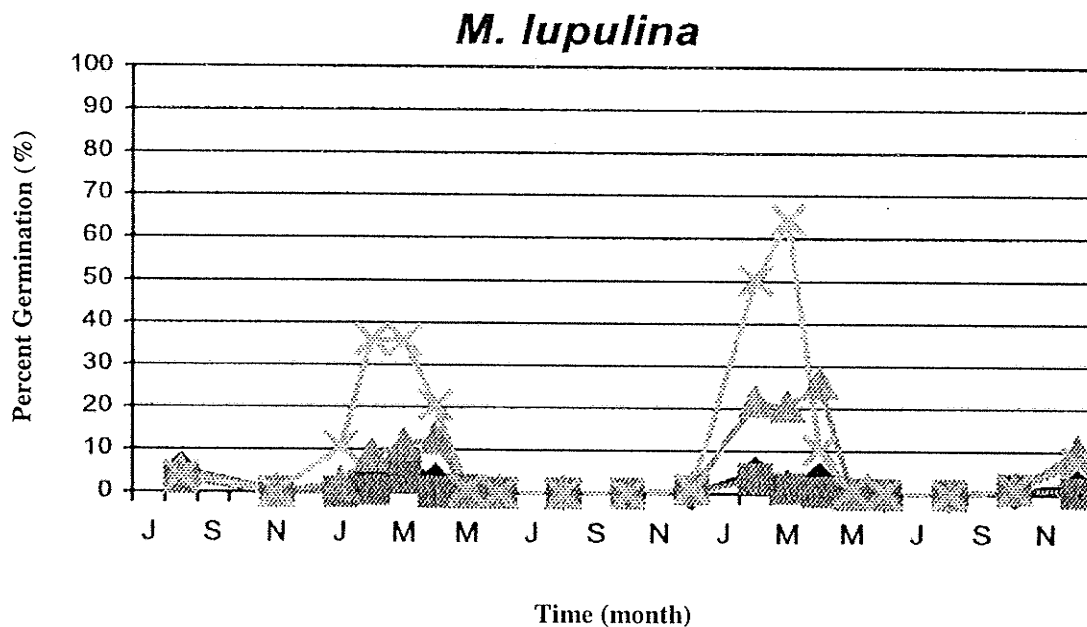
Dormancy loss of black medic was affected by temperature in a series of studies conducted in Belgium (Van Assche et al. 2003). Black medic seed was collected from along roadsides and in grasslands, and then reproduced to obtain a sufficient number of seeds for experimental purposes. In the first experiment freshly harvested seeds were subjected to various temperature regimes. Germination levels at temperature regimes of

5, 10, 23, 30, and 10/20 °C were 6.0, 2.3, 4.3, 3.0, and 4.3%, respectively. Low levels of germination and dormancy loss was observed in all treatments.

In a second experiment by Van Assche et al. (2003), the effect of seasonal temperature patterns on dormancy loss was examined under field conditions. Seeds were placed in nylon bags, buried to a depth of 7 cm in pots, and placed in the field. Every two months for a period of two years, seeds were exhumed and subjected to four temperature conditions: 30/20, 20/10 15/6, and constant 23 °C for 2 weeks. There were two major findings observed. First, the most favourable germination temperature was the 6/15 °C treatment, and to a lesser degree the 10/20 °C treatment (Figure 2.2). Little to no germination was observed in the 20/30 and 23 °C treatments. These results suggest that black medic germination in temperate climates is favoured either in early spring or late fall when a temperature regime of 6/15 °C is most common. The second major finding was that black medic exhibited a peak in germination when exhumed in spring, with almost no germination for seeds exhumed at other times of the year. Germination was limited to the period from January to March for all temperature treatments (Figure 2.2).

The work by Van Assche et al. (2003) helped clarify black medic recruitment dynamics in temperate regions. Baskin (2003) recognized the two-stage softening process in the experiment by Van Assche et al. (2003). The first chilling stage occurs during the winter period. The second stage is marked by cool temperature fluctuations that provide a signal to the seed that spring has arrived. This cue is very important because the spring period is the best possible germination time in terms of moisture availability and reduced competition from other plant species. The second stage softening process also indicates to the seed its vertical position in the soil profile.

Temperature fluctuations are more prominent at the surface and muted with depth. Therefore recruitment is only possible for seed near the surface. Another observation from the study by Van Assche et al. (2003) is that black medic seeds lose the ability to respond to low alternating temperatures. Van Assche et al. (2003) studied this dormancy cycling phenomena in *Melilotus*, finding that after two months of low temperature, the seeds germinated when exposed to alternating temperatures. However, within one month at room temperature, the seeds lost their ability to respond to alternating temperatures. Therefore, germination would only be possible shortly after the chilling period in spring.



**Figure 2.2:** Percentage germination of *M. lupulina* seeds incubated under different temperature regimes following 0-28 months burial in the soil. The first point in the graphs represent the percentage germination of fresh seeds. Diamonds, 23 °C; squares, alternating 12 h at 30 °C, 12 h at 20 °C; triangles, alternating 12 h at 20 °C, 12 h at 10 °C; crosses, alternating 12 h at 15 °C, 12 h at 6 °C (Van Assche et al. 2003).

Black medic dormancy loss in Canada appears to be affected by temperature.

The main contribution to this field of knowledge is based on a study of black medic

ecology by Sidhu (1971) in southern Ontario. Dormant black medic seed held under constant temperatures between 1 and 35 °C for three weeks reached a germination level of 4%. Temperature did not affect germination percentage in this experiment. Under fluctuating temperature regimes (0/10,5/10,10/20,10/30, 20/30, 20/35, 25/35 °C) and two light conditions (alternating light and dark, or constant darkness), the percent germination ranged from 0.7 and 4.7 % after a 3 week period. These differences were also not significant, likely because the treatments were not preceded with a chilling process (i.e.: the first step in the two-stage softening process). When extreme temperatures (40 to 80 °C) were used as a dry pre-treatment for time periods between 30 minutes and 12 hours, followed by a 7 day germination test at 20 °C, dormancy loss was observed, except for the 40 °C treatment. A maximum 35% germination was obtained for the 3 hour 50 °C treatment.

Sidhu (1971) also examined the effect of cold temperatures on dormancy. Black medic seed was stored for 23 and 218 days at (a) 1 °C under moist and dry conditions, (b) -5 °C under dry conditions, and (c) 20 °C in a storage room, and then germinated at 20 °C in darkness for 7 days. None of the treatments significantly affected dormancy loss and the prolonged freezing at -5 °C caused a 39% drop in seed viability. In light of the study by Van Assche et al. (2003), the lack of dormancy loss was possibly due to the absence of the second fluctuating temperature phase.

In another study Sidhu (1971) compared softening of black medic seed stored in fibreglass bags on the surface of a cultivated field (July 13 to March 25) to seed held in a storage room at 20 °C after 281 days. Ranges in levels of softening under field conditions versus the storage room were 39 to 75% and 1 to 6%, respectively. The increased



softening under field conditions can be explained using the two-stage softening theory. The winter period provided the chilling treatment for pre-softening, and fluctuating temperatures in March caused the final softening. The seed in the storage room only experienced a constant temperature, and therefore no significant softening occurred. Seed viability was not affected in either treatment.

Sidhu (1971) also evaluated the effect of overwintering black medic seed at different soil depths. Seed was collected in fall, mixed with sterilized soil, and sown into a tube made of fibreglass screening, and then placed in the soil on November 26 at 0, 7.5, 15, and 30 cm depths. The tubes were excavated on March 31, emptied onto a flat, and set 5 cm deep in the ground under field conditions. Percent germination of seed exhumed from 0, 7.5, 15, and 30 cm was 11, 19, 32, and 26%, respectively, indicating that dormancy loss was greatest at 15 cm.

The results from this overwintering experiment by Sidhu (1971) also support the two-stage softening theory. The over winter exposure satisfied the chilling requirement for the first stage. The seeds were then moved to the soil surface in spring where they were exposed to low fluctuating temperatures and softened. In addition to validating the two-stage softening theory, this experiment also tested for the effect of variable first stage softening conditions on dormancy loss. The differences in softening are likely related to temperature responses at the different depths during the overwintering chilling period. The low cumulative percentage at the 0 cm depth was possibly caused by low seed viability from the freezing temperatures. Another explanation may be that varying moisture conditions at the different depths may have interacted with temperature to cause varied dormancy losses. The results from Sidhu (1971) and Van Assche et al. (2003)

both support the idea that a two-stage softening process leads to the successful recruitment of black medic seedlings in temperate regions.

Pavone and Reader (1982) also found that winter temperatures were critical for black medic dormancy loss. They studied the dynamics of dormancy loss of black medic from the natural seed bank and introduced seeds in an abandoned pasture hollow in southern Ontario, Canada. The dormancy level of black medic seed in the natural seedbank and introduced seeds dropped from over 90% in mid summer to 68% and 25%, respectively, on March 31 of the following year. No reason was given for greater dormancy loss of introduced seeds. Possibly, the seed depth varied between the two seed groups resulting in different temperature regimes and subsequent softening patterns. The natural seedbank likely was distributed throughout the top 5 cm of the soil surface by insect activity and soil cracking. On the other hand, introduced seed was placed at the surface with a 1 mm soil covering. This introduced seed placed at the surface likely experienced greater temperature fluctuations within the range required for the second stage softening process and thereby reduced dormancy.

The relationship between dormancy loss and seasonal temperature variations is well supported in the scientific literature. For annual species, dormancy is either lost or reduced during the season prior to a favourable period for seedling development and growth (Benech-Arnold et al. 2000). Therefore, seed populations of summer annuals have highest dormancy levels in fall and lowest dormancy in spring after the low temperature winter period. Winter annuals undergo dormancy loss as a result of high summer temperatures to favour fall germination. Low temperatures later in the growing

season will induce dormancy, thereby ensuring that winter annual seedlings do not emerge too late in the season.

Temperature regulated seed dormancy changes are cues that not only relay information to the seed regarding time of year, but they also provide valuable information about the microsite, specifically seed position in the soil profile and the presence of vegetation at the surface. Grime et al. (1981) concluded that buried seed will experience greater temperature fluctuations if located near the surface and positioned in a vegetation gap. The dormancy loss mechanism is useful to synchronize germination with the time period to maximize seedling survival.

Although temperature drives dormancy loss, it is important to also recognize the role of moisture in dormancy loss (Benech-Arnold et al. 2000). Sidhu (1971) examined the effect of moisture on black medic dormancy immediately after the separation of the seed from the parent plant. Seeds of different populations were sown in soil on July 30 under soaked and unsoaked conditions and exhumed at various intervals up to 260 days after sowing. The trend observed was greater dormancy loss of unsoaked seeds after overwintering. Sidhu (1971) suggested that soaking the seeds affected the physiological state of the seeds which prevented dormancy loss over the winter period. This experiment by Sidhu (1971) is the only black medic experiment in which the effect of conditions during the chilling period on dormancy loss is examined. Studies by Van Assche et al. (2003) and Zeng (2001) recognize the importance of moisture during the second stage of the seed softening process (fluctuating temperatures), but not during the chilling period. These findings are logical because dormancy loss under moist conditions

could be a positive adaptation for germination. Further research is required to determine the effect of moisture on dormancy loss during the chilling stage.

The two-stage softening process of medicis in Australia (Mediterranean climate) and Canada (temperate climate) appears to be similar. The first stage preconditioning process requires extreme temperatures in both regions. In Australia, high temperatures are required and in Canada, low temperatures are required. The second stage in both regions requires a fluctuating temperature under moist conditions. The similarity in the softening process is surprising given the contrast between the two climates. The possibility of such similar softening processes from an evolutionary perspective is unexpected unless the two temperature extremes are having the exact same preconditioning effect. An interesting question coming out of this discussion is whether black medic seed would respond similarly to both high and low temperatures (first stage), followed by fluctuating temperatures under moist conditions. If this were the case, then the similarity in the softening process between the two climates would be well supported.

#### **2.4.3.2 Physiological**

Physiological seed dormancy caused by a light event or exposure to specific wavelengths of light will influence seed dormancy (Bewley and Black 1985; Taylorson 1987). The mechanism within seeds mediating this response involves phytochrome which acts as a receptor for red and far-red light. Seed germination is promoted by red light and inhibited by far-red light (Taylorson 1987; Dyer 1995). For some species a flash of light, such as would happen during tillage, is sufficient to break the dormancy and allow germination to progress (Wesson and Wareing 1969). The light requirement is

also viewed as an adaptation for small seeded species that would not germinate if they were located more than a few centimetres below the soil surface (Buhler et al. 1997). The ratio of far-red light to red light also influences dormancy loss. A higher ratio is common under the crop canopy which in turn inhibits germination. It is important to note that light requirements change over time and may interact with moisture and temperature (Buhler et al. 1997). In black medic, light effects were linked to physiological dormancy. Using a range of germination temperatures, Sidhu (1971) found that black medic germination was inhibited by light at low and high temperatures. This light sensitivity complements the germination sensitivity at low and high temperatures when usually there is a risk of freezing and desiccation from elevated temperatures.

Soil nitrate concentrations also affect seed dormancy, although the mechanisms involved are largely unknown (Benech-Arnold et al. 2000). In general, higher N concentrations in the microsite stimulate dormancy loss (Fawcett and Slife 1978; Bischoff and Mahn 2000). Since higher soil nitrate concentrations are often associated with higher soil temperatures and mineralization rates, N response could act as a seasonal emergence signal (Benech-Arnold et al. 2000) or a gap detection mechanism (Pons 1989). No studies are available on the effect of nitrate concentrations on black medic dormancy loss. However, field observations of black medic as a self-regenerating cover crop in North Dakota indicate that black medic seed positioned within 4 cm of an ammonium nitrate fertilizer band reduced black medic density by greater than 50%. It was not clear if the high nitrate concentration affected dormancy loss, germination, or both.

#### 2.4.4 Germination

The significance of germination was described in detail by Angevine and Chabot (1979) who personified a seed, suggesting that when germination occurs, the individual has, in a sense, 'bet its life' on the probability of favourable environmental conditions for seedling establishment. In the case of hard-seeded legumes, this occurs only after physical dormancy is broken. At times, the conditions required for dormancy loss are identical to the seedling requirement for germination. In these situations, distinguishing between the two processes is difficult. However, once the germination process begins, there is no turning back.

A number of factors control rate and timing of germination. First, the chemical environment must be adequate with proper amounts of water, oxygen, and the absence of inhibitory chemicals (Bewley and Black 1985). Secondly, the physical environment must satisfy the germination requirements of the seed and often include adequate temperature and light. When these conditions are met, germination can proceed.

Germination is the interaction of various factors causing changes within the seed, culminating with the protrusion of the radicle from within the seed (Forcella et al. 2000). Where dormancy is overcome and germination begins is not easily distinguished. The commonly accepted sequence of events during germination is (a) imbibition of water by the seeds, (b) activation of hormones and enzymes, and (c) embryo growth and development (Hartmann and Hartmann 1988). Germination has been studied extensively in controlled environments, but information on germination in natural environments is limited (Forcella et al. 2000). The primary factors that influence seed germination are temperature, moisture, and air quality (Blackshaw et al. 1981; Forcella et al. 2000).

Germination rates vary within seed populations, according to the interaction between the environment and unique genetic makeup of seeds (Forcella et al. 2000).

Temperature is an important factor in seed germination, affecting both the rate and quantity of germination. The use of growing degree days (GDD), a measure of soil thermal time, has commonly been used to predict germination of weed species. The temperature range at which germination occurs varies between species and is influenced by dormancy intensity. For green foxtail, the optimum temperature range for germination is between 15 and 35 °C with maximum germination at 35 °C (Vanden Born 1971). Wild oat germination is limited below 15 °C and above 25 °C (Sharma and Vanden Born 1978). Alfalfa seed germination is optimum at 19 to 25 °C, although germination is observed between 2 and 40 °C (McElgunn 1973). In addition, the rate of alfalfa seed germination depends on temperature, but the final germination percentage after 7 days is not influenced significantly over the 5 to 35 °C range (Townsend and McGinnies 1972). Sidhu (1971) examined the germination response of scarified black medic seed at constant temperatures ranging from 1 to 35 °C. The percent germination for the 35 °C treatment was significantly lower compared to the other treatments, indicating the inhibitory effect of high temperature. At temperatures ranging from 5 to 30 °C there was no significant difference in percent germination after the 7 day test. However, at 5 °C, the germination was slow. Only after 4 days did these seeds begin to germinate.

Moisture plays an important role in seed germination. Typically, as moisture decreases, so does seed germination (Weaver et al. 1988). Alfalfa seed germination was reduced at 15 atmospheres of osmotic pressure, the theoretical permanent wilting point

(Uhvits 1946). Germination response to moisture levels varies among plant species (Hoveland and Buchanan 1973). Black medic germination response to varying moisture levels has not been studied.

Germination is strongly influenced by temperature and moisture interactions at the microsite (Forcella et al. 2000). A problem with many recruitment models is that they fail to account for the interactions between temperature and moisture. Germination is reduced, if not halted, when the moisture tension in the microsite drops below the water absorption threshold of a particular species, regardless of temperature (McGinnies 1960). To account for this, some researchers have used hydrothermal time to describe the dynamics at the microsite level influencing germination (Forcella et al. 2000). In the hydrothermal time equation, both water potential and temperature are used to predict germination (Roman et al. 1999). Unfortunately, measuring precise changes in temperature and moisture at the microsite level is limited because of the variability between microsites across the landscape. The importance of the temperature by moisture interaction is critical when seeds at or near the soil surface are subjected to temperature fluctuations and cycles of hydration and dehydration on a daily basis and over the long term (Cavers 1995).

#### **2.4.5 Shoot Elongation**

In epigeous plant species, germination is followed by shoot elongation as the cotyledons are pulled to the soil surface. Like other components of recruitment, shoot elongation affects emergence timing, although to a lesser degree than other factors such



as hard seed coat breakdown or germination. Germination and shoot elongation usually happen in quick succession.

The timing of shoot elongation is strongly correlated with the germination process that results in radicle formation. Roman et al. (1999) found that shoot elongation of common lambsquarters (*Chenopodium album*) required a 3 °C higher base temperature than radicle formation. As a result, shoot elongation would closely follow germination. The usefulness of this timing mechanism is most apparent in early spring when soil temperatures are gradually increasing and environmental conditions are not yet ideal for seedling emergence. By delaying shoot elongation, emergence can be timed to better coincide with appropriate environmental conditions. The effect of this timing mechanism is not apparent when soil temperature exceeds the minimum temperature for both germination and shoot elongation.

The rate of shoot elongation is a thermal dependent process. Roman et al. (1999) developed a quadratic model to describe incremental increases in hypocotyl length on an hourly basis as a function of temperature. Moisture content, on the other hand, did not significantly affect shoot elongation. Therefore, prediction of shoot elongation rate in field studies depends on temperature data. Moisture becomes important in shoot elongation in soils with higher clay contents that are prone to crusting after a rainfall. The ability of the seedling to push through the surface crust will depend on the depth and strength of the crust as well as upward force and seed reserves of the germinated seed.

Shoot elongation duration also depends on the position of the seed in the soil profile (soil depth). Vertical position in the soil profile is especially important for small seeded species like black medic since they lack energy reserves to reach the soil surface.

## 2.5 The Impact of Management on Seedling Recruitment

Crop type, tillage system, and nitrogen fertilizer are management practices that indirectly alter moisture and temperature conditions in the seed microsite by changing soil characteristics around the seed or by physically moving the seed to a different microsite. As shown in the previous section, moisture and temperature are important components in the recruitment process. Therefore, knowledge of management effects on moisture and temperature is necessary to understanding black medic recruitment ecology.

### 2.5.1 Crop Type

Crop type affects seedling recruitment by altering the amount of light penetrating into the canopy. For example, an oat (*Avena sativa* L.) crop intercepts more light than a flax (*Linum usitatissimum* L.) crop due to a greater leaf area index. Lower light interception by a flax crop increases soil temperature and widens the diurnal temperature fluctuation (Chancellor 1985). As a result, seedling recruitment is affected because of environmental changes at the seed microsite caused by light penetration differences (Dyer 1995). These changes influence the temperature at the microsite level and thereby affect dormancy loss, germination, and shoot elongation. Dormancy loss in black medic results from temperature fluctuations in the spring period as shown by Van Assche (2003). Increased interception by the oats versus the flax will reduce the range of the temperature fluctuations. As a result, less spring recruitment may be observed in oats as compared to flax. Soil temperature differences between the crops also affects the rate of germination and shoot elongation. Therefore, black medic recruited under flax may emerge sooner than under oats. Another important consideration is the black medic

sensitivity to light during germination (Sidhu 1971). Therefore, under a flax crop, black medic germination may be inhibited by light exposure during low and high temperatures.

Moisture use differences between crops changes the soil volumetric water content (VWC) and subsequently the conditions required for recruitment. As discussed in the previous section, moisture plays a key role in dormancy loss and germination. Since oats requires more moisture than flax, recruitment under oats may be reduced because of lower soil VWC.

Another important factor influencing seedling recruitment is varied herbicide selection between crops. A comparison of crops in a Manitoba weed survey attributed differences in weed populations between crops to herbicide selection (Van Acker et al. 2000). Weed density was the highest in oats since herbicides were rarely used. Relatively low weed densities in canola and flax fields were linked to intensive herbicide use. Plant species also varied with respect to herbicide tolerance. Black medic recruitment has been shown to be influenced by herbicide residue which vary among crops (Wilson et al. 1993). In addition, black medic is relatively tolerant to low rates of glyphosate and MCPA amine, especially beyond the 3 leaf stage, and was sensitive to bromoxynil (Entz and Bamford 2003). Therefore, varied levels of black medic recruitment are expected among crops since herbicide use patterns differ among crops.

The sequence of crops (rotation) may also affect seedling recruitment. Many studies suggest that long-term crop rotation characteristics are important factors influencing weed recruitment (Dyer 1995; Leeson et al. 2000; Derksen et al. 2002). Seed abundance and composition data collected over 30 years under tillage and rotation treatments in Ohio was influenced by the long-term effect of rotation rather than the

effect of the most recent crop (Cardina et al. 1998). A ten year crop rotation trial at the Glenlea Research Station in Manitoba also attributed weed community dynamics to crop rotation effects (Entz 2002). Therefore, crop type and crop rotation will have long-term effects on seedling recruitment for many species, including black medic.

Seedling recruitment under an existing cover crop is also affected by the microclimate conditions resulting from the cover crop. For example, a red clover crop grown in relay with winter wheat moderates late summer and fall surface air temperatures and reduces soil moisture availability (Thiessen - Martens et al. 2001). Similarly, a self-regenerating cover crop of black medic may affect the microclimate surrounding the black medic seeds in the soil seedbank. The main factors would be increased light interception and soil moisture use resulting in reduced maximum soil temperatures, lower average soil temperature, and lower soil volumetric water content (VWC). Thus, recruitment of the self-regeneration cover crop would be affected.

### **2.5.2 Effect of Tillage System on the Seed Microsite**

Tillage alters (a) the vertical distribution of seed, (b) soil surface residue properties, (c) soil structure, and (d) organic matter content of the soil. These changes modify temperature, moisture, and light conditions at the seed microsite. Any shift in environmental conditions immediately surrounding the seed affects the components of recruitment.

### 2.5.2.1 Vertical Redistribution of Seeds

The most obvious effect of tillage is the redistribution of seeds within the soil profile. Burial depth is a function of tillage type (Cousens and Moss 1990; Yenish et al. 1992), depth of operation (Chepil 1946a), and intensity (Cousens and Moss 1990). Zero tillage (ZT) systems tend to leave the majority of seeds at the surface or in the top 1 cm of the soil profile (Froud-Williams et al. 1981; Yenish et al. 1992; Dyer 1995).

Moldboard plowing preferentially distributes seed deep within the soil profile, and reduced tillage and chisel plowing distributes the majority of the seeds at an intermediate depth (Dyer 1995).

The movement of seed by tillage to a different vertical position within the soil profile will affect the average temperature and magnitude of the temperature fluctuation at the seed microsite. For example, the average soil surface temperature under zero tillage (ZT) was greater than the average soil temperature at a 2.5 cm depth under conventional tillage (CT) (Malhi and O' Sullivan 1990). According to Table 2.1, minimum soil temperatures during the winter at various depths were lower under CT as compared to ZT. As well, soil temperature fluctuations deeper in the soil profile are muted, regardless of tillage system. Similar findings were observed by Reid (2003). Therefore, tillage systems that favour seed placement near the surface of the soil will increase the average temperatures at the seed microsite during the growing season and increase the magnitude of temperature fluctuations throughout the year. Related research in Australia noted the impact of soil tillage on soil temperature at the microsite. Taylor and Ewing (1996) studied the effects of extended (4-12 years) burial on seed softening in subterranean clover and annual medics. Significant differences in response to seed burial

between species were explained in terms of the effects of varied responses to different soil temperatures at different depths. Vertical seed movement as a result of tillage will also affect the moisture conditions around the seed. Differences in soil moisture within the profile are the result of evaporation at the soil surface by solar radiation, rainfall events, and the extraction of soil moisture by plant roots. Occasionally, higher moisture levels are observed at the soil surface than at depth (Malhi and O' Sullivan 1990). However, soil moisture is typically greater at deeper depths (Fu et al. 2003). In general, the magnitude of moisture fluctuations decreases with depth as a result of wetting and drying cycles caused by rainfall events and solar radiation (Fu et al. 2003).

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**Table 2.1: Minimum temperatures recorded during the winter at 0, 1, 2.5, 5, 10, and 20 cm under CT and ZT in southern Manitoba (Adapted from Gauer (1980)).**

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Depth	CT	ZT	Difference
0	-21.1	-12.8	8.3
1	-20.2	-10.2	10.0
2.5	-19.2	-9.8	9.4
5	-17.8	-8.5	9.3
10	-16.1	-7.2	8.9
20	-13.8	-7.0	6.8

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Lastly, seed movement within the vertical profile by tillage will affect the seed exposure levels to light. As soil is moved by a tillage implement, light penetrates into the soil. This phenomena has been extensively documented by many researchers who linked light exposure to dormancy loss (Wesson and Wareing 1969; Scopel et al. 1994).

### 2.5.2.2 Surface Residue

Tillage systems affect the amount of surface residue left on the soil surface. As tillage intensity decreases, percent groundcover increases (Potter et al. 1985; Guy and Cox 2002). Increased groundcover affects the exchange of energy at the soil surface during the day by increasing the surface albedo (Bullock and Lafond 2002). Higher albedos caused by increased surface residue result in increased reflection of incoming short wave radiation. For example, dry plant material will reflect 2-5 times as much of the incoming radiation as a bare black soil (Shaykewich 1980). Therefore, increased residue levels lower soil temperature since less of the radiation is absorbed to heat the soil. As a result, ZT systems generally have lower soil temperatures than CT systems early in the growing season, before the ground is shaded by the crop (Potter et al. 1985; Arshad and Azooz 1996).

In addition to affecting mean soil temperatures, surface residues also affect the magnitude of temperature fluctuations. Surface residues usually reduce the magnitude of temperature fluctuations, caused primarily by lower daily maximum temperatures under zero till systems during the growing season (Grant et al. 1990). Minimum temperatures are quite similar between ZT and CT systems during the crop growth period. During the winter period, soil temperatures remained higher and fluctuated less under ZT compared to CT (Table 2.1).

Groundcover differences between tillage systems also affect soil moisture conditions. The primary processes involved are evaporation, infiltration, and surface runoff (Blevins and Frye 1993). According to Borstlap and Entz (1994), seasonal evapotranspiration was either reduced or unaffected by ZT. Another study found no

difference between soil moisture between zero, reduced, and CT systems at the 0-10 cm depth (Arshad and Azooz 1996). However, there was a tendency towards increased soil moisture levels by the zero and reduced till systems compared to the conventional till system. Similar results were obtained by Potter et al. (1985) who observed no significant difference in soil VWC at the 2.5 to 15 cm depth for CT, chisel plow, and ZT systems with an average residue cover of 7, 37, and 66% respectively. Brandt (1992) compared ZT with CT over three rotation phases and 12 years for a total of 36 comparisons. ZT increased spring soil moisture in 9 comparisons and the remainder showed no difference. In addition to reducing evaporation, surface residue improved water infiltration. As a result of improved infiltration rates, surface run-off will be reduced which also will increase soil moisture content.

### **2.5.2.3 Soil Structure**

Soil structure refers to the size and distribution of soil aggregates. Tillage disturbs soil and therefore alters the soil structure, changing the physical properties of the soil which impact moisture and thermal properties. In turn, conditions at the seed microsite are affected.

Bulk density is used to measure the impact of tillage on soil structure. Boyd and Van Acker (2003) linked higher bulk density resulting from compaction with a roller to better soil-seed contact and improved soil water uptake. Studies comparing the effects of ZT and CT systems indicated that bulk density was (a) unaffected (Azooz et al. 1996; Arshad et al. 1999), (b) greater in ZT than CT (Wu et al. 1992), and (c) lower in ZT than CT (Voorhees and Lindstrom 1984). This disagreement suggests that bulk density is not



especially useful in comparing the effect of tillage systems on microsite conditions.

However, bulk density is still a useful measure for describing changes at the microsite.

Tillage systems change soil structure by altering pore size distribution. Azooz et al. (1996) observed a shift to greater numbers of micropores (<0.75  $\mu\text{m}$ ) and fewer macropores (>15 $\mu\text{m}$ ) under ZT than under CT. Blevins and Frye (1993) observed similar effects. Micropores retain more plant-available water than the macropores which tend to drain rapidly after rainfall (Hill et al. 1985). Arshad et al. (1999) attributed higher water retention of unsaturated soil under ZT to the greater volume fraction of micropores under ZT than under CT. Furthermore, water infiltration rate is influenced by pore size distribution. Improved pore continuity and enhanced earthworm activity under zero till systems contributed to greater saturated hydraulic conductivity (Blevins et al. 1983). Arshad et al. (1999) also attributed higher water infiltration rates under ZT compared to CT to soil structural improvements associated with improved aggregate size distribution and stability. In addition to soil moisture, thermal conductivity may also be affected by soil structure. Potter (1985) observed similar soil volumetric heat capacities between tillage treatments. However, the thermal diffusivity and conductivity were greater in ZT versus CT. Yet, Potter (1985) concludes that percent surface residue cover had a greater effect on soil temperature and soil heat flux than soil thermal properties.

#### **2.5.2.4 Organic Matter**

Tillage systems alter the distribution and stratification of organic matter in the soil profile. Numerous studies have shown an increase in soil organic matter under ZT compared to CT (Franzluebbers and Arshad 1997; Hernanz et al. 2002). This shift was

caused by accelerated decomposition rates of organic matter as tillage intensity increased (Hernanz et al. 2002). Soil organic carbon under ZT tends to accumulate in the surface soil layer. Since this region is also the recruitment zone for seeds, understanding the effect of organic matter on the seed microsite is important. The most important effect of increased soil organic matter in this regard may be increased water holding capacity.

#### **2.5.2.5 Temperature by Moisture Interactions**

Tillage alters the temperature and moisture conditions in the seed microsite by affecting the temperature of the soil. Incoming short wave radiation is absorbed and conducted at different rates through the soil depending on the moisture content of the soil which influences the thermal diffusivity of soil. Thermal diffusivity is directly proportional to thermal conductivity and inversely proportional to volumetric heat capacity. Dry soil has very low thermal conductivity. Therefore, incoming short wave radiation will cause the surface of dry soil to increase sharply, but the heat will not be conducted through the soil. Increasing soil moisture will increase soil thermal conductivity and allow the heat from short wave radiation to be conducted deeper into the soil. Thermal conductivity is the rate at which soil conducts thermal energy and is maximized at soil VWC between 8 and 20% (Azooz et al. 1996). Therefore, up to this soil VWC, a soil warms more quickly with additional water. As soil moisture is increased further, the thermal conductivity drops. The reason for declining thermal conductivity is increased soil volumetric heat capacity. The volumetric heat capacity of soil increases with more soil water. More energy is required to heat soil as soil VWC increases. When the effects of increased thermal conductivity with additional soil water

are offset by increased soil volumetric heat capacity, the thermal diffusivity of soil is reduced and therefore soil warms more slowly. A practical example of this interaction is found in tillage studies. For example, Arshad and Azooz (1996) observed that ZT had higher heat storage capacity than CT, resulting from higher soil water content. This higher heat capacity under ZT reduced thermal diffusivity of ZT during the growing season. As a result, the temperature was lower in the ZT system. Another implication of this principle relates to the diffusion of heat energy in soils and effect on temperature fluctuations. Low diffusivities will result in large temperature fluctuations within a relatively thin layer of soil since the heat diffuses more slowly. High diffusivities will mute temperature fluctuations since the rate of heat diffusion is greater.

### **2.5.3 Nitrogen Fertilizer Application**

Nitrogen fertilizer application can affect soil conditions at the seed microsite. Fertilizer N application rates vary, depending on the previous crop, current crop, moisture conditions, and mineralization potential of the soil. In Manitoba, N fertilizer recommendations for continuously cropped oats and flax following stubble vary between 55 to 90 kg N ha<sup>-1</sup> and 40 to 60 kg N ha<sup>-1</sup> for oats and flax, respectively (Manitoba Agriculture 2003). The N fertilizer may be banded or broadcast in fall or spring. After N fertilizer is applied, N enters the soil solution as either ammonium (NH<sub>4</sub><sup>+</sup>) or nitrate (NO<sub>3</sub><sup>-</sup>). The result is an increased N concentration in the fertilizer band area. Nitrogen fertilizer also affects crop growth which can further influence environmental conditions at the seed microsite. Higher crop growth rates will reduce the photosynthetic active

radiation at the soil surface (Bischoff and Mahn 2000), reduce available moisture, and increase uptake of other (non N) soil nutrients.

Few studies have considered the effects of fertilizer management on black medic recruitment. Cavan et al. (2000) used long-term plots at Rothamstead to study the genetic diversity of black medic growing in continuous wheat under different nitrogen fertility regimes since 1843. At N fertilizer rates of 0, 48, 96, 144, and 240 kg N ha<sup>-1</sup> rates, the corresponding black medic scores were 22, 20, 8, 1, and 0 (each score represents the number of 0.1 m<sup>2</sup> quadrats out of a possible 25 per plot where the black medic was present), respectively. Therefore, black medic colonization was favoured by low nitrogen levels which affected plant population. Cavan (2000) also investigated the effect of N rate on black medic genetic diversity level, however, no differences were observed.

## **2.6 Summary and Objectives**

Cover crops improve the sustainability of cropping systems on the Canadian prairies. Their use is limited because of high reseeding costs. The use of self-regenerating cover crops can eliminate reseeding in order that the practice of cover cropping is more feasible. A candidate for a self-regenerating cover crop is black medic. Black medic is an annual legume which self-regenerates from the soil seed bank. Unfortunately, the process of black medic self-regeneration has not been studied within a continuous grain cropping system on the Canadian prairies. The success of black medic as a self-regenerating legume cover crop grown in association with continuous grain cropping systems will depend on a basic understanding of black medic recruitment ecology within the cropping system. Black medic recruitment is the culmination of seed

formation, dormancy development, dormancy loss, germination, and shoot elongation. Each of these processes operate on different time scales and often require different environmental conditions. Generally, however, temperature and moisture are the most important environmental conditions affecting black medic recruitment. Management practices indirectly affect these temperature and moisture conditions at the microsite and therefore will determine to a large extent recruitment success at the microsite level.

Laboratory and field experiments were designed to explore the recruitment ecology of black medic in an annual cropping system. The following general objective and four research questions provided foci and rationale for the experiments.

**General Objective:** To understand the recruitment ecology of black medic in a continuous grain cropping system.

**Research Question #1:** What is the effect of temperature on the germination of scarified black medic seed?

**Research Question #2:** What is the effect of temperature and moisture on dormancy loss of black medic seed?

**Research Question #3:** What is the effect of crop type, tillage system, and N fertilizer rate on black medic recruitment under field conditions?

**Research Question #4:** Is there a link between the two-stage softening process between medics grown in Australia (Mediterranean climate) and Canada (temperate climate)?

### 3. MATERIALS AND METHODS

#### 3.1 Black Medic Germination and Dormancy Characteristics

##### 3.1.1 Experiment #1: Temperature Effect on the Germination of Scarified Seed

This study was designed to determine the effect of temperature on the germination of scarified black medic seed. The seed was produced in Montana, scarified and packaged by Timeless Seeds in Montana and shipped to the University of Manitoba in April, 2000 where it was stored at room temperature. Prior to the experiment, brown shrivelled seed was removed from the seed lot by manually separating the brown seed with a ruler. The experiment was a completely randomized single factorial design with repeated measures.

Fifty seeds were evenly placed into four sets of six petri dishes lined with two layers of Whatman #1 filter paper. Each petri dish was then moistened with 4.5 mL of distilled water so that approximately 0.5 mL of free water was visible when the plates were tipped to one side. The four sets of six petri dishes were each placed into a plastic bag to reduce evaporation and then into aluminum foil baking plates covered with two layers of aluminum foil. Aluminum was chosen for its high thermal conductivity and light reflectivity. The concern for maintaining the seeds in the absence of light was based on work by Sidhu (1971) where light influenced physiological dormancy which inhibited the germination of black medic at temperature extremes. Prior to the experiment, light seepage into the covered tinfoil plates was measured using a model LI-1000 Quantum Sensor (Licor, Lincoln, NB). The sensor was placed inside the tinfoil plates and the plates were exposed to a light intensity of  $1400 \text{ } \mu\text{molm}^{-2}\text{s}^{-1}$  (full sunlight). The light

seepage into the plates was less than  $0.00417 \text{ umolm}^{-2}\text{s}^{-1}$ . This light level had no effect on cleavers (*Galium spurium*) germination, and cleavers is known to be a light sensitive species with respect to germination (Malik and Vanden Born 1987).

The four sets of tinfoil plates each containing 6 petri dishes were divided into four germination cabinets set at the following temperature regimes: a) constant 10 °C, b) constant 20 °C, c) constant 30 °C, and d) diurnal fluctuating 5/40 °C. For the constant temperature regimes, Controlled Environment Ltd. Model EF7H germination cabinets were used. An Econoair Model TC-19 germination cabinet was used for the diurnal fluctuating 5/40 °C treatment. The 5/40 °C temperature treatment was applied to provide a 12 hour diurnal shift between the two temperatures. Appendix A shows the temperature curve for the 5/40 °C temperature treatment. Prior to the experiment, Stow Away Tidbits (Onset Computer Corporation, Bourne, MA) were used to measure if there was a temperature difference between the interior of the incubator versus the inside of the tinfoil plates. The temperature readings were identical.

For the first week of the experiment, germination counts were made every day, and every week thereafter up to the end of week 8. Germination counts took place in a dark room using a green safe light (filter wavelength 500-570, 40 watt incandescent bulb) which is not considered inhibitory to germination for cleavers (*Galium* species), a light sensitive species (Malik and Vanden Born, 1987)(Reid 2003). Seed with a radicle greater than or equal to 2 mm was considered germinated and removed from petri dishes. Distilled water was added to petri dishes approximately every 1 to 2 weeks to maintain 0.5 ml of free water when set on the side.

The data from these experiments analyzed using SAS. Data were tested for homogeneity of variance using Bartlett's test. Analysis of variance was then performed using Proc GLM. A significance level of 0.05 was used.

### **3.1.2 Experiment #2: Temperature and Moisture Effects on Dormancy and Germination**

A factorial experiment was designed to examine the effect of temperature, moisture, time and seed lot on the dormancy and germination of black medic. Three temperature regimes were selected for the first factor: 20, 10/40, and 5/40 °C. The 5/40 and 10/40 °C temperature treatments were chosen to simulate temperature fluctuations a black medic seed positioned on the surface of the soil would experience during the spring in southern Manitoba. Stout et al. (1999) selected a temperature fluctuation of 5/35 °C to simulate spring temperature fluctuations for alfalfa seed placed at 1 cm depth in southern British Columbia. Southern Manitoba soil temperature data from May 17 to May 20 2002, under CT and a recently established ZT system, showed fluctuations of approximately 5/30 °C at a depth of 1.5 cm (Appendix B). Seed at the surface would undergo a larger fluctuation. For the purposes of this experiment, 5/40 and 10/40 °C treatments were used to simulate soil temperatures at the soil surface.

The second experimental factor was time. Two time periods were used: one week and eight weeks.

The third factor in the experiment was moisture. Fifty black medic seeds were placed on two layers of either moistened or dry Whatman #1 filter paper placed in petri dishes. The moistened petri dishes received 4.5 mL of distilled water so that



approximately 0.5 mL of free water was visible when the plates were tipped to one side. Germination counts were conducted weekly for 8 weeks. The dry treatments were in the incubator for either 1 week or 8 weeks, after which time they were removed, moistened as per above, and placed in a 20 °C incubator for an additional week.

The fourth factor in the experiment was seed lot. The seed lots were produced in 2002 in Goodrich, North Dakota (ND1) and Winnipeg, Manitoba (P02-W) under flax and oat crops, respectively. The ND1 lot of black medic seed was harvested with the flax on August 20, 2002 and stored in a grain bin until February, 2003 when it was separated and placed in a paper sack in a non-heated storage shed in Goodrich, North Dakota. The seed was transported to Winnipeg in April, 2003 and stored at room temperature. The P02-W lot was separated from harvested oats on October 30, 2002 and immediately placed in nylon mesh bags pinned onto the soil surface over flax stubble in Winnipeg for the winter. Hobo temperature loggers (Onset Corporation) were placed under the bags to record daily maximum and minimum temperatures. On April 6, 2003, the P02-W lot was removed from the field and stored at room temperature. Both lots were de-hulled using a belt roller and cleaned using a forage screen and air column in April, 2003.

For each factorial combination four petri dishes were prepared. The petri dishes were placed into plastic bags and then into aluminum foil baking plates covered with two layers of aluminum foil. Germination counts were done weekly for 8 weeks. As in the previous experiment, counts were made in a dark room using a green safe light and seed with a radicle greater than or equal to 2 mm was considered germinated and removed from each petri dish. Distilled water was added to the moist treatment petri dish every 1 to 2 weeks to maintain 0.5 ml of free water when the petri dishes were set on their side.

After each time period, the petri dishes were placed for 1 week at 20 °C (standard germination test) in an incubator and germination was recorded. Dry treatments were moistened prior to this standard germination test. Total germination was the sum of percent black medic germination during the allotted time and the germination percentage after the 1 week germination test.

Data was analyzed using SAS (1990). The data were tested for homogeneity of variance using Bartlett's test. Analysis of variance was then performed on the data using Proc GLM. There were two runs of this experiment. Since there was no significant difference between the runs, data were pooled. Means separation was carried out using a protected least significance test at a 5% level of significance.

## **3.2 Black Medic Field Recruitment Studies**

### **3.2.1 Carman 2002 and 2003**

#### **3.2.1.1 Site Description**

Black medic field recruitment experiments were conducted in 2002 and 2003 at the University of Manitoba Research Station at Carman, Manitoba. The soil type was a Hochfeld series with a very fine sandy loam texture.

The trial was initiated in 2001. Flax was seeded on May 28, 2001 at a rate of 45 kg ha<sup>-1</sup> and under-seeded to black medic (cv: 'George') at a rate of 15 kg ha<sup>-1</sup> from scarified seed obtained from Timeless Seeds in Montana in 2000. Since no herbicides were used for weed control, a patchy infestation of red root pigweed affected the uniformity of black medic seed production. On August 8, 2001, the flax was cut at a

height of 30 cm and removed from the field, leaving black medic pods to drop onto the ground as seed rain.

The experimental design was a randomized complete block design in a split-split plot arrangement imposed on the field site in September 2001. The main plot effect was crop species type, subplot effect was tillage system, and the sub subplot effect was nitrogen fertilizer amendment. Four replicates were used. See Appendix C for plot plan and measurements.

### **3.2.1.2 Management Effects**

#### **3.2.1.2.1 Crop Type**

The purpose of the main plot effect, crop type, was to determine the crop effect on black medic recruitment. Flax, oat, and winter wheat were the three crops used in this experiment as main plot effects in 2002 and 2003. Due to time constraints, data was gathered only from the flax and oat main plot treatments. In 2002, oat and flax were seeded on May 14 at rates of  $120 \text{ kg ha}^{-1}$  and  $45 \text{ kg ha}^{-1}$ , respectively. The oat variety was AC Assiniboia and oats were seeded at a depth of 3 cm. The flax variety was Bethune seeded at a depth of 2 cm. Phosphorous ( $\text{P}_2\text{O}_5$ ), potassium (KCl), and sulphur ( $\text{SO}_4$ ) were broadcasted on all treatments at rate of  $34 \text{ P}_2\text{O}_5$ ,  $40 \text{ KCl}$ , and  $16 \text{ SO}_4 \text{ kg ha}^{-1}$ . Additional phosphorous was drilled with the seed at a rate of  $24 \text{ kg ha}^{-1} \text{ P}_2\text{O}_5$ . Flax and oats were swathed on September 2 and harvested on September 12 and September 3, respectively. All harvested grain was air dried for 4 days and weighed to determine yield.

In 2003, oat and flax were seeded on May 5 and May 13 at rates of  $120 \text{ kg ha}^{-1}$  and  $45 \text{ kg ha}^{-1}$ , respectively. Varieties and non-N soil nutrient application rates and

methods were the same as in 2002. In the fall of 2003, the oat crop was straight combined on September 2, while the flax crop was first swathed on August 21 and combined on September 3. Both crops were dried for 4 days and weighed to determine yield.

#### **3.2.1.2.2 Tillage Regime**

The purpose of the subplot treatment (tillage regime) was to determine the effect of tillage on black medic recruitment. Two tillage treatments, ZT and CT, were applied. For the 2002 CT treatment, the first tillage pass was on October 9, 2001 with a deep till cultivator to a depth of approximately 10 cm. On May 14, 2002, one pass with a cultivator harrow packer was made for seedbed preparation and weed control. On October 2 and October 7, 2002, two cultivations were made using a deep till cultivator to a depth of approximately 10 cm. In 2003, the CT operation included one pass with a cultivator harrow packer on May 5 and May 13 for the oat and flax crops, respectively.

For the ZT treatment, no tillage was used. Weed control during seeding was done with herbicides (see section 3.2.1.3).

#### **3.2.1.2.3 Nitrogen Fertilizer Rate**

The purpose of the sub subplot treatment was to determine the effect of N fertilizer rate on black medic recruitment. The target N treatment levels were 30 and 70% of field test recommendations to compare a conventional and low-input N cropping system.

In 2002, actual N fertilizer application rates of 26 kg ha<sup>-1</sup> and 52 kg ha<sup>-1</sup> were applied as the low and high application rate treatments, respectively. They represented 37% and 73% of the recommended N application rate. This application was based on soil samples of the top 20 cm of soil taken in October, 2001. Four samples were taken from each block and combined immediately after collection and a sub sample was removed and sent to Norwest Labs (Winnipeg, MB) for determination of nitrogen, phosphorous, potassium, and sulphur requirements to achieve average yields based on the fertilizer recommendation.

In 2003, actual N fertilizer rates of 25 kg ha<sup>-1</sup> and 72 kg ha<sup>-1</sup> were applied as the low and high application treatments, respectively. For the 2003 trial, soil samples were taken in October, 2002 and also sent to Norwest Labs for analysis.

### **3.2.1.3 Herbicide Application**

Several herbicides were used to control weeds in the test plots at Carman. Herbicide regimes for the 2002 and 2003 site years are summarized in Table 3.1 and 3.2, respectively. A bicycle sprayer with a 4 m boom was used to apply the herbicides at 275 kPa with 80015 flat nozzles.

### **3.2.1.4 Air Temperature and Precipitation**

Air temperature and precipitation were measured using the Environment Canada weather station located less than 500 m from the trial site on the Carman Research Station.

**Table 3.1: Application date, herbicide type, herbicide rate, water volume, and application area of herbicides applied to Carman 2002 black medic recruitment trial.**

Application Date	Herbicide Type	Herbicide Rate	Water Volume	Application Area
Sept 7, 2001	Glyphosate †	879 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	ZT
May 17, 2002	Glyphosate †	623 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	ZT
June 8, 2002	MCPA amine + propanil	284 g a.i. ha <sup>-1</sup> 980 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	CT and ZT
June 24, 2002	Bromoxynil	280 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	CT and ZT
Oct. 7, 2002	MCPA + Glyphosate ‡	140 g a.i. ha <sup>-1</sup> 316 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	CT and ZT

† Roundup Original

‡ Roundup Transorb

**Table 3.2: Application date, herbicide, herbicide rate, water volume, and application area of herbicides applied to Carman 2003 black medic recruitment trial.**

Application Date	Herbicide	Rate	Water Vol. (l/hectare)	Application Area
May 9, 2003	Glyphosate †	879 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	Oat ZT
May 16, 2003	Glyphosate †	879 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	Flax ZT
June 5, 2003	MCPA amine + propanil	284 g a.i. ha <sup>-1</sup> 980 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	ZT and CT
Oct. 7, 2003	Glyphosate ‡ + 24-D amine	879 g a.i. ha <sup>-1</sup> 140 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	ZT

† Roundup Original

‡ Roundup Transorb

### **3.2.1.5 Measurement of Dependent Variables**

#### **3.2.1.5.1 Black Medic Recruitment**

Black medic recruitment in 2002 was measured beginning on April 28 and continued until September 10. The number of plants, both seedlings and established plants, were counted within a 0.25 m<sup>2</sup> quadrant randomly placed five times in each sub subplot. These five measurements were averaged to produce a sub subplot density value for use in statistical analysis. The frequency of measurements were weekly during the peak recruitment period in spring and every two to three weeks thereafter.

Black medic recruitment in 2003 was measured beginning on April 20 and continued until September 8. The number of plants, both seedlings and established plants, were counted within a 0.16 m<sup>2</sup> quadrant randomly placed five times in each sub subplot. These five measurements were averaged to produce a sub subplot value for use in statistical analysis. The frequency of measurements were weekly during the peak recruitment period in spring and approximately every two to three weeks thereafter.

#### **3.2.1.5.2 Soil Temperature**

In both 2002 and 2003, hourly soil temperature was recorded during the period from late to early May until the end of August. The hobo sensors were inserted into the soil at a 30° degree angle so that the sensor was 1.5 cm below the surface of the soil. The 1.5 cm depth was chosen because the majority of recruitment was assumed to occur within the top 2 cm of soil (Du Croix Sissons et al. 2000). The temperature probes were placed only in the low N treatment for all crop and tillage combinations. Special care was taken to minimize soil disturbance in the ZT site by cutting a 10 cm wide slice of soil with a flat

shovel and carving an indentation where the probe could be inserted. The soil was then placed over the probe. To assure that the depth of the probe was constant throughout the year, a fishing line was attached to the sensor end and marked with a piece of tape at 1.5 cm. Routinely the probes were checked to make sure the depth had not changed. The soil probes were removed before and replaced immediately after tillage and seeding operations.

Additional soil temperature data was recorded from September, 2001 to August, 2003 at the weather station in Carman. The soil temperature sensor for this station was located 2 cm under a permanent grass sward.

#### **3.2.1.5.3 Light Penetration**

Light penetration measurements into the crop canopy were made on all treatments from May 24 to August 22, 2002 using a 1 m Line Quantum Sensor with a LI-1000 data logger. Output units were  $\text{mol m}^{-2}$  and light penetration values were based on full light conditions above the crop canopy. Measurement frequency was approximately every two weeks.

#### **3.2.1.5.4 Soil Volumetric Moisture**

In 2002, soil volumetric moisture ( $\text{cm}^3 \text{cm}^{-3}$ ) was measured at two depths, 0-2 cm and 2-4 cm from May 7 to September 10 in flax - ZT and flax - CT treatments in the low N treatments. During peak recruitment in spring, 2-3 samples were taken each week and later only 1 sample was taken each week. Two samples per sub subplot were taken at both depths using a 8.3 cm diameter copper ring pressed into the soil and removed with a



trowel. The sample was placed directly into a heat resistant plastic container and sealed. The 2-4 cm sample was taken directly below the 0-2 cm sample. Samples were weighed and dried at 95 °C for 48 hours. Soil VWC was calculated based on soil GWC and dry soil bulk density.

$$\text{VWC} = \text{GWC} \times \text{dry soil bulk density}$$

For the 2003 site year, soil volumetric moisture at only the 0-2 cm depth was measured from May to July, approximately 1 to 3 times per week in the same treatments as in 2002. The extraction, drying, and calculation approaches for soil volumetric moisture were identical to 2002.

#### **3.2.1.5.5 Soil Moisture Measurement Using Neutron Probe**

In 2002, volumetric surface soil moisture ( $\text{cm}^3 \text{ cm}^{-3}$ ) at the 0-10 cm depth was measured using a neutron moisture gauge (Troxler model 4300 moisture gauge, Troxler Electronic Laboratories, Inc., Triangle Park, NC) with a surface shield. Bullied (1997) calibrated the gauge and developed an equation for conversion to soil VWC.

#### **3.2.1.5.6 Grain Yield**

Grain was harvested using a Massey Ferguson Kircaid XP8 small plot combine. A 2 m width was combined the length of each sub subplot, emptied into a bag, dried for 3 days, and weighed. Yield was calculated based on individual length measurements of each plot.

### **3.2.2 Winnipeg 2003**

#### **3.2.2.1 Site Description**

Field experiments were conducted at the Department of Plant Science Field Research Station at Winnipeg, MB in 2003 on a Riverdale, silty clay soil. The experimental design was a randomized complete block design with three replications using a split plot arrangement. Main plot was nitrogen fertilizer amendments and subplot was crop type.

The site was established in 2000 to examine the potential of black medic (cv. 'George') as a self-regenerating cover crop. A flax-winter wheat-oat rotation was imposed on the field trial. In 2000, black medic was broadcast at a rate of 10 kg ha<sup>-1</sup> and harrowed. In 2001, an additional 10 kg ha<sup>-1</sup> of black medic seed from Timeless Seeds (Montana) was broadcast on the plots in spring to increase the seed bank and subsequent seed production. Broadleaf herbicides were not used in 2000 and 2001 and additional black medic seed was not added in 2002 or 2003.

#### **3.2.2.2 Management Effects**

##### **3.2.2.2.1 Nitrogen Fertilizer Rate**

Nitrogen fertilizer rate was the main plot effect and the application rate was based on soil test results of soil samples taken in October, 2002. Four samples at a depth of 0-20 cm were taken from each block and combined immediately after collection and a sub sample was removed and sent to Norwest Labs (Winnipeg, MB) for determination of nitrogen, phosphorous, potassium, and sulphur requirements to achieve average yields

based on the fertilizer recommendation. The target application rate was 10 and 70% of N fertilizer recommendations. Nitrogen fertilizer was applied at seeding at a rate of 5 kg ha<sup>-1</sup> actual N on all plots and on June 5, 34 kg ha<sup>-1</sup> and 50 kg<sup>-1</sup> actual N was applied as ammonium nitrate (46-0-0) on the oat and flax plots, respectively, for the high N treatment only.

#### **3.2.2.2.2 Crop Type**

Flax, oat, and winter wheat were the three crops used in the experimental design as subplot treatments from 2000 to 2003. In this study, measurements were only taken in the flax and oat main plot treatments. In 2002, oat and flax were seeded on May 14 at rates of 120 kg ha<sup>-1</sup> and 45 kg ha<sup>-1</sup>, respectively. The oat variety was AC Assiniboia seeded at a depth of 3 cm. Flax (cv. Bethune) was seeded at a depth of 2 cm. Phosphorous was applied at a rate of 25 kg ha<sup>-1</sup> P<sub>2</sub>O<sub>5</sub> with the seed.

#### **3.2.2.3 Herbicide Application**

Herbicides were used to control weeds in the trial and the herbicide regime is summarized in Table 3.3. Spray solution at 110 L ha<sup>-1</sup> at 275 kpa using 80015 flat fan nozzles was used to apply the herbicides.

#### **3.2.2.4 Air Temperature and Precipitation**

Air temperature and precipitation were measured using a Campbell Scientific weather station located at the Department of Plant Science Field Research Center approximately 200 m from the trial site.

**Table 3.3:** Application date, herbicide type, herbicide rate, water volume, and application area of herbicides applied to the Winnipeg black medic recruitment trial.

<u>Application Date</u>	<u>Herbicide</u>	<u>Herbicide Rate</u>	<u>Water Volume</u>	<u>Application Area</u>
May 14, 2003	Glyphosate †	879 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	All crops
June 10, 2003	Propanil ‡	980 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	Oat
June 17, 2003	Sethoxydim ¥	500 g a.i. ha <sup>-1</sup>	110 L ha <sup>-1</sup>	Flax

† Roundup Original

‡ Stampede EDF

¥ Poast Ultra

### 3.2.2.5 Measurement of Dependent Variables

#### 3.2.2.5.1 Black Medic Recruitment

Black medic recruitment measurements in 2003 began on April 20 and continued until September 8. The number of plants, both seedlings and established plants, were counted within a 0.16 m<sup>2</sup> quadrant randomly placed five times in each sub subplot. These five measurements were averaged to produce a sub subplot value for statistical analysis. The measurement frequency was weekly during the peak recruitment period in spring and every two to three weeks thereafter.

#### 3.2.2.5.2 Soil Temperature

Hourly soil temperature at two depths, 0 cm and 1.5 cm, was recorded with Hobos (Onset Corporation) from April 22 to August 22. The Hobos at 0 cm depth were placed with the sensor flush with the soil surface and covered by trash. These hobos were only placed in the low N flax treatments. The 1 Hobos placed at 1.5 cm were only in the low

N flax and oat treatments. Precautions were taken in the placement and maintenance of Hobos as per the methods described for the Carman site.

#### **3.2.2.5.3 Soil Volumetric Moisture**

Soil volumetric moisture at the 0-2 cm depth was measured from May 2 to August 6 on the low N treatment in the oat and flax crop. During peak recruitment in spring, 2-3 samples were taken per week and approximately one sample per week was taken during the remaining part of the season. An 8.3 cm diameter copper ring was pressed into the soil to a depth of 2 cm and removed with a trowel. The sample was placed directly into a heat resistant plastic jar and sealed. The samples were taken per plot and bulked. The soil sample was dried by removing the lid and placing the jars for 48 hours in an oven set at 95 °C. Volumetric soil moisture was calculated based on gravimetric soil moisture and dry soil bulk density (See equation in section 3.2.1.5.4).

#### **3.2.2.5.4 Grain Yield**

The oat and flax crops were straight combined on August 19 and September 8, respectively, using a Massey Ferguson Kircaid XP8 small plot combine. A 2 m width was harvested the length of each sub subplot, emptied into a bag, dried for 3 days, and weighed. Yield in  $\text{kg ha}^{-1}$  was calculated based on individual length measurements of each plot.

### **3.3 Statistical Analysis**

SAS statistical software was used for all analyses (SAS Institute 1990). Data were checked for homogeneity of variance using the Levene test. Black medic recruitment for both site years at Carman was transformed using a log base 10 to achieve homogeneity of variance. Transformed data was subjected to ANOVA using Proc GLM in SAS and transformed treatment means were separated on the basis of a protected least significant test with a 5% level of significance. Means presented in the figures are actual means, not transformed means.

## 4.0 RESULTS AND DISCUSSION

### 4.1 Black Medic Germination and Dormancy Characteristics

#### 4.1.1 Introduction

Two laboratory experiments were conducted to study black medic dormancy and germination characteristics. These distinct and consecutive recruitment processes depend on temperature and moisture conditions at the seed microsite. The physical dormancy mechanism of black medic, a water impermeable seed coat, is fractured under certain temperature conditions as demonstrated by Sidhu (1971) and Van Assche et al. (2003). A non-dormant seed can then germinate over a range of temperature conditions in the presence of moisture (Sidhu 1971). In order to fully understand the principles underlying black medic recruitment under field conditions, more information is required about the role of temperature and moisture conditions for dormancy loss and germination. Therefore, the purpose of the following experiments was to (a) examine the effect of various temperature treatments on the germination of scarified black medic seeds, (b) determine the effect of extreme fluctuating temperatures on dormancy loss in black medic seeds, and (c) examine the interaction of moisture and temperature on dormancy loss in black medic seeds.

#### 4.1.2 Experiment #1: Temperature Effects on the Germination of Scarified Seed

After 3 days, the germination percentage for the 20 °C treatment was significantly higher than for the 10 °C treatment (Table 4.1). By the end of week 1, this difference had disappeared indicating that germination was delayed but not inhibited by the 10 °C

treatment. No further germination was observed after week 1 for the 10 and 20 °C treatments. To test the viability of the ungerminated seed, the remaining seeds were scarified by piercing the seed coat with a scalpel and placing them on moistened filter paper. All seeds germinated at 20°C after 3 days indicating that the ungerminated seed was dormant and viable. The 10 and 20 °C constant temperatures did not result in softening of the dormant seeds between week 1 and week 8.

The constant 30 °C and fluctuating 5/40 °C treatments inhibited germination compared to the constant 20 °C treatment after week 1 and week 8, although over time the differences between treatment means decreased. A proportion of the scarified seeds responded favourably to the 30 and 5/40 °C treatment, given the initial rapid germination after 3 days. Over time the remainder of scarified seeds began to germinate. This result suggested that the 30 and 5/40 °C temperature regimes were less ideal for germination than the 10 and 20 °C temperature regimes.

**Table 4.1:** Germination percentage of scarified black medic seed exposed to 10, 20 , 30, and 5/40 °C temperature regimes measured at 3 days, 1 week, and 8 weeks.

Temperature (°C)	Time		
	3 days	1 week	8 weeks
10	0.3 c†	82.0 a	82.0 ab
20	87.6 a	88.0 a	88.7 a
30	53.7 b	61.0 b	71.3 c
5/40	59.3 b	61.7 b	76.0 b
LSD (0.05)	7.7	8.28	10.0

† Values followed by a different letter within a column are significantly different at P<0.05



The results from this experiment indicate that black medic is capable of germinating over a wide range of temperature conditions when moisture is not a limiting factor. Sidhu (1971) studied scarified black medic seed germination over a wider range of temperatures and found similar results. Inhibition of germination in the Sidhu study was only observed at high temperature (35 °C) after a 25 day germination test.

In our study, no inhibition, only delayed germination occurred at 10 °C compared to the 20 °C treatment. Sidhu (1971) also observed delayed germination at 5 °C and alternating 0/10 °C temperatures. Black medic germination at low temperatures is an important ecological adaptation which allows for early spring germination under field conditions. This characteristic has not been investigated in the context of black medic use as a self-regenerating cover crop. When used as a cover crop, early spring recruitment may allow black medic to benefit the cropping system before spring seeding. A disadvantage of rapid early spring germination, however, may be a significant decline in the soil seed bank, a significant portion of which may be needed for recruitment later in the season.

Relating these germination studies to annual soil temperature variations (Table 4.2) suggests that in northerly climates like southern Manitoba, non-dormant black medic seed could germinate throughout the growing season. Significant diurnal and annual variations in soil and air temperature are apparent during the growing season. These temperature ranges are not an obstacle for black medic germination. This adaptation to the range of actual environmental conditions is supported by Sidhu (1971) who noted that black medic germination under field conditions in southern Ontario was a continuous event beginning in the last week of March and ending the first week of November. The

longer germination period in Ontario compared to Manitoba is a reflection of the longer growing season.

**Table 4.2: Monthly average air and soil (5 cm depth) maximum and minimum temperatures from Carman, MB in 2002.**

Month	Air Temperature		Soil Temperature	
	max	min	max	min
	----- °C -----			
Jan	-8.2	-17.5	-3.3	-4.0
Feb	-2.7	-14.0	-3.0	-4.6
Mar	-5.1	-15.2	-3.8	-6.2
April	8.2	-3.7	2.9	0.4
May	15.8	0.5	9.8	5.4
June	23.6	12.0	20.0	15.7
July	27.1	13.5	25.1	19.9
Aug	24.0	11.5	21.0	17.5
Sept	19.7	7.0	16.3	13.7
Oct	5.2	-5.0	5.5	3.7
Nov	-0.3	-9.9	-0.4	-1.4
Dec	-3.4	-12.4	-2.6	-3.4

The delayed and inhibited germination of black medic observed in this experiment at low and high temperatures is a valuable self-protection mechanism. Germination during early spring and late fall, when unexpected freezing temperatures are most likely to occur, will result in seedling death. Black medic has a built-in safeguard against these false breaks via delayed germination during periods of low temperatures. Conversely, high temperatures may cause desiccation of recently emerged seedlings. In response, black medic germination is delayed at high temperatures as a protection mechanism. A parallel protective mechanism for black medic at low and high

temperature conditions is light sensitivity. Sidhu (1971) found that light inhibited the germination of scarified seeds of black medic at low and high temperatures. If black medic is located near or on the surface of the soil in the presence of light, germination will be inhibited under low and high temperature conditions.

#### **4.1.3 Experiment #2: Temperature and Moisture Effects on Dormancy and Germination**

Since there was a temperature by moisture interaction, each temperature and moisture combination was treated as a separate treatment for statistical analysis. Significant differences in germination percentages between treatments were observed for both populations after week 1 and 6. For the ND1 black medic population, the 5/40 °C moist treatment germination percentage was higher than all other treatments at week 1 and 6. After one week, germination levels for the 5/40 °C and 20 °C moist treatments were 29.8 and 21.3%, respectively. After six weeks, the germination levels for the 5/40 and 20 °C moist treatments were 49.0 and 21.5%, respectively. Germination increased in the 5/40 °C moist treatment between week 1 and 6, whereas no change in germination level over time was observed for the other treatments. It is possible, therefore, that the 5/40 °C caused physical dormancy loss in contrast to the 20 °C moist treatment where only the non-dormant cohort of the population germinated.

In the PO2-W population, there was no significant difference between seed germination between the 20 and 5/40 °C moist temperature treatments after week 1. However, after week 6, a significantly greater portion of the hard seed germinated in the

5/40 °C moist temperature treatment compared to the 20 °C moist temperature treatment. This finding indicates that a similar temperature effect on black medic seed softening was present after 6 weeks for both black medic seed lots.

**Table 4.3 : Percent germination of two black medic populations, ND1 and P02-W, exposed to temperature treatments of 20, 10/40, and 5/40 °C in the presence and absence of free water. ‡**

Temp. (°C)	Moisture	Black Medic Population			
		ND1		P02-W	
		Week 1	Week 6	Week 1	Week 6
-----Percent Germination-----					
20	Dry	20.8 b†	23.5 b	11.0 ab	9.0 b
	Moist	21.3 b	21.5 b	8.5 b	8.8 b
10/40	Dry	17.3 b	20.3 b	8.3 b	12.0 b
	Moist	15.3 b	17.0 b	-----	-----
5/40	Dry	-----	23.5 b	-----	-----
	Moist	29.8 a	49.0 a	14.3 a	28.3 a
<b>LSD</b>		6.9	6.4	4.3	5.0

† Values followed by a different letter within a column are significantly different a P<0.05.

This experiment validates the existence of a two-stage softening process in black medic. Both seed lots were exposed to a chilling period followed by fluctuating temperatures in the presence and absence of moisture. It appears that physical dormancy was only lost after the second stage when the black medic seed was exposed to the 5/40 °C fluctuating temperature treatment in the presence of moisture. This study confirms the

findings of Van Assche et al. (2003), Zeng (2001), and Taylor (1981) who describe the black medic hard seed coat breakdown as the result of two distinct temperature and moisture regimes (see section 2.4.3).

Another important observation based on this study is the apparent similarity of the two-stage softening process of legumes in two distinct climates. In this experiment, a chilling period and appropriate temperature fluctuation were requirements for softening. These conditions are common in a temperate climate like Canada. However, in the Mediterranean climate where Taylor (1981) worked, a warm summer conditioning period followed by cooler fluctuating temperatures in the presence of moisture was required for seed softening. The evolution of a similar softening process in two distinct climatic regions is significant and warrants further study.

Our study also builds on the work of Van Assche et al. (2003) regarding the critical time period between the two stages of the softening process. Van Assche et al. (2003) observed that black medic seed loses the ability to overcome dormancy if the period between the chilling and fluctuating temperatures is greater than 2 months. In our study, a time period of 4 months lapsed between exhumation of the seeds from the chilling treatment and exposure to fluctuating temperatures. Despite this longer time period, seed softening still occurred. This observation highlights the importance of the second period of fluctuating temperature which completes the two-stage process. In the present study, the fluctuating temperature was 5/40 °C whereas Van Assche et al. (2003) used 6/15 °C. Benech-Arnold et al. (2000) explained that dormancy loss is gradual and best understood as a continuum. It is logical to assume that the environmental conditions necessary for dormancy loss vary with time. The 5/40 °C treatment used in this

experiment was likely within the realm of fluctuating temperatures that are capable of causing black medic dormancy loss 4 months after the chilling period. Further studies comparing several temperature fluctuation regimes are necessary to confirm how dormancy intensity varies over time.

The magnitude of the temperature fluctuation may determine the proportion of seed which will recruit from a seedbank. A greater temperature fluctuation may be more effective in softening seeds in the second stage of the softening process. Therefore, a greater proportion of seeds in a seedbank may be released from dormancy under extreme fluctuating temperatures. Environmental conditions and management practices that favour greater fluctuations in soil temperatures may be more effective for softening a greater portion of black medic seed.

Our study also showed the importance of moisture for softening to occur during the 5/40 °C temperature treatment. Seed softening may occur in a moist soil environment when black medic seed is positioned very near or on top of the soil surface and exposed to solar radiation. The current body of literature is limited regarding information on temperature effects on dormancy loss, and there is very little information on the role of moisture in dormancy loss in hard seeded legumes. The biochemical interactions that are believed responsible for softening the seed coat lipids and the role of water in the process requires more investigation, as suggested by Zeng (2001).

The present study did not examine the number of 5/40 °C cycles required for dormancy loss. Van Assche et al. (2003) reported that most black medic seeds germinated within 5 days under the 6/15 °C treatment. Therefore, seed softening brought on by the 6/15 °C temperature regime resulted in immediate germination. In contrast, the

present study showed continuous germination over a 6 week period under the 5/40 °C treatment. However, based on the results in Table 4.1, the 5/40 °C temperature regime inhibited germination. Therefore, immediate softening may have resulted from the 5/40 °C treatment, but germination was inhibited because of the extreme temperature fluctuation. These effects need to be researched further by exposing the seeds to the 5/40 °C treatment for a short period of time (ie: 3 days), and then moving the seeds to more favourable germination temperature regime such as 20 °C. An experiment of this type would better simulate actual field conditions since an extended period (greater than a week) of fluctuating temperatures in the range of 5/40 °C is unlikely. This hypothesis is supported by seed softening studies in Australia that show final softening in the second stage is the result of only a few appropriate diurnal temperature fluctuations (Taylor 1996b).

The data presented in Table 4.3 clearly indicates that the proportion of hard seeds in a population varies between medic populations. The ND1 and P02-W populations had initial germination levels (after 1 week) of 20.8% and 11.0%, respectively. These differences can be the result of (a) genetic differences caused by unique selection pressures of the cropping system where each population was grown, (b) environmental conditions during seed production, and (c) over-wintering and or storage conditions. Despite these differences in the initial proportion of seed in each population, the effect of the 5/40 °C moist treatment resulted in an approximately 2.5 fold increase in percent germination compared to an average of the other treatments after 6 weeks.

#### **4.1.4 Summary**

Characteristics of black medic dormancy loss and germination were studied in two laboratory experiments. Germination inhibition of non-dormant black medic seed at high and low temperatures reduces the risk of freezing seedlings by low temperatures or desiccation of seedlings by high temperatures. The two-stage softening process provides valuable cues for black medic germination timing during the spring. Varied dormancy loss responses to different fluctuating temperatures may be an effective mechanism for black medic population response to environmental cues as dormancy intensity increases. Moisture is necessary to complete the second stage of the softening process. Black medic populations will have varied dormancy loss and germination characteristics depending on genetic differences and environmental conditions in order to adapt more effectively to the surrounding environment. All of these findings suggest that black medic is an adaptable species. Not surprisingly, black medic has spread across North America in a very short time. Understanding these characteristics of black medic are necessary to develop a regenerating cover crop system and to explain the effect of management practices on the recruitment of black medic. Most important are the effects of temperature and moisture on black medic recruitment. These variables are the focus in the following section in which we examine the effect of management practices on black medic recruitment.

## **4.2 Black Medic Field Recruitment Studies**

### **4.2.1 Influence of Management Practices on Black Medic Recruitment**

Black medic recruitment under field conditions was affected by management practices. The management practices applied to the test plots were crop type, tillage

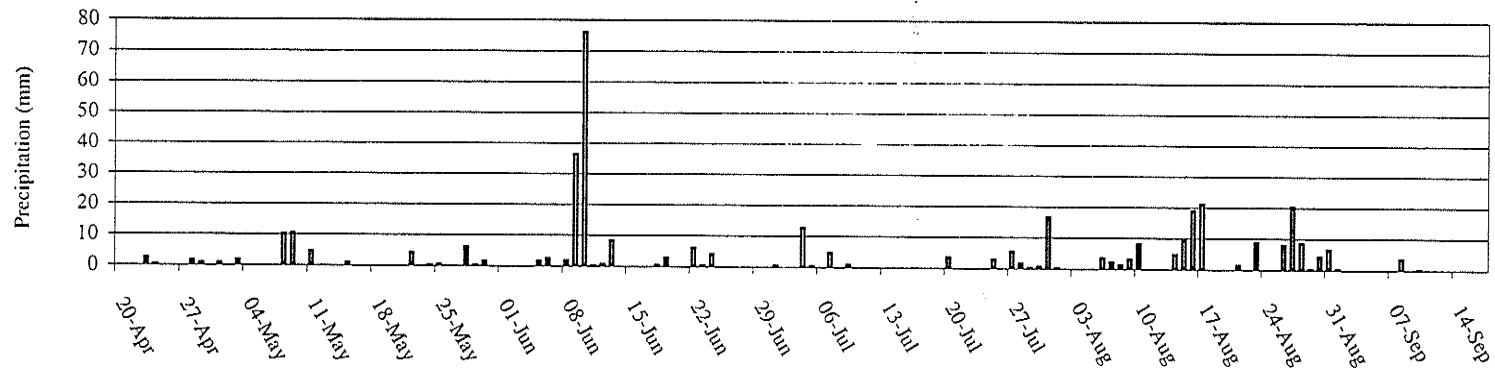


practice, and N fertilizer rate. These main effects altered moisture and temperature conditions at the black medic seed microsite which in turn influenced recruitment by influencing the processes of dormancy loss and germination. As a result, black medic recruitment varied among treatments.

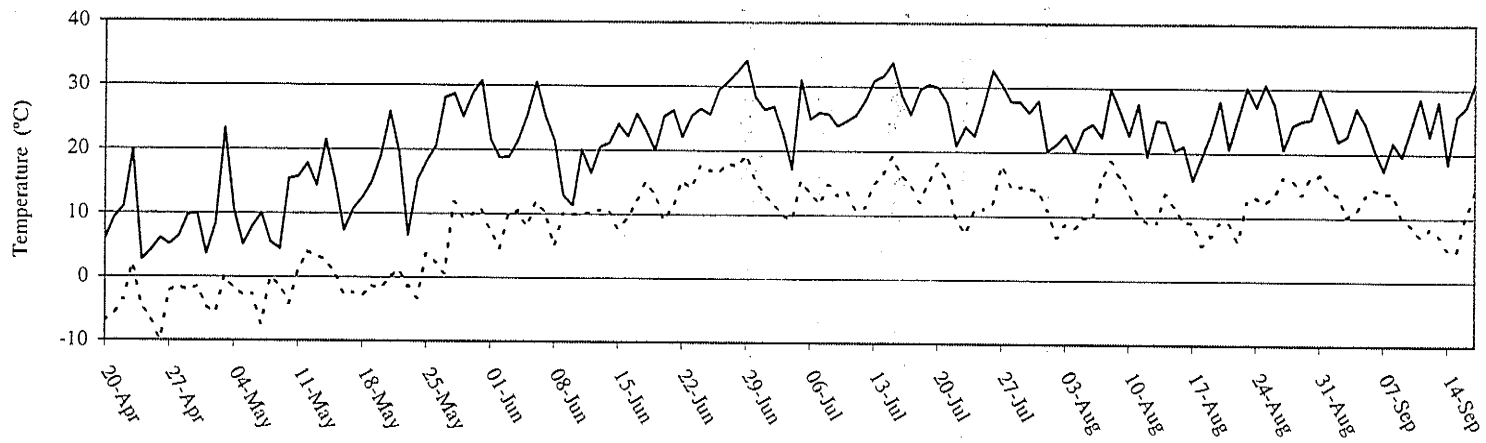
#### **4.2.1.1 Carman 2002**

Black medic recruitment at Carman in 2002 was observed in a field trial using a randomized complete block design with a split split plot arrangement. The main effects were crop (flax or oats), tillage (ZT or CT), and N fertilizer (high and low treatments). ANOVA was performed on all main effects and interactions. No significant interactions between main effects occurred, and therefore the main effects are discussed individually.

Precipitation and air temperature data during the growing season are found in Figures 4.1 and 4.2, respectively. In general, increases in black medic recruitment occurred when black medic seed was non-dormant and moisture and temperatures conditions were suitable for germination. The most significant jump in black medic recruitment occurred after an extended dry period (April 20 to June 9) which ended on June 10-11 with precipitation amounts greater than 110 mm (Figure 4.1). Sharp decreases in black medic recruitment were associated with (a) seedbed preparation in spring that consisted of tillage in CT treatments on May 14 and glyphosate application in ZT treatments on May 17 and (b) in-crop herbicide treatments (MCPA amine and propanil) on June 8 and 24.



**Figure 4.1:** Daily precipitation (mm) during the growing season at Carman in 2002.



**Figure 4.2:** Maximum temperature (solid line) and minimum temperature (dotted line) during the growing season at Carman in 2002.

#### 4.2.1.1.1 Crop Type

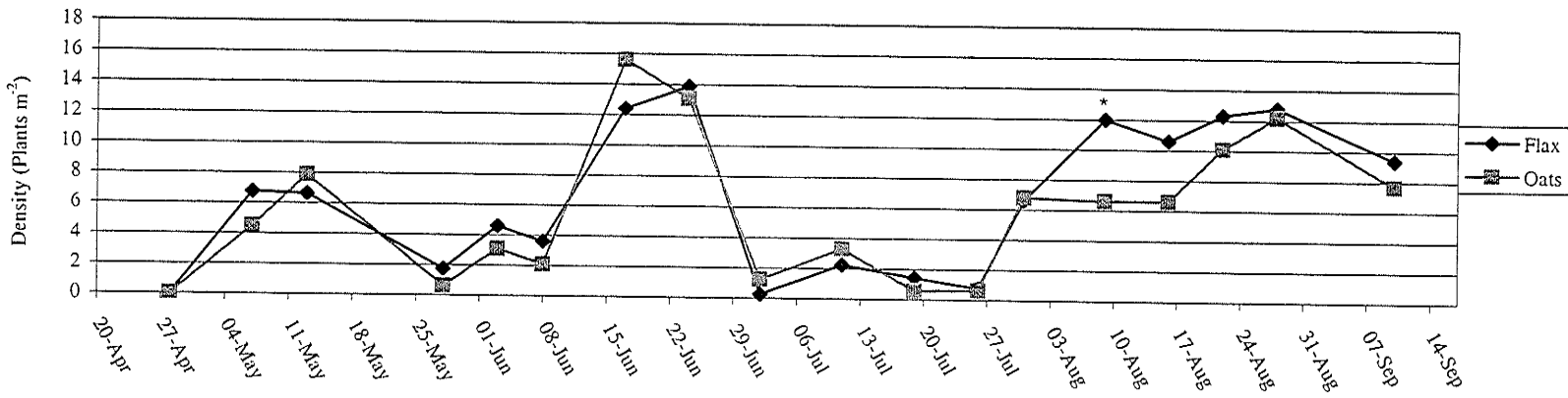
Black medic recruitment in flax and oat crops was observed at Carman in 2002. The results of the field trial are presented in Figure 4.3. Black medic recruitment was not significantly affected by crop type, except on August 9 when black medic recruitment was 12.0 and 6.6 plants m<sup>-2</sup> in the flax and oats, respectively.

The presence of early spring recruitment of black medic under both crops confirms that the two-stage softening process was functional in the field. Low winter temperatures followed by appropriate fluctuating temperatures in early May (Figure 4.4) most likely contributed to seed softening. The soil temperature during this time period fluctuated between approximately 4 and 15 °C. These temperatures were identical to the temperature that Van Assche et al. (2003) noted as ideal for seed softening during the second stage of the softening process.

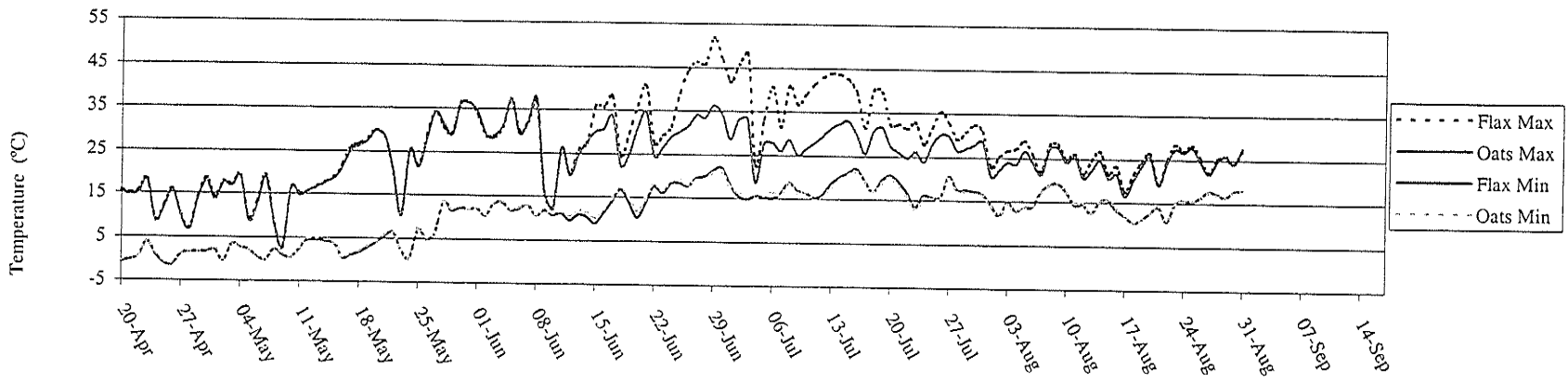
The period following seeding on May 14 until June 10 represented a period of low black medic recruitment under both crop types. Tillage, herbicide applications, below average rainfall (Figure 4.1), and low soil VWC (Figure 4.5) contributed to this lack of recruitment.

Significant rainfall events after June 10 caused a sharp increase in soil moisture (Figure 4.5) and black medic recruitment (Figure 4.3), and a decline in soil temperature (Figure 4.4) in both treatments. The in-crop herbicide on June 24 caused a sharp decline in black medic recruitment in both the flax and oat crops.

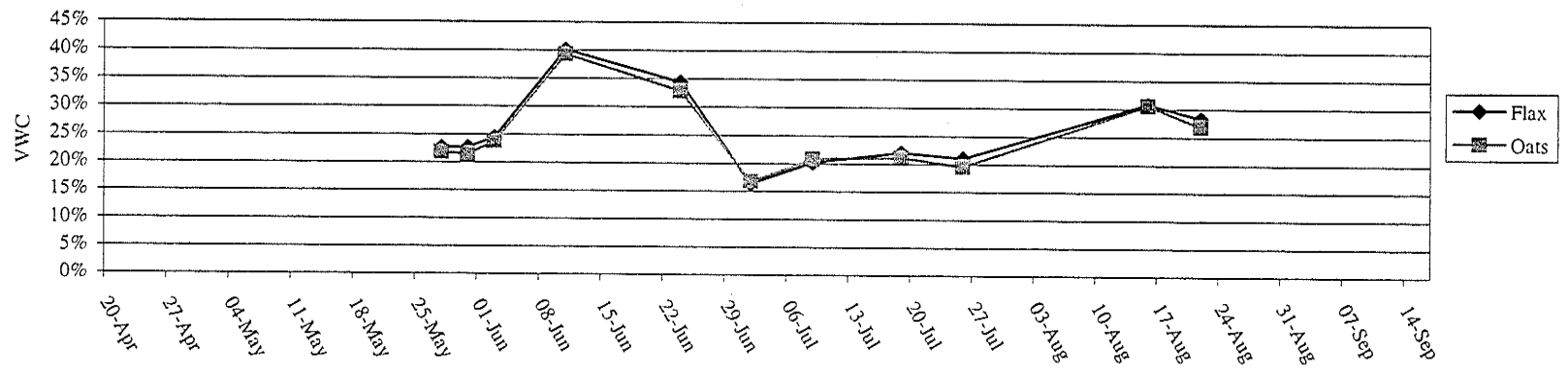
Between June 24 and July 27, limited black medic recruitment was observed in both crops. However, after July 27, a sharp increase in recruitment occurred in both crops occurred, followed by a significant difference on Aug 9 when black medic



**Figure 4.3:** Black medic recruitment as influenced by crop type (flax and oats) at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at  $P < 0.05$ .



**Figure 4.4:** Soil temperature (maximum and minimum) as influenced by crop type (flax and oats) at Carman in 2002.



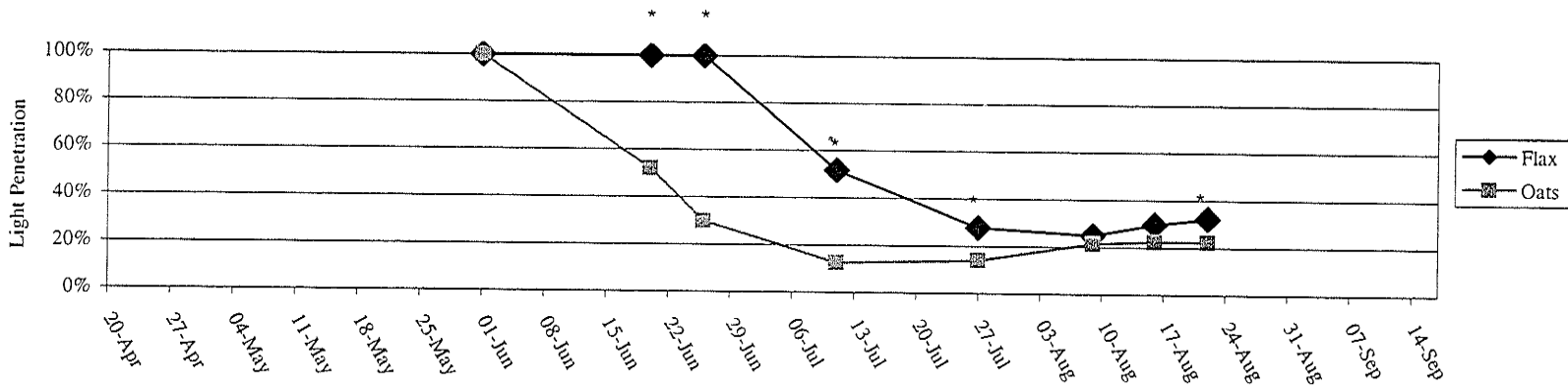
**Figure 4.5:** Soil volumetric water content (VWC) from 0-10 cm as influenced by crop type (flax and oats) at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at  $P < 0.05$ .

recruitment was greater in the flax versus the oat crop. Although black medic recruitment did not vary between crops from June 24 to August 9, maximum soil temperature under flax was significantly higher on all except 5 days. In addition, maximum soil temperature under flax was usually greater than 35 °C and on June 27-30 and July 2-3, the maximum soil temperature in the flax crop exceeded 45 °C. In contrast, the maximum soil temperature in the oat crop exceeded 35 °C on only one day during the same period.

Higher soil temperature under flax was attributed to better light penetration of the canopy in flax versus oats prior to July 27 (Figure 4.6). On June 20 and 26, light penetration in flax was 100% whereas in oats it was 52 and 30%, respectively. Herbicides applied on June 8 and 24 stunted the flax. As the season progressed, the flax recovered from the herbicide treatment and the light penetration differences decreased, but remained significant up to July 27.

Based on the two-stage softening process, black medic dormancy loss is the result of a chilling period followed by fluctuating temperatures. However, this process does not account for mid to late season softening and recruitment as observed after July 27 (Figure 4.3). According to Van Assche et al. (2003), black medic failed to respond to fluctuating temperatures (5/16 °C) if two months had elapsed since the chilling period. In our study, we observed a recruitment response to fluctuating temperatures four months after the winter chilling treatment. Therefore, another softening mechanism may be responsible for the late season softening and recruitment.

An explanation for late season softening of black medic in a temperate climate may be linked to the two-stage softening process that occurs in a Mediterranean



**Figure 4.6:** Light penetration (%) as influenced by crop type (flax and oats) at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at  $P < 0.05$ .

climate. In our study, elevated maximum soil temperatures (35 to 50 °C) followed by cooler fluctuating temperatures (15/30 °C) in the presence of moisture characterized the conditions leading up to the recruitment flush in August (Figure 4.3 and 4.5). These conditions are identical to the temperatures causing seed softening and recruitment in Australia under a Mediterranean climate. This represents the first report of medic from a temperate climate responding to the two-stage softening conditions typical for Mediterranean conditions. Laboratory studies are needed to further confirm that black medic from a temperate climate will soften and germinate following the two stage softening process common in a Mediterranean climate. Verifying this link will be a significant advancement to increase the understanding of the softening process.

The significant difference in black medic recruitment between crops on August 9 (Figure 4.3) can be explained on the basis of the two-stage softening process common in a Mediterranean climate. Higher black medic densities in flax on August 9 compared to oats may be related to a combination of higher soil temperature in the flax during June and July, followed by cooler fluctuating temperatures under moist conditions. In the present study, soil temperature at 1.5 cm exceeded 45 °C on several days, and reached 50 °C on June 29 (Figure 4.4). Surface soil was 5 to 10 °C warmer than at 1.5 cm based on several soil temperature measurements taken with an infrared thermometer (data not shown). Therefore, black medic seed located near the soil surface encountered temperatures in excess of 50 °C. These high temperature conditions were more common in flax versus oats and likely contributed to greater dormancy loss in flax in this study. Similar findings were found in Australia where seed softening was optimal when



maximum temperature in the first stage of softening was increased (Taylor and Ewing 1988).

The temperature requirement in the second stage of the softening process for black medic seed may vary depending on the temperature treatment in the first stage. Based on Experiment 2 of this study and the work by Van Assche et al. (2003), a critical minimum temperature of 5 °C is required for softening during the second stage when the first stage is a chilling treatment. However, when the first stage is an elevated temperature regime, a higher diurnal fluctuation in the second stage may cause greater softening. Based on soil temperature readings between July 27 and August 9 (Figure 4.4), temperature fluctuations of approximately 15/30 °C in the presence of moisture appeared to be sufficient to cause softening.

The elevated temperatures and enhanced black medic recruitment observed in the flax crop are related to the gap detection mechanism described by Benech-Arnold et al (2000). A sudden increase in seed temperature during the day will provide a cue to the seed of its relative position in the soil profile as well as the presence of gaps in the vegetation. The latter of the two cues was most important in the present study. The effect of the vegetation gap was decreased black medic dormancy. This change positioned some of the seed in a state of non-dormancy for quick germination in the presence of moisture.

In the present study, light penetration in flax was significantly greater in oats for each measurement before August 9. On August 9, black medic recruitment was significantly higher in flax. Therefore, increased light penetration resulted in greater black medic recruitment. These results are in contrast to those of Sidhu (1971) who found that germination of scarified black medic seed was inhibited in the presence of light under high

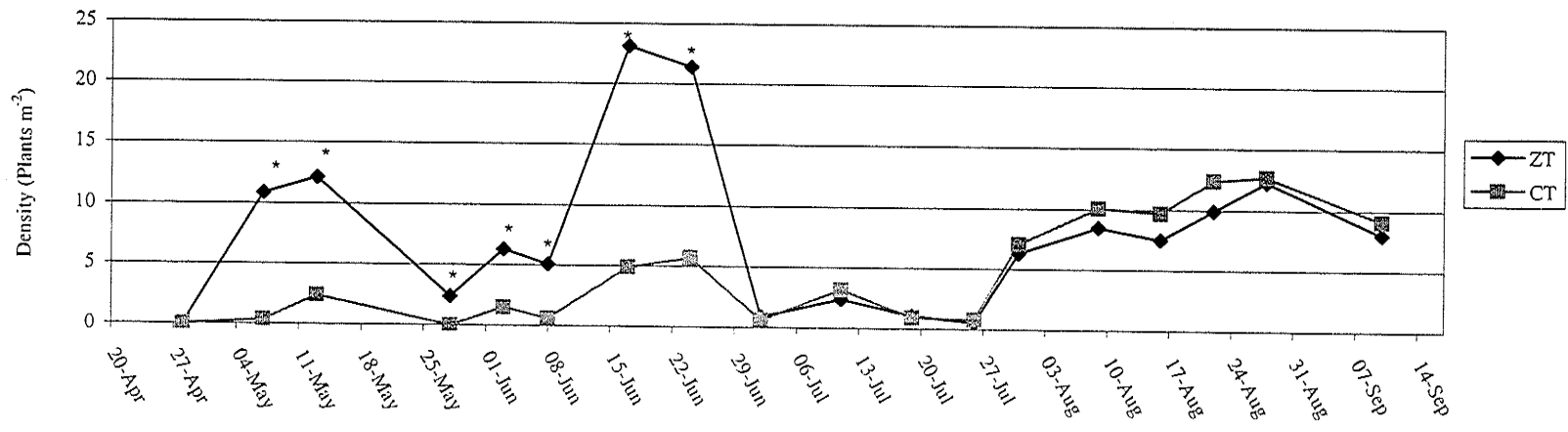
temperatures. However, the observations by Sidhu (1971) are not relevant in this study since the temperatures during germination for the August 9 cohort were within the acceptable range. The effect of light on black medic recruitment is not as critical as temperature or moisture conditions at the microsite. More important was the relationship between light penetration of the crop canopy and soil temperature. Increased light penetration of the crop canopy in flax versus oats caused higher soil temperatures and consequently increased recruitment in flax.

#### 4.2.1.1.2 Tillage

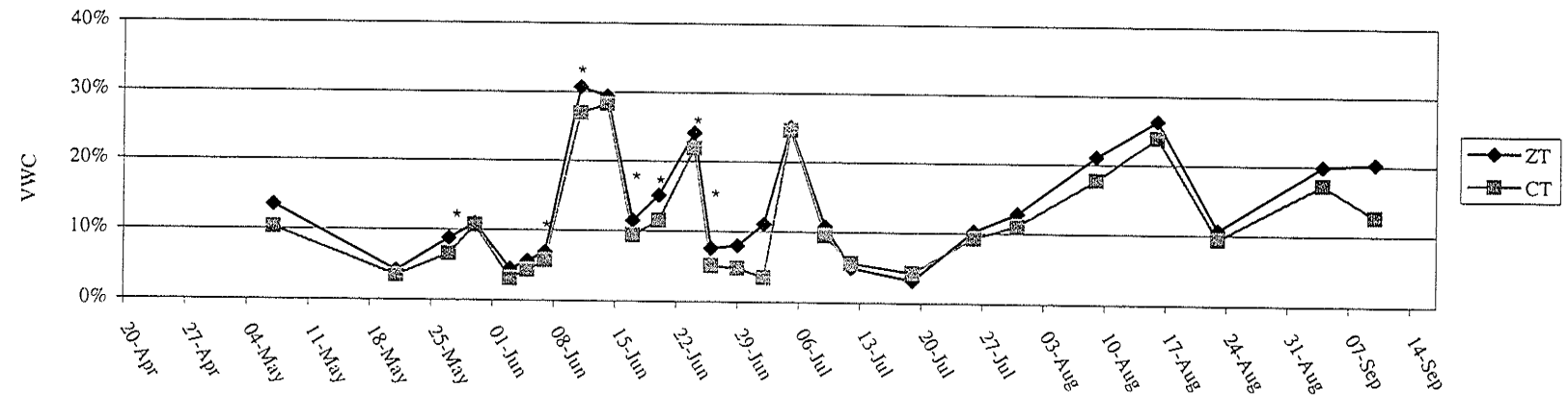
During the first half of the growing season up to the end of June, black medic recruitment was significantly greater in ZT compared to CT (Figure 4.7).

Soil VWC differences at 0-2 cm depth (Figure 4.8) between the two tillage systems likely accounted for some of the difference in recruitment between tillage systems. In general, soil VWC under ZT tended to be higher than under CT. Since increasing soil moisture results in increased germination (Roman et al. 1999), it follows that recruitment will be greater under ZT. Whether the slight differences recorded in VWC between tillage systems were the cause of the dramatic differences in black medic recruitment between tillage systems (Figure 4.7) is not known and requires further study.

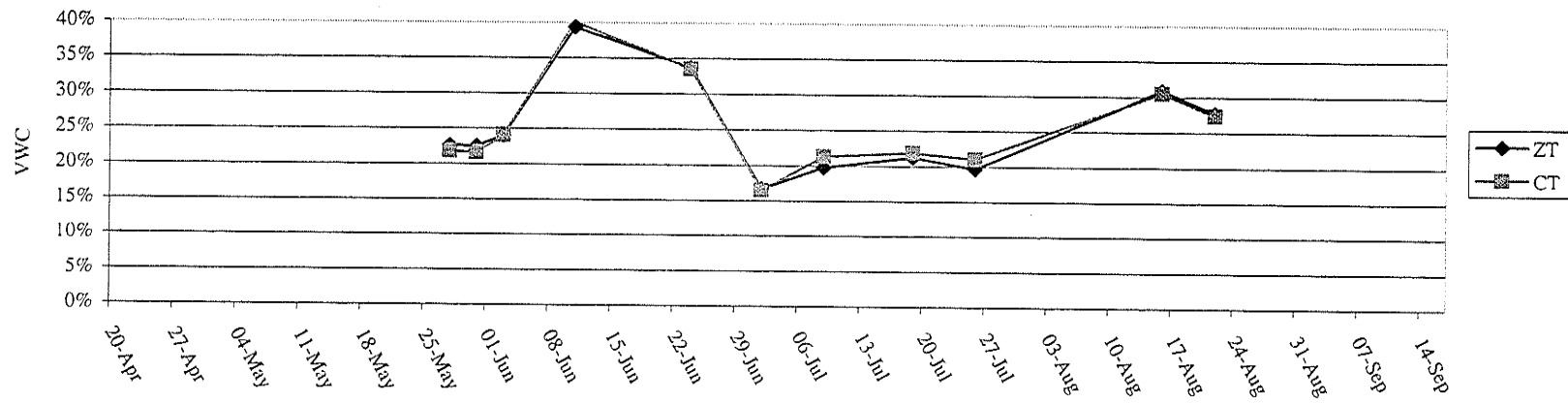
There was no clear association between soil VWC at 0-10 cm and black medic recruitment between tillage systems because soil VWC was not significantly different between tillage treatments (Figure 4.9). Measurement of soil VWC in the top 10 cm would likely mask slight differences in VWC at the surface where the majority of recruitment occurs.



**Figure 4.7:** Black medic recruitment (plants m<sup>-2</sup>) as influenced by zero tillage (ZT) and conventional tillage (ZT) at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



**Figure 4.8:** Soil Volumetric Water Content (VWC) (0-2 cm) as influenced by by zero tillage (ZT) and conventional tillage (CT) in flax at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



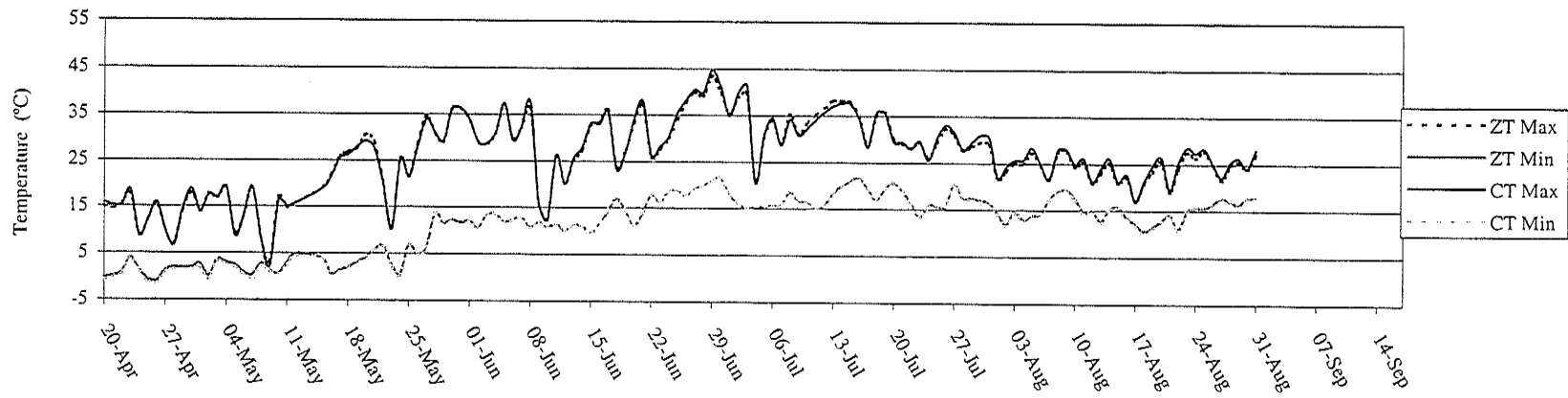
**Figure 4.9:** Soil Volumetric Water Content (VWC) from 0-10 cm as influenced by zero tillage (ZT) and conventional tillage (CT) at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at  $P < 0.05$ .

Maximum and minimum soil temperatures were not helpful for explaining differences in black medic recruitment between the tillage systems. During the growing season, the temperatures between the ZT and CT were almost identical (Figure 4.10).

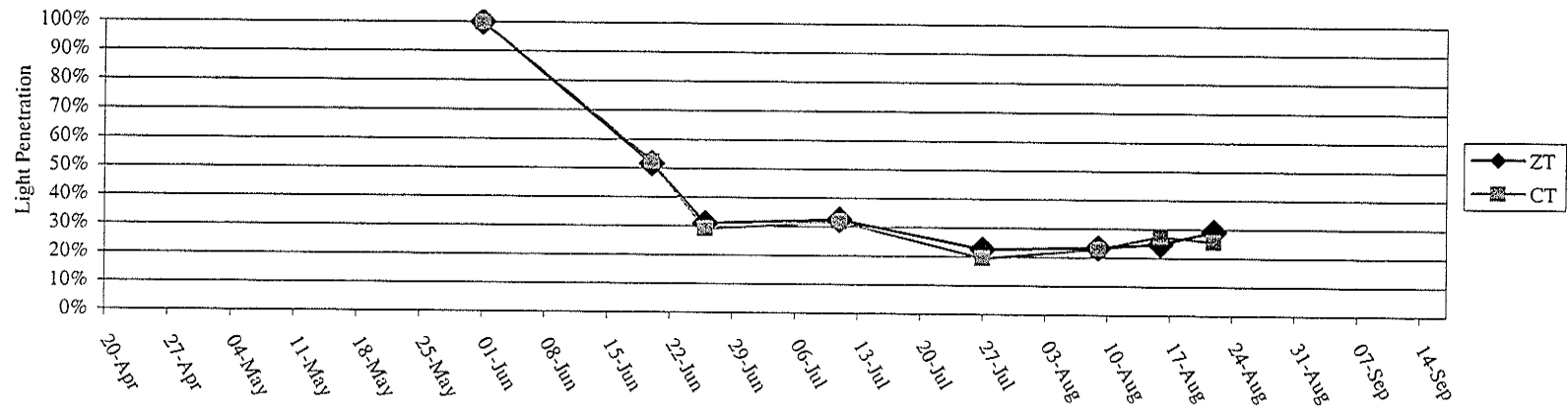
Light penetration differences by tillage were also not significant (Figure 4.11). This observation was expected since there were no differences in soil temperature between tillage treatments. Increased light penetration would increase soil temperature.

In summary, the majority of the dependent variables measured did not explain the significant differences in black medic recruitment caused by tillage, except VWC at the 0-2 cm depth. Differences in soil temperature between tillage systems were expected, but in the present study, on only one day was there a significant difference. The lack of association was related in part to the duration of the tillage effects and the time period for the tillage effects to reach an equilibrium. The tillage treatments in our study began only in September, 2001 and would need at least 3-5 years to reach an equilibrium. Therefore, conclusions based on ZT and CT soil properties of a developing system should be approached with caution. Long-term trials are necessary to allow soil characteristics to equalize before making definitive statements about tillage effects.

In the context of our tillage treatments, the effect of tillage on black medic recruitment prior to June 24 was likely related to the re-distribution of seeds in the soil profile. We did not measure seed distribution, but many studies show that as tillage intensity increased, seed burial depth also increased (Chepil 1946a; Cousens and Moss 1990; Du Croix Sissons et al. 2000; Reid 2003; Van Acker et al. 2004). For example, a simulated seed burial study using beads indicated that a single tillage pass with sweeps buried the majority of the beads in the top 4 cm of soil (Reid 2003). As a result, the



**Figure 4.10:** Soil temperature (maximum and minimum) as influenced by zero tillage (ZT) and conventional tillage (CT) at Carman in 2002.



**Figure 4.11:** Light penetration (%) as influenced by zero tillage (ZT) and conventional tillage (CT) at Carman in 2002.

seeds in the ZT tillage system would be expected to remain largely in the top 1 cm of the profile, whereas in the CT tillage system, the seeds would be redistributed throughout the top 4 cm of soil. The ideal recruitment zone for most small seeded species like black medic is approximately the top 2 cm of soil (Du Croix Sissons et al. 2000). Therefore, the seed distribution effect caused by tillage likely reduced the number of seeds within the zone in the CT treatment. This may have reduced recruitment in the CT treatment. In addition, the vertical location of black medic seed associated with different tillage systems may have led to differences in microsite conditions, particularly temperature fluctuations. As soil depth increases, the magnitude of daily soil temperature fluctuations usually decreases (Reimer and Shaykewich 1980). Therefore, the surface positioned black medic seeds in ZT may have experienced greater diurnal temperature fluctuations. Since appropriate temperature fluctuations appear to be necessary for dormancy loss (Van Assche et al. 2003), greater black medic recruitment in the ZT treatments may be explained by the difference in microsite conditions at the surface of the soil versus 2-10 cm below the soil surface.

Tillage treatment also affected recruitment timing of the first spring flush of black medic. Early spring recruitment under ZT and CT reached a maximum on May 6 and 13, respectively (Figure 4.7). A much larger proportion of the black medic seed bank germinated at an earlier date in the ZT treatments. Under ZT, the majority of the seeds would be near the soil surface (0-2 cm depth), whereas under CT, seeds would be more evenly distributed in the top 10 cm. Seeds buried deeper in CT treatments would require more time for recruitment (Bullied et al. 2003). In addition, average soil temperatures in spring are greatest at the soil surface (Malhi and O' Sullivan 1990). Since germination

and shoot elongation are temperature dependent processes (Roman et al. 1999), black medic emergence would be earlier under a ZT system. Furthermore, earlier black medic germination under ZT may be related to the two-stage seed softening process. Seeds lying at the surface of the soil in a ZT system would be exposed to appropriate low temperature fluctuations (second stage of softening) earlier than seeds buried deeper in the soil profile in the CT system. As the appropriate low temperature fluctuation penetrated deeper into the soil profile as the soil warmed, more seeds in the CT system likely overcame dormancy and began to germinate. A 'catching-up' effect resulted that was likely related to seed depth. In conclusion, rapid early spring germination under the ZT system may have been affected by a combination of shoot elongation distance, soil temperature effects, and dormancy loss mechanisms that expedited the recruitment of seeds at the surface of the recruitment zone.

The lack of significant difference in black medic recruitment levels between tillage treatments after June 24 is difficult to explain, especially in light of the large differences prior to June 24. As mentioned earlier, a higher concentration of black medic seed in the recruitment zone under ZT would lead to increased recruitment in comparison to CT. However, this was not observed after June 24 since the recruitment shift was almost identical between ZT and CT. It is possible that seed losses in the ZT treatments were greater. One possible explanation would be greater predation of black medic seeds on the soil surface in the ZT versus the CT treatments. This explanation is supported by Pavone and Reader (1982) who attributed significant black medic seed loss on the soil surface to predators. Seed buried in the CT treatments but still within the recruitment zone would be less vulnerable to predation. Predation may have equalized the quantity of



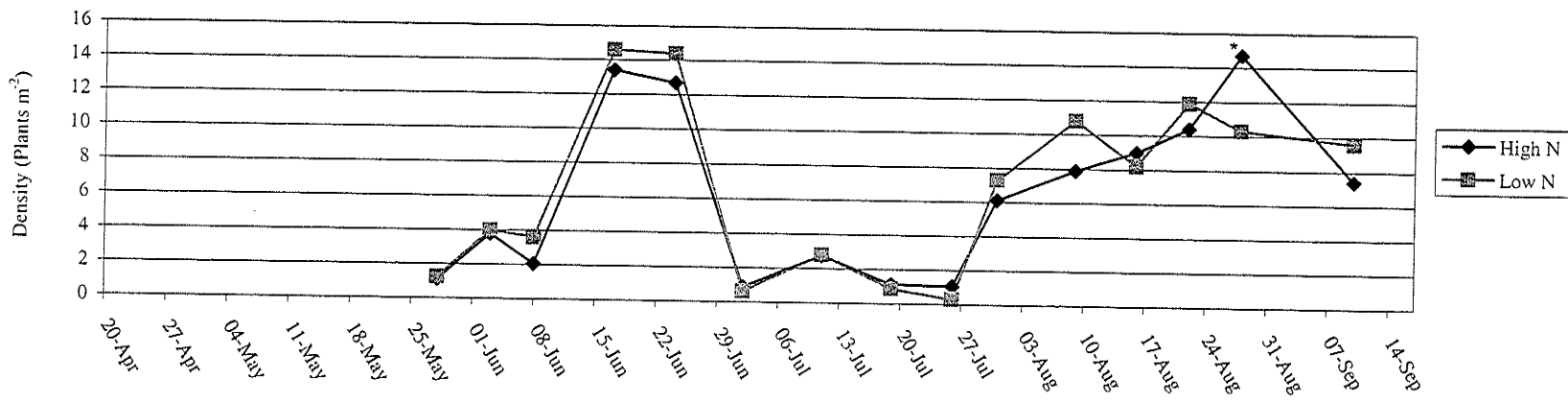
seeds germinating in each treatment. Another possible explanation may be related to the differing risks of desiccation of recruiting black medic seedlings between the two treatments. Black medic seed germinating in the ZT treatment would be more vulnerable to fluctuations in soil moisture and subsequent desiccation at the soil surface because of (a) the proximity of seed to the soil surface and (b) the fact that the ZT treatments had little surface residue. Light rain showers after June 24 (Figure 4.1) may have led to germination of the surface positioned seeds in the ZT treatments and these seedlings may have succumbed to desiccation.

#### 4.2.1.1.3 Nitrogen Fertilizer Rate

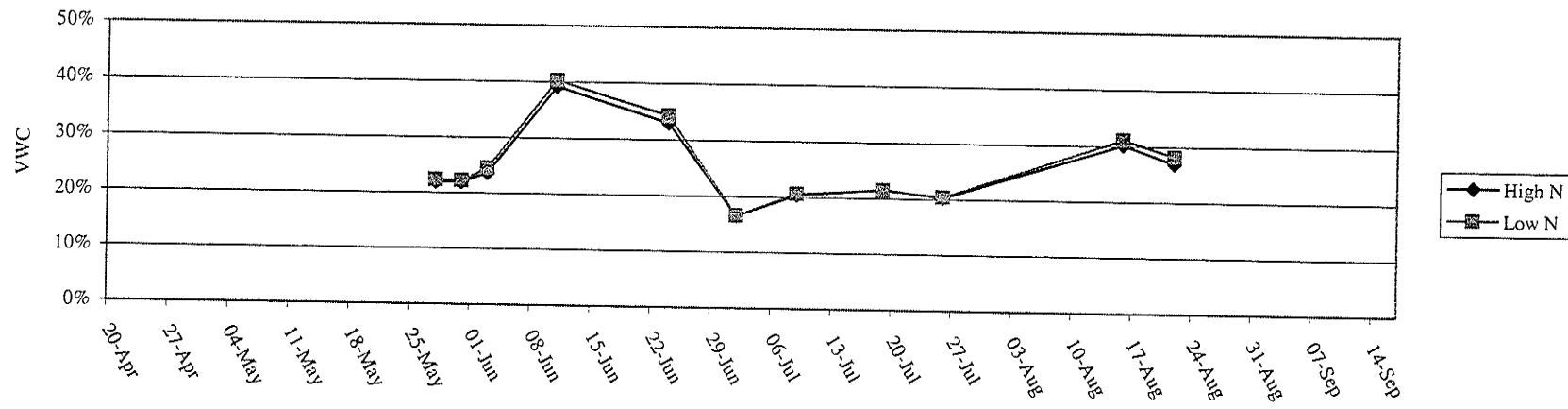
There was a trend towards greater black medic recruitment under the low N treatment (Figure 4.12). Soil VWC in the top 10 cm of soil was not significantly different between the N fertilization treatments (Figure 4.13). Light penetration was significantly greater in the low N treatment on two sampling dates (Figure 4.14). We conclude that N fertilization rate does not affect black medic recruitment.

#### **4.2.1.2 Carman 2003**

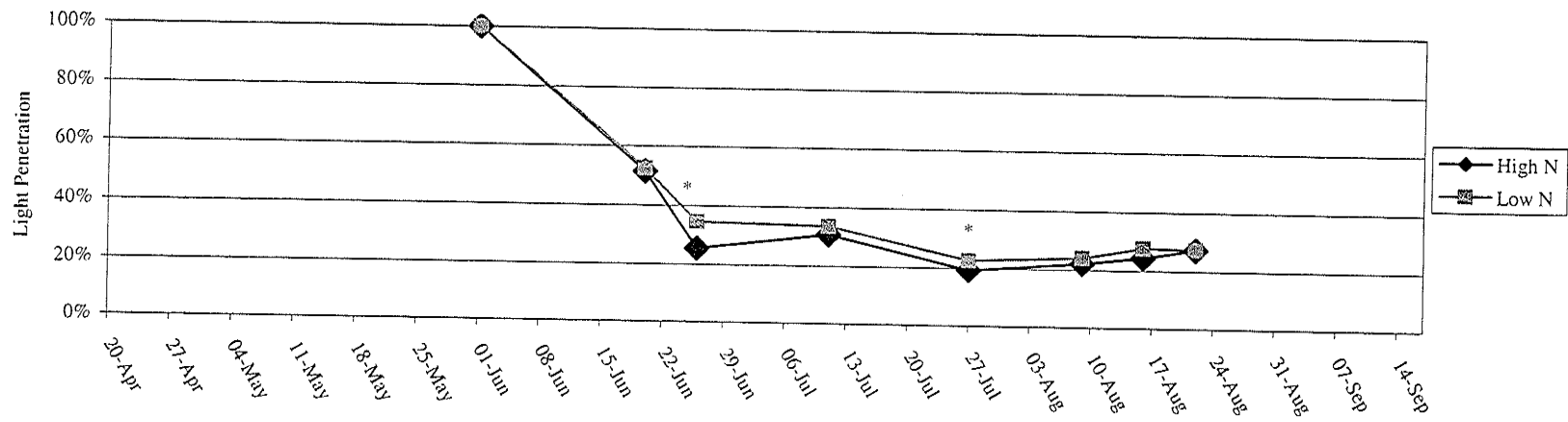
There were no significant interactions between main effects at Carman in 2003 and therefore the main effects are discussed individually. Precipitation and temperature data during the growing season are found in Figure 4.15 and 4.16, respectively. As in 2002, increases in black medic recruitment occurred when black medic seed was exposed to conditions conducive to dormancy loss and suitable germination temperatures. The initial flush of black medic during the spring began on April 28, approximately one week



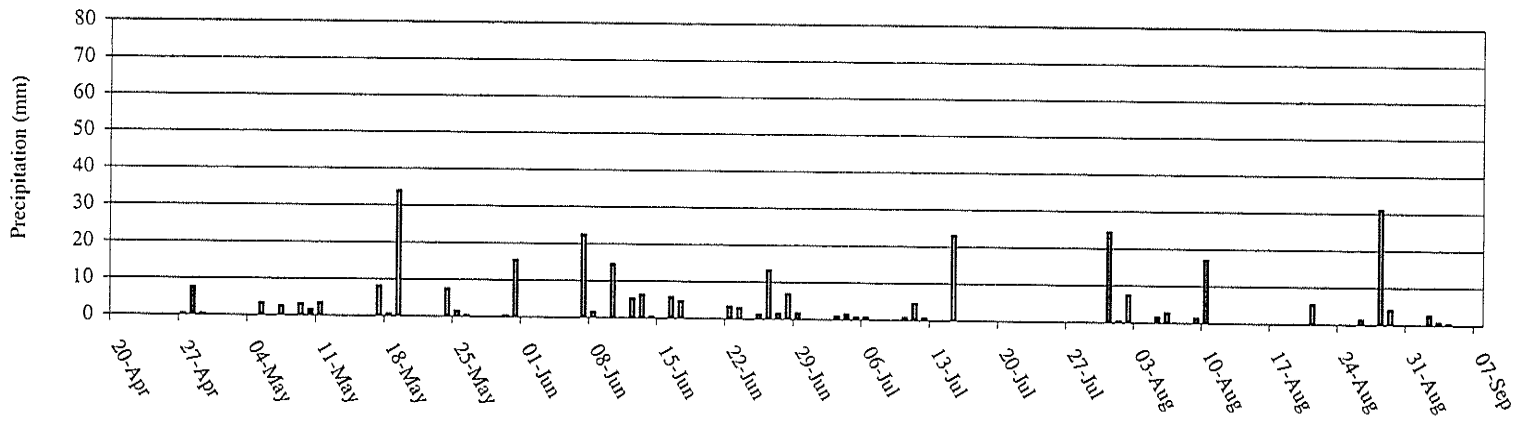
**Figure 4.12:** Black medic recruitment (plants m<sup>-2</sup>) as influenced by high nitrogen (High N) and low nitrogen (Low N) fertilizer application rates at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



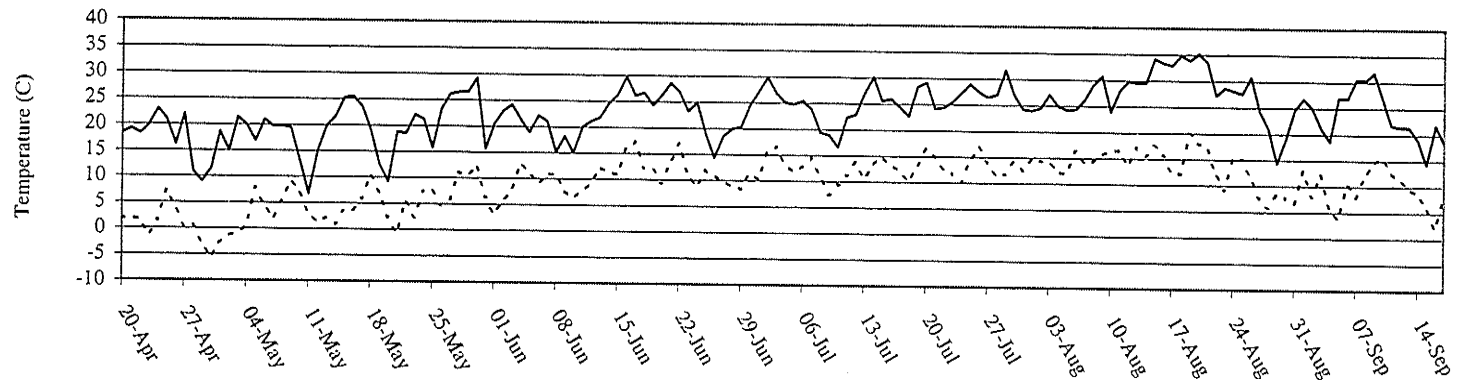
**Figure 4.13:** Soil volumetric water content (VWC) at 0-10 cm as influenced by high nitrogen (High N) and low nitrogen (Low N) fertilizer application rates at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



**Figure 4.14:** Light penetration as influenced by by high nitrogen (High N) and low nitrogen (Low N) fertilizer application rates at Carman in 2002. Sampling dates with an asterisk (\*) are significantly different at  $P < 0.05$ .



**Figure 4.15:** Precipitation (mm) at Carman in 2003.

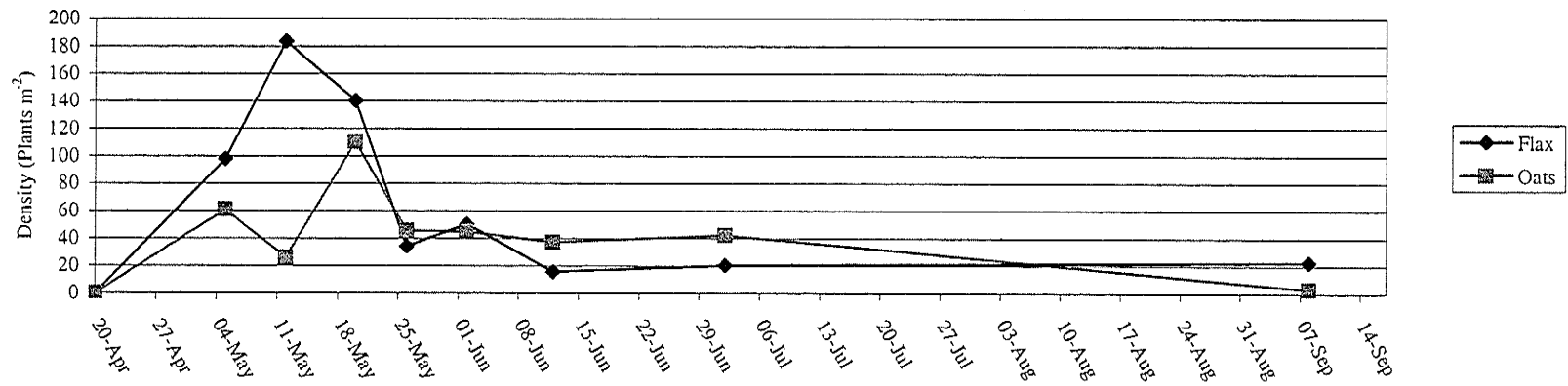


**Figure 4.16:** Maximum temperature (solid line) and minimum temperature (dotted line) during the growing season at Carman in 2003.

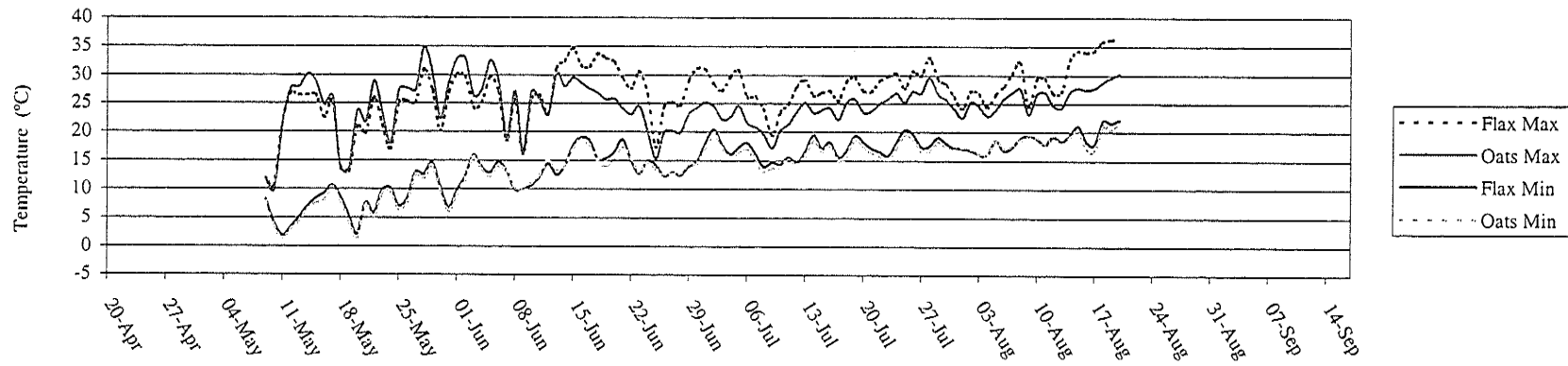
earlier than in the previous year. Several evenly spaced rain events in early May created ideal soil moisture conditions for spring recruitment (Figure 4.15). Sharp decreases in black medic recruitment were associated with spring tillage and herbicide treatment for seedbed preparation. The oat CT treatments were cultivated on May 5 and the ZT treatments were sprayed with glyphosate on May 9. The flax CT treatments were cultivated on May 13 and the ZT treatments were sprayed on May 16. Since the crops were treated differently in May, the crop main effect was only considered after the June 5 application of MCPA amine and propanil which eliminated the majority of black medic seedling recruited up to that date. Tillage and N fertilizer effects were analyzed separately for flax and oats because the crops were tilled at different times.

#### 4.2.1.2.1 Crop Type

After June 5, there was no significant difference in black medic recruitment between crops and black medic recruitment was reduced from August 1 to September 8 (Figure 4.17). This decline in recruitment is different than in 2002 when significant recruitment was observed in August. Black medic recruitment at Carman in August 2002 was attributed to a Mediterranean type two-stage softening process which appeared to require soil temperatures in excess of 35 °C. In 2003, soil temperatures never exceeded 35 °C in either crop (Figure 4.18). Black medic may require a higher critical maximum temperature for the first stage of softening in the late season recruitment process.



**Figure 4.17:** Black medic density (plants m<sup>-2</sup>) as influenced by crop (flax and oats) at Carman in 2003. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



**Figure 4.18:** Soil maximum (Max) and minimum (Min) temperature as influenced by crop (Flax and Oats) at Carman in 2003.

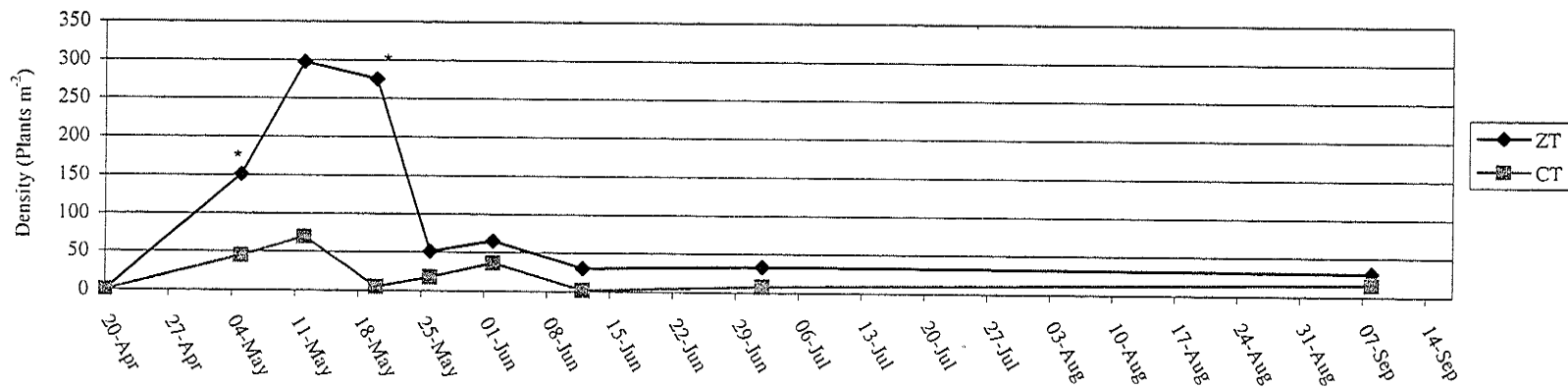
#### 4.2.1.2.2 Tillage

##### *Flax*

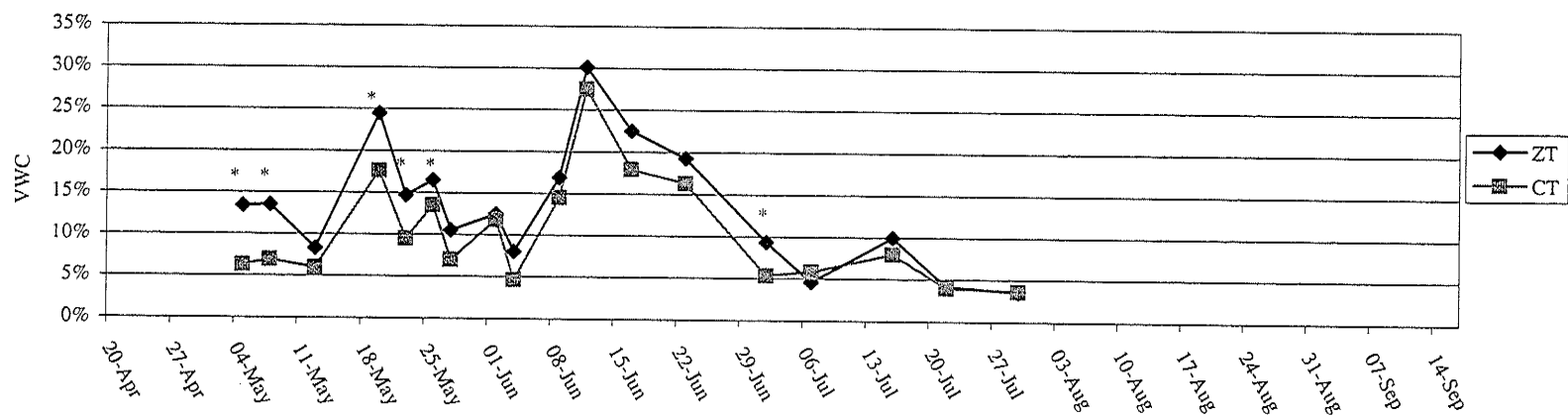
In flax, tillage system affected black medic recruitment in May (Figure 4.19). The first significant difference occurred on May 5 when black medic recruitment was 151 and 45 plants  $m^{-2}$  in ZT and CT, respectively. Higher soil VWC under ZT prior to this date (Figure 4.20) may have contributed to significant differences in recruitment. However, as in 2002, the main factor likely causing recruitment differences between tillage systems was the movement of seed below the recruitment zone caused by tillage in the CT treatment. The lag effect in early spring recruitment observed in 2002 under CT was not observed in the flax tillage treatments in 2003.

The significant difference in recruitment between tillage systems on May 20 should be interpreted with caution. The tillage treatment in the CT treatment occurred on May 13 whereas the glyphosate application in the ZT treatment occurred on May 16, as noted on Figure 4.19. The effect of tillage on black medic recruitment in the CT treatment was immediate, and therefore a decline to 5 plants  $m^{-2}$  on May 20 accurately reflected black medic recruitment. However, under ZT, the effects of the glyphosate application were only beginning to affect plant desiccation by May 20 and the counts on May 20 may have included dying seedlings. The glyphosate effect was more obvious on May 26 when black medic recruitment dropped to 51 plants  $m^{-2}$ .

After May 25, black medic recruitment remained very low and did not differ between tillage treatments (Figure 4.19). Low recruitment can be attributed to the same factors mentioned in the discussion of crop effects. High soil temperatures necessary for the first stage of a Mediterranean two-stage softening process were not observed in either



**Figure 4.19:** Black medic density in flax as influenced by zero tillage (ZT) and conventional tillage (CT) at Carman in 2003. Sampling dates with an asterisk (\*) are significantly different at  $P < 0.05$ .



**Figure 4.20:** Soil volumetric water content (VWC) in flax as influenced by zero tillage (ZT) and conventional tillage (CT) at Carman in 2003. Sampling dates with an asterisk (\*) are significantly different at  $P < 0.05$ .



of the tillage treatments (Figure 4.21). Even though there was a trend towards higher soil moisture conditions under ZT versus CT (Figure 4.20), this difference appeared to have no impact on black medic recruitment..

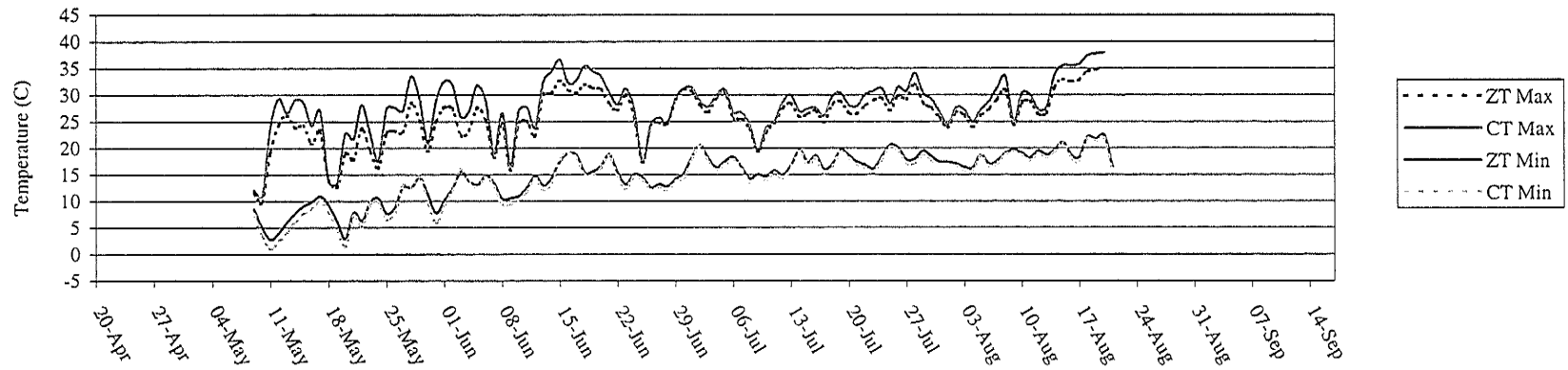
### *Oats*

In the oat crop, a significant difference between tillage systems occurred only on May 12 (Figure 4.22). Black medic recruitment in ZT and CT treatments was 52 and 0 plants m<sup>-2</sup>, respectively. As in the flax crop, this significant difference was likely caused by a lag in the effect of glyphosate on black medic seedling in the ZT treatment. On May 5 the CT treatment was tilled and on May 9 the glyphosate was applied in the ZT treatment. By May 12, the effects of the glyphosate on the black medic were just beginning to appear.

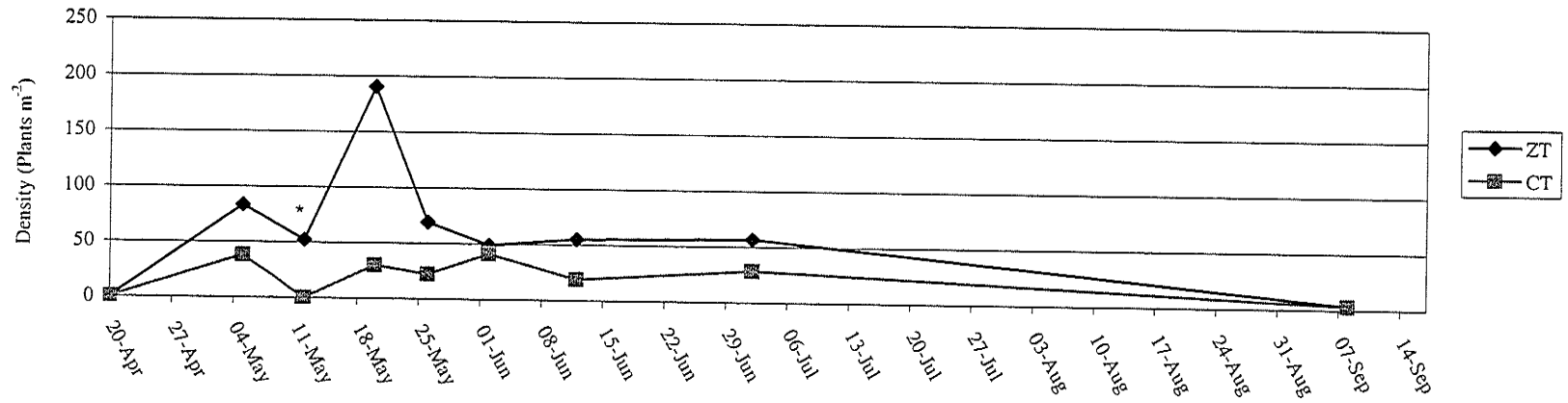
No other significant differences were observed during the remainder of the growing season despite differences in maximum soil temperature between the tillage systems (Figure 4.23). As in the flax crop, the very high soil temperature conditions required for dormancy loss were not met (Figure 4.23). However, there was a trend towards greater black medic recruitment in the ZT versus the CT treatments. This difference was likely the result of seed placement differences between tillage treatments.

#### 4.2.1.2.3 Nitrogen Fertilizer Rate

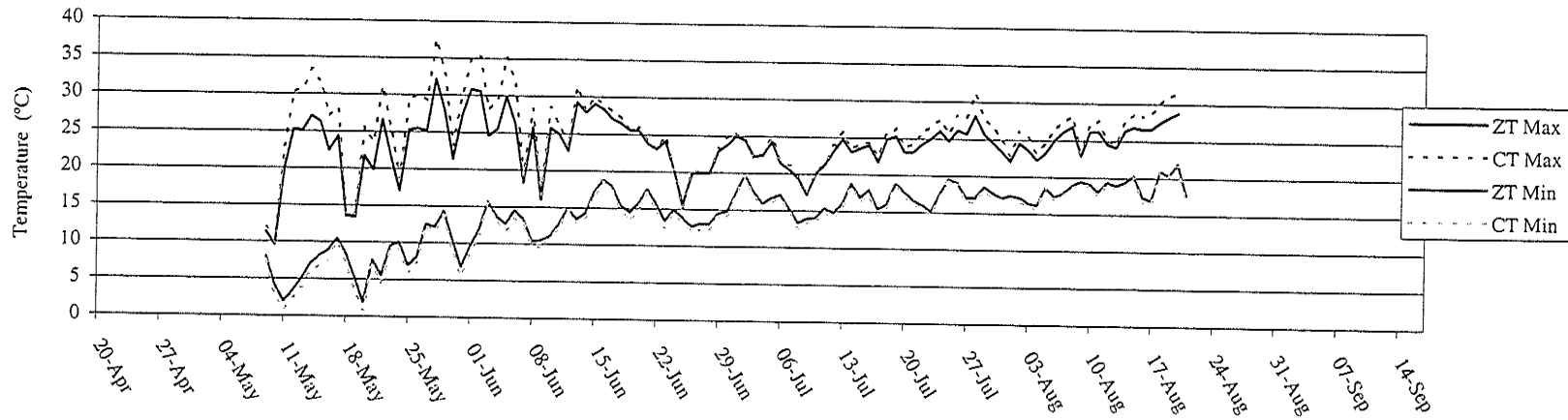
Figure 4.24 and 4.25 summarize the effect of N fertilizer rate on black medic recruitment at Carman in 2003 for flax and oats, respectively. No significant differences were observed, although there was a trend towards greater black medic recruitment in the low N treatments. Long term trials at Rothamsted showed significant increases in black medic recruitment in treatments without additional N as compared to additional N



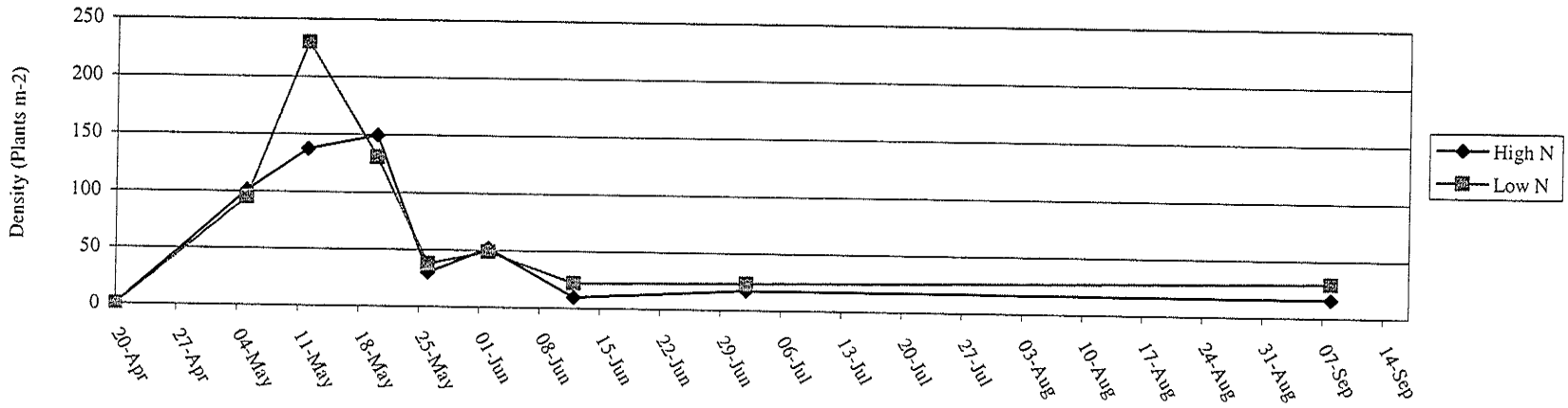
**Figure 4.21:** Maximum and minimum soil temperatures in flax as influenced by zero-tillage (ZT) and conventional tillage (CT) at Carman in 2003.



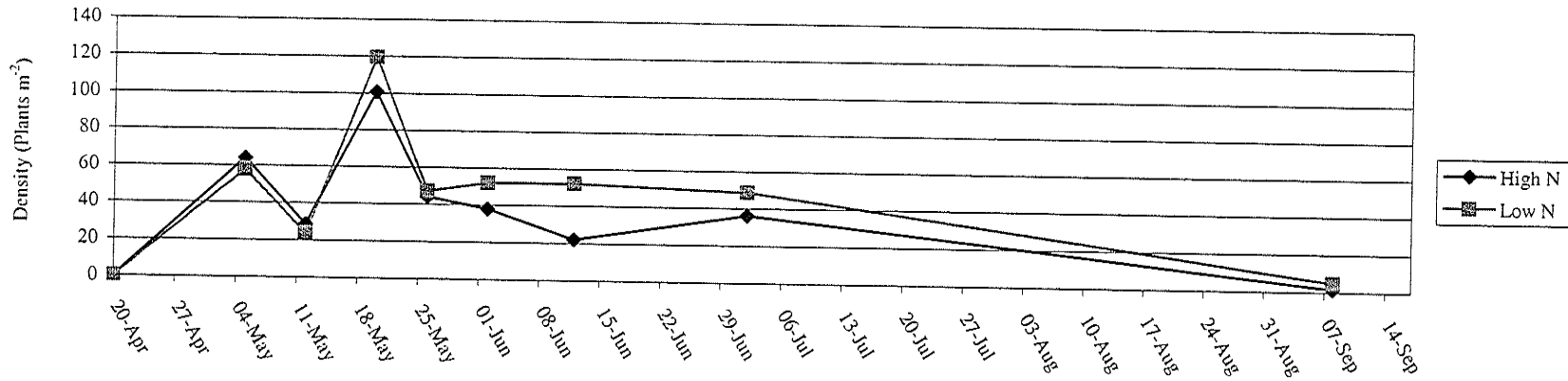
**Figure 4.22:** Black medic recruitment (plants m<sup>-2</sup>) in oats as influenced by zero tillage (ZT) and conventional tillage (CT) at Carman in 2003. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



**Figure 4.23:** Maximum (Max) and minimum (Min) soil temperature in oats as influenced by zero tillage (ZT) and conventional tillage (CT) at Carman in 2003



**Figure 4.24:** Black medic recruitment (plants m<sup>-2</sup>) in flax as influenced by high nitrogen (High N) and low nitrogen (Low N) fertilizer application rates at Carman in 2003. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



**Figure 4.25:** Black medic density (plants m<sup>-2</sup>) in oats as influenced by high nitrogen (High N) and low nitrogen (Low N) fertilizer application rates at Carman in 2003. Sampling dates with an asterisk (\*) are significantly different at P<0.05.

(Cavan et al. 2000). Important to remember is that the trials at Rothamsted have been in place for over 100 years and the differences in N rate are much larger than those in our study. N effects on recruitment are likely long term effects caused by gradual changes in the N level over time until an equilibrium is reached.

#### **4.2.1.3 Winnipeg 2003**

##### 4.2.1.3.1 Crop Type

The effect of oat and flax crops on black medic recruitment was also observed in Winnipeg in 2003. Precipitation and air temperature data is presented in Figure 4.26 and 4.27. Black medic recruitment in flax was generally higher than in oats with significant differences on May 7, May 12 (prior to glyphosate application), May 21, and September 8 (Figure 4.28).

Significantly greater black medic recruitment in flax than in oats on May 7, 12, and 21 may have been due to higher soil VWC in flax versus oat plots (Figure 4.29). The positive effect of greater soil moisture on black medic recruitment is supported in the scientific literature. For example, higher soil moisture under a moss covered soil compared to a bare soil increased the recruitment of black medic in one study in southern Ontario (Pavone and Reader 1985).

Differences in crop residue from the previous year influenced soil VWC between crop treatments. The previous crop for flax in 2003 was oats and the previous crop for oats in 2003 was winter wheat. Based on visual observations in the spring of 2003, crop residue levels were 4 times greater for the oat stubble than the winter wheat stubble.

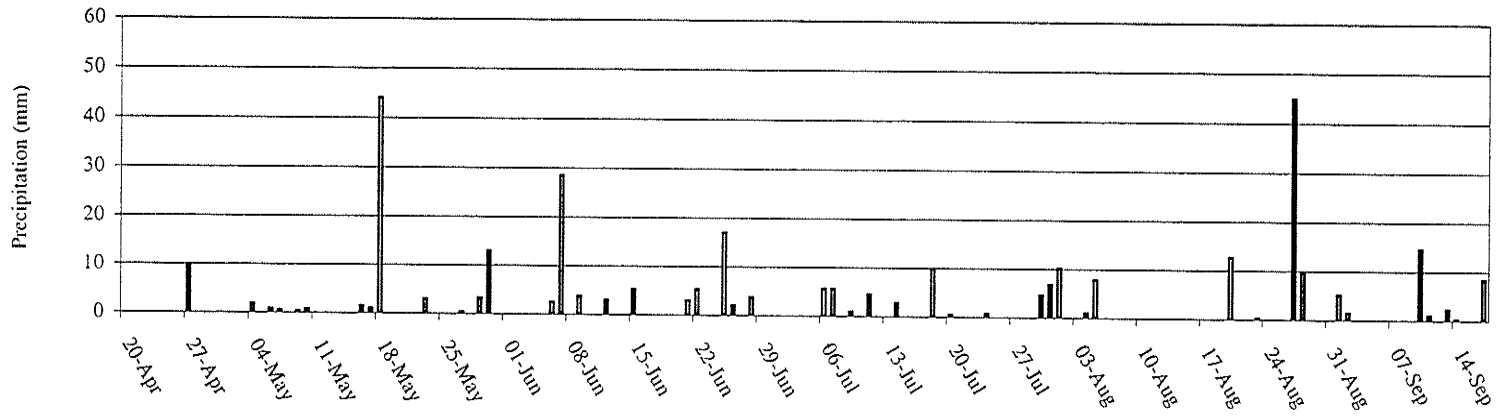


Figure 4.26: Precipitation (mm) at Winnipeg in 2003

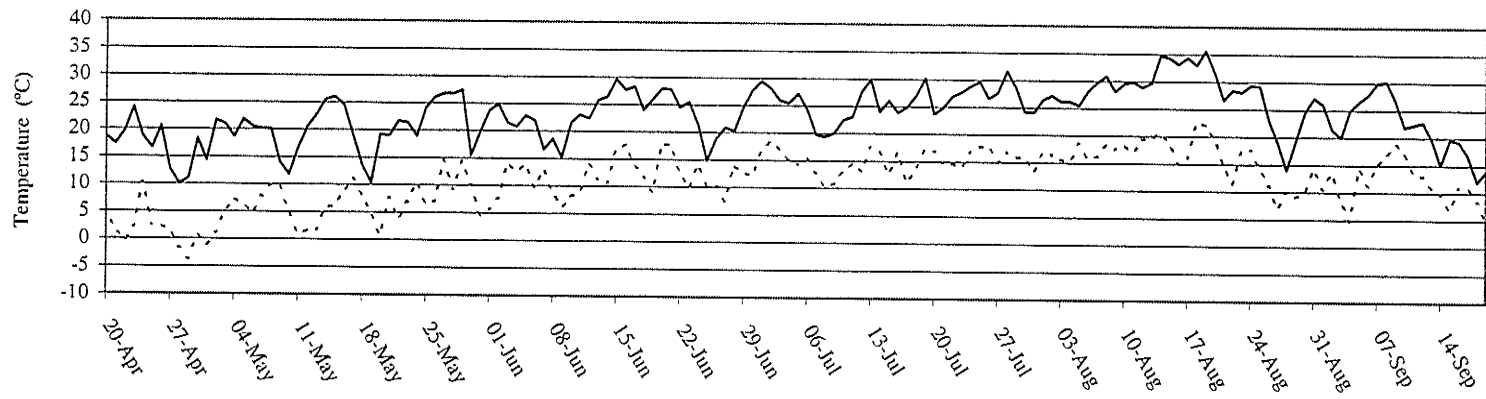
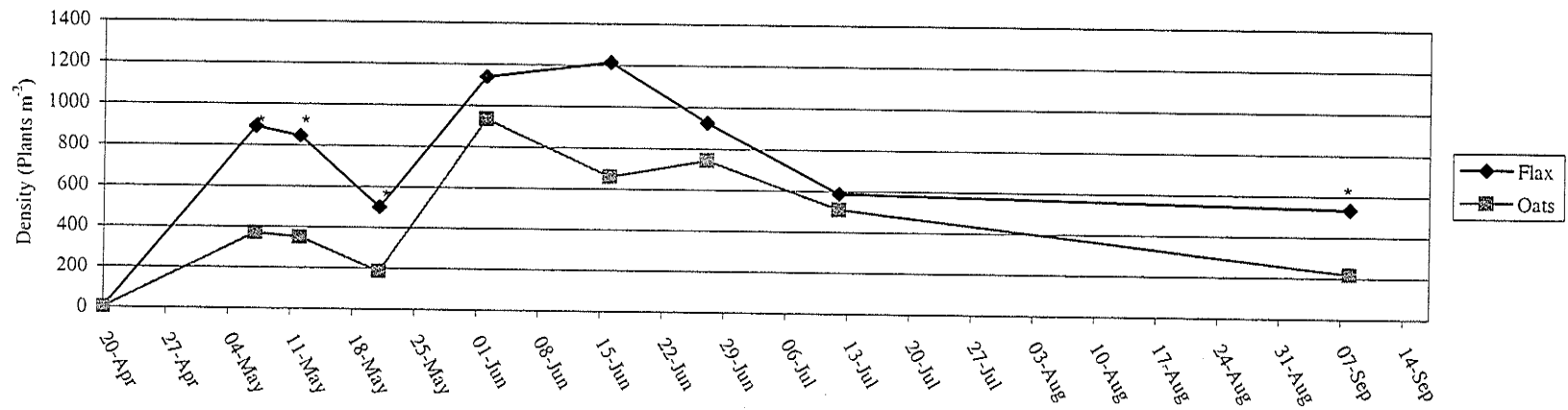
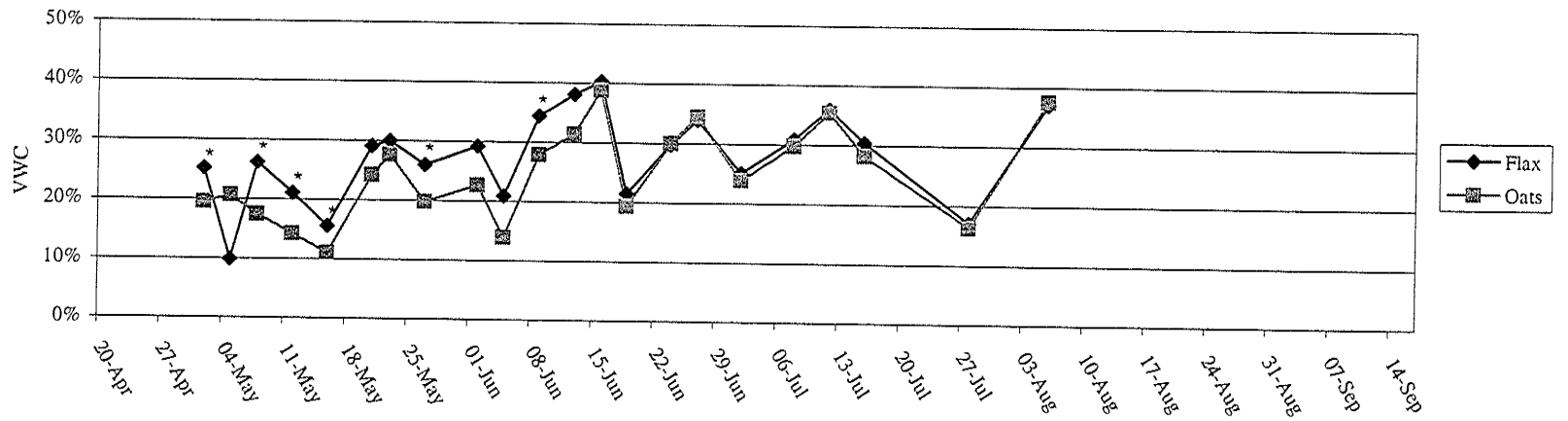


Figure 4.27: Daily maximum (solid line) and minimum (dotted line) temperature at Winnipeg in 2003.



**Figure 4.28:** Black medic recruitment (plants m<sup>-2</sup>) as influenced by crop (flax and oats) at Winnipeg in 2003. Sampling dates with an asterisk (\*) are significantly different at P<0.05.



**Figure 4.29:** Soil volumetric water content (VWC) from 0-2 cm as influenced by crop (flax and oats) at Winnipeg in 2003. Sampling dates with an asterisk (\*) are significantly different at P<0.05.

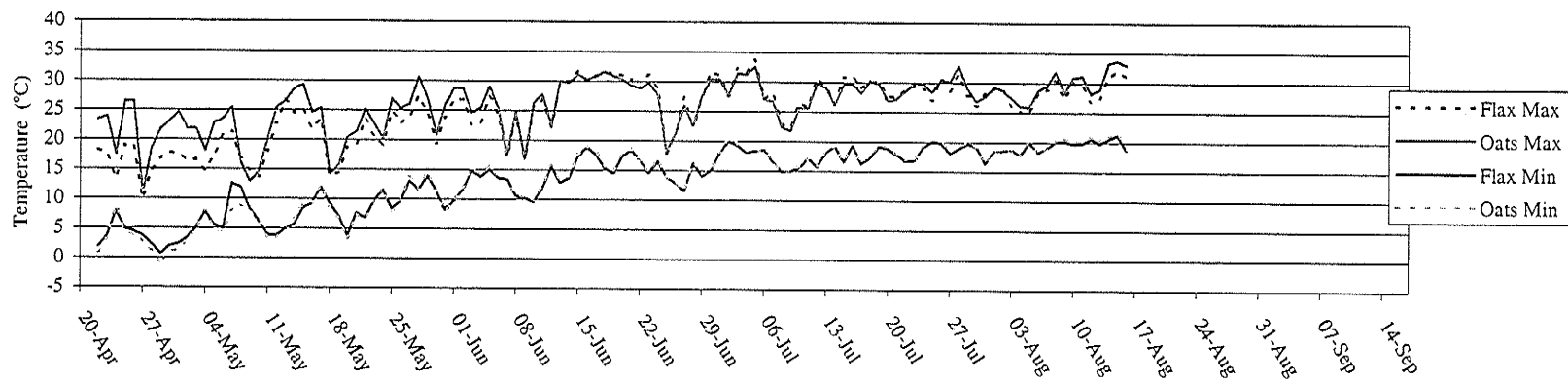
Differences in crop residue level may have caused the greater soil VWC levels in flax compared to oats prior to May 10 (Figure 4.29) by limiting soil moisture evaporation from the surface soil.

Higher minimum soil temperature and lower maximum soil temperature from April 22 to May 6 (Figure 4.30) may have also been the result of greater residue in the flax versus the oat crop. The minimum soil temperature in the flax crop was significantly higher than in the oat crop on all days except two and the maximum soil temperature in the flax crop was significantly lower than in the oat crop for all days. The average minimum soil temperature in the flax and oat crop from April 22 to May 6 was 4.1 and 3.3 °C, respectively, and the average maximum soil temperature was 16.6 and 21.6 °C, respectively.

Although there were differences in maximum and minimum soil temperatures between treatments (Figure 4.30), the differences do not appear to be biologically significant. Van Assche et al. (2003) proposed that a fluctuation of 5/16 °C was sufficient for the second softening stage of black medic. This range is similar to temperature fluctuations observed in both crops at the Winnipeg site. Therefore, despite significant differences in maximum and minimum temperatures between crop type, black medic would have been exposed to appropriate low temperature fluctuation cycles in both flax and oats in order to ensure the completion of the second stage of the softening.

Black medic recruitment levels were not significantly different between crops on June 2, 16, 27, and July 12 (Figure 4.28), although there was a trend towards greater recruitment in flax. During this same time period, soil VWC was significantly greater under flax than under oats on June 9 (Figure 4.29), but was not significantly different





**Figure 4.30:** Soil maximum (Max) and minimum (Min) temperatures as influenced by crop (Flax and Oats) at Winnipeg in 2003.

between crops on the remaining days. Small differences in soil temperatures were observed during this same time period (Figure 4.30). The trend towards higher black medic recruitment in flax is therefore not explained by differences in microsite conditions.

Black medic recruitment ended on approximately June 2 and June 16 in the oat and flax crop, respectively (Figure 4.28). This observation may have been caused by a termination in recruitment or suicidal germination. Unfortunately, our measurement of recruitment by monitoring changes in black medic density does not specifically focus on germination, but rather measures germination by counting the recruited seedlings. Oats likely competed more than flax for available resources (i.e.: nutrients and water) which caused suicidal germination of black medic due to increased competition.

The black medic recruitment pattern was similar for flax and oats. A large initial germination flush from approximately April 20 to May 8 established 891 and 370 plants  $m^{-2}$  in the flax and oat treatments, respectively (Figure 4.28). Further recruitment was prevented by low soil VWC in both flax and oats (Figure 4.29). On May 12, soil VWC dropped near the wilting point. For example, on this date, the soil VWC in the flax and oats was 15 and 11%, respectively. Soil VWC increased significantly after the rainfall event on May 18 which initiated another germination flush similar in size to the early spring flush for both crops. Although soil moisture conditions were adequate for additional recruitment after June 8, no further recruitment occurred in either crop.

The black medic recruitment trend observed in flax and oats in Winnipeg provides further evidence of the operation of the two-stage softening process. Black medic recruitment began after the winter chilling period when the seeds were exposed to

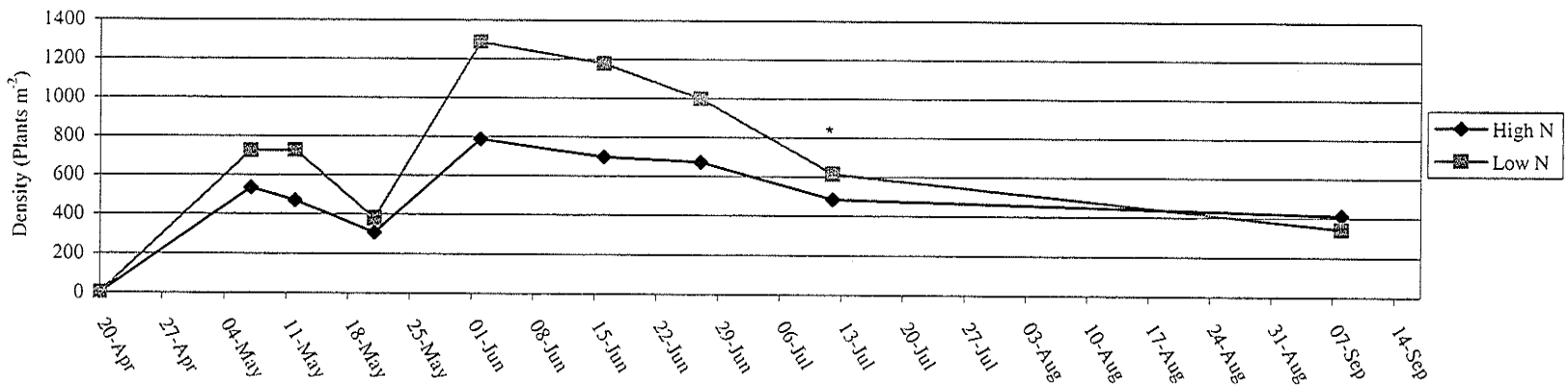
fluctuations of approximately 5/16 °C (Figure 4.30). Recruitment continued until approximately May 20 when the minimum temperature exceeded 5 °C. Based on the results in Experiment 2 of our study, this cessation of recruitment would be expected at this time since there appears to be a critical minimum softening temperature of between 5 and 10 °C. Further studies are required to identify the critical minimum softening temperature.

#### 4.2.1.3.2 Nitrogen Fertilizer Rate

Figure 4.31 summarizes the effect of N fertilizer rate on black medic recruitment at Winnipeg in 2003 . Similar to results in both years at Carman, there was a trend towards greater black medic recruitment under the low N treatment. However, the difference was significant only on July 12. Vigorous black medic and main crop growth in the high N treatment may have reduced black medic recruitment in comparison to the low N treatment. How these effects influenced conditions at the microsite are not known.

Based on visual observations up until mid June, black medic seedlings were stunted and chlorotic in the low N treatments versus the high N treatments. However, black medic recruitment was not different between the N treatments. Reduced soil nitrate concentrations may have contributed to the reduced growth of black medic, and only when the rhizobium bacteria were able to satisfy the N demand of the black medic did the seedlings begin to grow more vigorously.

As mentioned earlier, the Rothamstead trials show significant differences in black medic recruitment between fertilizer N treatments. Whether the same N effects will become apparent in this trial can only be determined over the long-term.



**Figure 4.31:** Black medic recruitment (plants m<sup>-2</sup>) as influenced by high nitrogen (High N) and low nitrogen (Low N) fertilization rates at Winnipeg in 2003. Sampling dates with an asterisk (\*) are significantly different at P<0.05.

## 4.2.2 General Characteristics of Black Medic Recruitment

### 4.2.2.1 Growing Degree Days and Black Medic Recruitment

Accumulated GDD's are often used to forecast the recruitment period for weed species (Bullied et al. 2003). Table 4.5 presents black medic recruitment data based on the accumulated GDD's up until the cessation of the spring recruitment flush. The range in total GDD's for the three site years was from 650 to 766 GDD. This variation was likely the result of microsite differences, characteristics of the soil seed bank, the effect of variable moisture levels during the spring period, and variation in the complex interaction between hard seed coat breakdown and specific temperature fluctuations for breaking dormancy. To reduce this variability, a hydrothermal model similar to that presented by Forcella et al. (2000) would be useful to predict the recruitment of black medic. Such a model would account for the effect of fluctuating soil VWC on recruitment. However, specific temperature regimes, not just GDD accumulations, would be necessary to improve the accuracy of the prediction.

**Table 4.5: Growing degree days required to complete the spring recruitment flush at Carman in 2002 and 2003 and Winnipeg in 2003**

Date	Site Year	GDD†
June 24	Carman 2002	766
June 5	Carman 2003	697
June 2	Winnipeg 2003	650

† GDD based on air temperature using a base temperature of 0 °C.

#### 4.2.2.2 Black Medic Recruitment Level Comparisons Between Site Years

Maximum recruitment comparisons between site years provides additional information about black medic recruitment ecology. Black medic recruitment from the seed bank in Carman was greater in 2003 than in 2002 for both crops (Table 4.6). This increase in recruitment cannot be attributed to additional seed production in 2002 because no seed was produced. Higher recruitment in 2003 was caused by either (a) environmental conditions being more suitable for germination in 2003 or (b) variable softening characteristics (i.e.: longer time period required for softening to occur) of the black medic seedbank cohort that enhanced germination in 2003. Studies of annual *Medicago* species in Australia indicated that relatively few seeds lose dormancy and germinate the first year after production (Lloyd et al. 1997). However, increased softening and germination were observed after the second year. Black medic emergence also reached a maximum in the second year as noted by Chepil (1946b) and Roberts and Feast (1973). Therefore, the additional recruitment observed at Carman in 2003 appears to be due to genetic effects which delay softening until the second year after production.

**Table 4.6: Black medic maximum recruitment under ZT flax and oats at Carman in 2002 and 2003 and Winnipeg in 2003**

Site Year	Crop	Black Medic Maximum Recruitment (plants m <sup>-2</sup> )
Carman 2002	Flax	20
	Oats	26
Carman 2003	Flax	298
	Oats	190
Winnipeg 2003	Flax	1214
	Oats	743

Greater black medic recruitment at Winnipeg versus Carman may be due to differences in site characteristics and management systems. Soil type may have also had an effect. The Winnipeg site was a silty clay and the Carman site was a very fine sandy loam. Soil texture may have contributed to differences in recruitment. Most significant, however, may be the difference in management between the two sites. The Winnipeg site was established a year earlier than the Carman site (see section 3.2.2.1) and during the second year an additional 10 kg ha<sup>-1</sup> of black medic seed was spread on the plots. At Carman, no additional black medic seed was added to the plots after the initial establishment year in flax in 2001. In addition, there were differences in black medic seed production between the two sites (Bamford 2003). Excellent black medic seed production in 2001 and 2002 at Winnipeg may also have been due to the fact that no broadleaf herbicide was applied. At Carman in 2002, the application of broadleaf herbicides eliminated the possibility of black medic seed production.

#### **4.2.2.3 Effect of Herbicide**

Broadleaf herbicide application caused a decline in black medic recruitment. Therefore, maintaining an adequate black medic recruitment level will require careful herbicide selection and timing. Varied degrees of tolerance of black medic to different herbicides was observed in our study. Black medic showed greatest tolerance to the MCPA amine and propanil tank mix. This in-crop herbicide for broadleaf and grassy weed control killed 100% of the top growth of black medic, and approximately 5-10% of the seedlings recovered.

Glyphosate application reduced black medic seedling populations more than the MCPA amine and propanil mix. In general, 100% top growth kill resulted from applications of glyphosate at 623 and 879 g a.e. ha<sup>-1</sup>. Some tolerance to glyphosate was observed at these rates since a small portion (less than 5%) of seedlings survived the treatment and began to form new leaves at the base. Established plants with 5 or more true leaves were more resistant to glyphosate than seedlings with fewer than 5 true leaves. These findings are in agreement with preliminary trials at the University of Manitoba (Entz and Bamford 2003).

Black medic showed little tolerance to bromoxynil. The black medic population at Carman in 2003 was totally eliminated using 280 g a.i. ha<sup>-1</sup> of bromoxynil, the recommended rate for in-crop application in flax and oats. Excellent growing conditions (moisture and temperature) during the bromoxynil application at Carman in 2003 may have improved the efficacy of bromoxynil.

#### **4.2.3 Summary**

In 2 of the 3 site-years, black medic recruitment was influenced by crop type. Black medic recruitment was significantly greater in flax than in oats at Carman in 2002 on only one observation day and in general, was greater at Winnipeg in 2003 during the majority of the growing season. Higher soil temperature and greater surface residue levels under flax compared to oats were the main factors contributing to these differences in recruitment levels.

Important characteristics about the recruitment ecology of black medic in different tillage systems were observed. The most significant effect of tillage on black



black medic recruitment appeared to be the vertical redistribution of seeds in the profile. Soil depth influenced temperature and moisture conditions, both of which were critical factors in recruitment. Furthermore, tillage effects on black medic recruitment highlighted the microsite recruitment limitations. Due to the juvenile state of the tillage systems, relatively small impacts on soil properties and microsite conditions between tillage systems were observed. In the future, greater effects of tillage on black medic recruitment caused by changes in organic matter content, pore distribution, and bulk density may become apparent.

Nitrogen fertilizer rate affected black medic recruitment only marginally. The greatest effect was seen at the Winnipeg site where there was greater black medic recruitment under low N fertilizer applications.

Dormancy loss in spring was evidence of the operation of the two-stage seed softening process. Fluctuating soil temperatures in spring with minimum temperatures in the 5 °C range were likely the reason for dormancy loss. A time-lag effect between dormancy loss and germination was attributed to low soil VWC.

Late season dormancy loss and subsequent recruitment was attributed to the operation of a Mediterranean two-stage softening process. Elevated soil temperatures in summer and lower fluctuating temperatures in the presence of moisture likely caused dormancy loss late in the season for a portion of the seeds.

## 5.0 GENERAL DISCUSSION

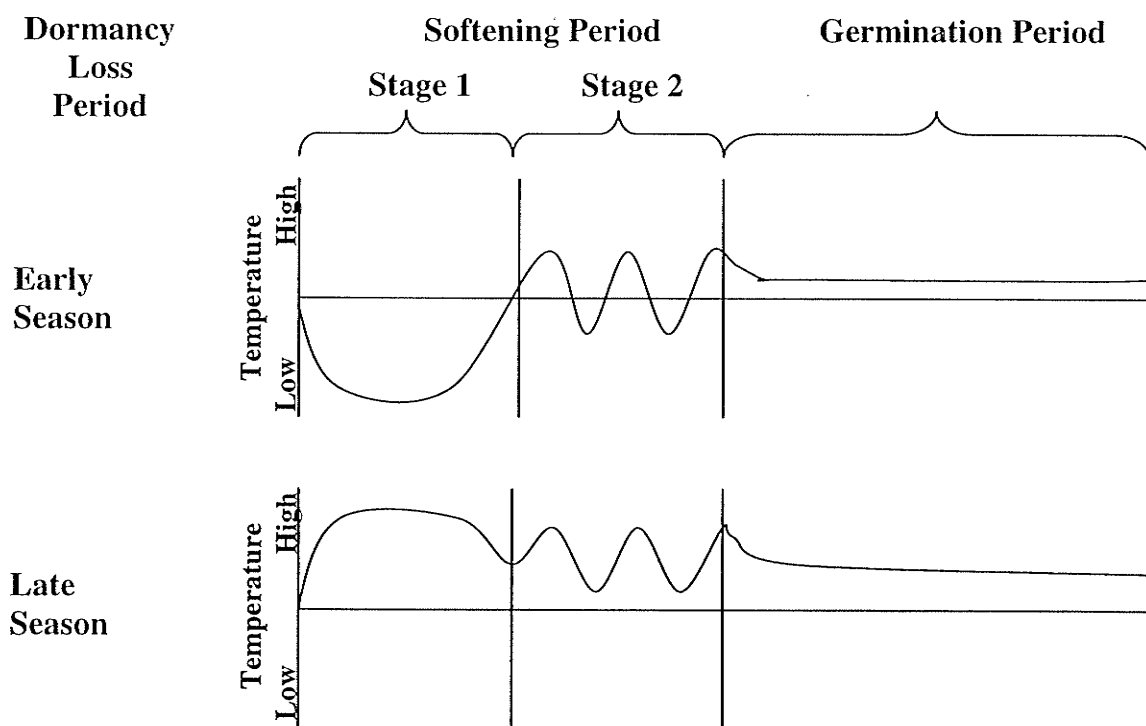
### 5.1 Black Medic Dormancy Loss and the Two-Stage Softening Process

The two-stage softening process in medics was first described by Taylor (1981) to explain the effect of temperature on dormancy loss in medic seeds in a Mediterranean climate. Recently, Van Assche et al. (2003) discovered that medic dormancy loss in black medic seeds in a temperate climate was also controlled by a two-stage process requiring different temperature regimes. In our study, early season dormancy loss of black medic in a continuous grain cropping system in Manitoba was explained based on the temperate climate two-stage softening process presented by Van Assche et al. (2003). In addition, late season recruitment of black medic in the same cropping system was explained using the Mediterranean climate softening process presented by Taylor (1981).

Black medic dormancy loss is the result of a two stage softening process which fractures the seed coat in order that moisture may enter the seed and begin the germination process. In our study, dormancy loss occurred both early and late in the growing season as a result of distinct temperature regimes during each period (Figure 5.1). Early season dormancy loss during the spring recruitment period resulted when black medic was exposed to a chilling period during the winter period followed by higher fluctuating temperatures in the spring. During the late season recruitment period, elevated temperatures followed by cooler fluctuating temperatures resulted in dormancy loss.

This study has made the following contributions to the understanding of black medic dormancy loss: (a) confirmed the discovery by Van Assche et al. (2003) of the

two-stage softening process in a temperate climate, (b) highlighted the importance of moisture during the second stage of the early season softening process in a temperate environment, (c) linked the medic two stage softening process in a Mediterranean climate to late season medic softening in a temperate climate, (d) identified a medic species that appears to respond to both Mediterranean and temperate two-stage softening processes, and (e) studied the management effects on black medic dormancy loss under a continuous grain cropping system.



**Figure 5.1: Conceptual model of the black medic two-stage softening process during early and late season dormancy loss periods.**

The precursors to dormancy loss that cause seed coat softening are not well understood. The theory developed by Zeng (2001) (see section 2.4.3.1) provides an explanation based on physical and chemical seed coat changes that may result under varied temperature and moisture regimes. However, based on the information presented in this thesis, the theory by Zeng (2001) needs to be re-evaluated as there are critical questions which Zeng's theory does not address. First, can a chilling first stage temperature regime have the same physical weakening effect in the seed coat as compared to an elevated first stage temperature regime? An alternative theory may be that temperature treatments during the first stage may not have a physical weathering effect, but rather trigger cellular changes within the seed which affect the nature of the chemical bonds in the lipids. Further study is required of the impact of different environmental conditions at the seed microsite during the first softening stage on physical and chemical conditions in the seed coat. Second, why do temperature requirements during the second stage differ depending on the temperature treatment in the first stage? Based on the results of the present study and work by Van Assche et al. (2003), a fluctuating temperature with a minimum temperature of approximately 5 °C was necessary for final seed coat softening and germination after a low temperature first stage treatment. In contrast, a higher second stage fluctuating temperature (15/30 °C) was appropriate for final softening under a late season softening regime in this study. The current understanding of physical and chemical changes in the seed coat during the first stage of softening do not provide an adequate explanation for the need for varied temperature requirements during the second softening stage. Third, why are fluctuating temperatures important in the second softening stage? Zeng (2001) emphasizes the

importance of water (chemical) to hydrolyze the seed coat lipids, but does not explain the importance of fluctuating temperatures. Are the seed coat changes strictly the result of physical and chemical changes within the seed coat? Perhaps there are responses at the cellular level to moisture and temperature conditions in the microsite. This alternative interpretation would suggest that both physical and physiological dormancy mechanisms are involved. Additional research is required comparing the effects of different two-stage softening regimes. This information may be instrumental in furthering our understanding of the dormancy loss processes in black medic.

## **5.2 Adding Diversity to Cropping Systems with Self-Regenerating Cover Crops**

Cropping systems on the Canadian prairies have been simplified during the past 50 years. Before intensive herbicide use, cropping systems usually had a variety of weed species growing simultaneously in the main crop. Herbicides reduced this diversity by reducing the presence of other species aside from the main crop. Nutrient management was also simplified with the introduction of chemical fertilizers. Soil fertility was traditionally maintained with legume forage crops, green manures, summerfallow, and livestock manure inputs. Chemical fertilizers replaced the need to rely on fertility building crops and thereby simplified the rotation by decreasing temporal diversity. In addition, a simplified weed management system was adopted that promoted reliance on fewer herbicide types for weed control. Accompanying these changes in production practices was a fundamental shift towards a management system that emphasized simplified decisions. Understanding cropping system ecology dynamics to solve production problems was replaced by a heavy reliance on chemical inputs and advice

from the agronomist selling the inputs. Each of these simplifying effects have combined to produce an agriculture production system that now lacks biodiversity and the ability to respond to production problems out of the conventional input driven agri-business system.

The use of self-regenerating legume cover crops in annual cropping systems on the Canadian prairies represents one approach to increase the diversity of cropping systems. The self-regenerating cover crop will grow simultaneously with the main crop enhancing spatial diversity. Nutrient management will be diversified, relying not only on chemical fertilizers, but also on the nitrogen fixation benefits from the legume cover crop. The overall management approach will also require diversification. In order to manage the self-regenerating crop, a broad knowledge of ecosystems and their interactions will be required.

### **5.3 The Self-Regeneration Potential of Black Medic**

The present study has provided knowledge about black medic as a self-regenerating legume cover crop. The specific purpose of our study was to understand the recruitment ecology of black medic within a cropping system. Understanding this characteristic was key to develop a cover crop that would not require re-seeding year after year. Three site years of field trials and two laboratory experiments showed that dormancy loss and germination (two vital recruitment processes) were related to moisture and temperature conditions in the black medic seed microsite. Conditions favouring dormancy loss and germination were met primarily during the spring period resulting in black medic recruitment from the end of April to mid June. In general, black medic

recruitment was greater in ZT versus CT treatments. Maximum black medic recruitment in ZT was 243 and 1214 plants m<sup>-2</sup> at Carman in 2003 and Winnipeg in 2003, respectively. These levels of recruitment were considered sufficient to develop a cover crop. Therefore, black medic satisfied the self-regenerating characteristic required to function as a self-regenerating legume cover crop in ZT systems.

#### **5.4 Characteristics of an Ideal Cropping System for Black Medic**

Black medic as a self-regenerating legume cover crop is best adapted to cropping systems where low levels of N are applied. At Winnipeg in 2003, the flax yield on the low N treatments with medic was 21% higher than the low N plots without medic (Appendix D). However, flax yield on high N treatments was significantly greater in the absence of medic. Reduced N availability also reduces the competitive effect of the main crop on black medic. Organic cropping systems often have an N deficit and therefore may benefit from a self-regenerating cover crop such as black medic.

A cropping system located in a region or an area of the landscape where moisture deficits seldom occur is best suited for a self-regenerating cover crop such as black medic. Moisture competition between the crop and the black medic may reduce yields. The thin black soil zone of the Canadian prairies is likely the best location to include medics in a cropping system.

Farm systems that integrate livestock and annual crops are also an ideal choice to utilize black medic as a self-regenerating cover crop. This allows the producer to use the fall black medic growth for grazing.

## 5.4 Understanding Recruitment Ecology with Laboratory and Field Experiments

Recruitment ecology deals with the interaction of biotic and abiotic factors that result in seedling recruitment. Understanding the process of seedling recruitment has been difficult given the wide range of factors and complex interactions involved in recruitment. To understand the process of seedling recruitment, an in depth knowledge of seed biology, the ecosystem, and the interactions between the two is required.

Laboratory and field experiments are commonly used to understand seedling recruitment. Each approach has advantages and disadvantages. Laboratory experiments allow researchers to control environmental conditions and examine the interaction of several factors that eventually lead to seedling development. These studies are useful and relatively simple and provide detailed information on recruitment ecology and biology of a species. However, these experiments fail to capture the range of interactions and environmental conditions that influence recruitment under field conditions.

While field experiments capture the range of environmental conditions that influence the recruitment of a seedling, there are many problems associated with field experiments. The most obvious shortcomings include understanding (a) which factors are influencing recruitment, (b) the relative importance of each factor, (c) the interactions between the factors, and (d) the inability to obtain an exact measurement of many of the environmental conditions at the seed microsite level given the current technology.

Accurate measurement of soil moisture at the field level is difficult to accomplish with current technology. In the present study, soil VWC was measured based on bulk density and water volume in soil samples taken at a depth of 0-2 cm at a specific point in time. As a result, soil VWC across many microsites was averaged disregarding (a) the



heterogeneous nature of soils in terms of soil texture and organic matter across the landscape (vertical and horizontal) and (b) temporal fluctuations that occur over an hour, a day, or a week, and (c) the variability of VWC with depth. Another soil moisture measurement technique that captures soil moisture fluctuations over time is time domain reflectometry and frequency domain reflectometry. The major shortcoming of this technology is that (a) the sphere of influence (10 to 15 cm diameter) is much larger than the recruitment zone and (b) the amount of moisture in the soil affects the volume being tested (Bullock et al. 2003). The use of near-infrared spectroscopy for determining soil water in small volumes may improve the ability to measure soil microsite conditions over time (Bullock et al. 2003). However, the accuracy of measuring specific variables like soil moisture will depend on the sensitivity of a particular species to the variable. This scale will then determine the level of accuracy required in the measurement of the environmental conditions.

In light of the advantages and disadvantages of laboratory and field studies, both methods must be used at the same time to further our understanding of recruitment ecology. This combined approach was used in past studies related to the recruitment of black medic (Sidhu 1971; Pavone and Reader 1982; Hogenbirk and Reader 1989; Van Assche et al. 2003). In the present study, laboratory and field studies complemented each other, and often field observations were validated or explained with results of simple laboratory experiments. For example, the laboratory experiment that compared the effect of fluctuating 5/40 and 10/40 °C explained the cessation of spring recruitment when minimum temperatures in field trials exceeded 5 °C.

Unique to our study was the recruitment environment. Previous black medic recruitment studies did not examine recruitment in an annual cropping system. Therefore, understanding recruitment in an annual cropping system required information about the microclimatic effects of the cropping system. Measurements at the microsite provided information on the unique seed microsite environment within a cropping system under several management treatments. Some of these environmental conditions were then transferred to the laboratory for further study under controlled conditions. This model utilizing both laboratory and field studies is necessary to understand the ecology and regeneration of all plant species.

### **5.5 Black Medic as a Weed Problem**

Reports of black medic as a weed by some producers raises concern regarding the future of black medic as a self-regenerating legume cover crop. There are several situations when black medic may become overly competitive with the main crop. If post-emergence broadleaf herbicides are not applied in the crop, especially in a ZT system, black medic will become very competitive with the main crop. At Winnipeg in 2003, this effect was observed since broadleaf herbicides were not applied. Yields of flax with high N, oats with high N, and oats with low N were reduced by 27, 18, and 11%, respectively, when grown with black medic as a self-regenerating cover crop (Appendix D). This competition may occur when post-emergent herbicides are not applied during a drought. Rains later in the season may cause a flush of black medic that will become very competitive with the crop. However, this situation can be reversed based on yield data from Winnipeg in 2003. Flax grown with low N inputs was 21% higher with black

medic as a self-regenerating cover crop. Therefore, finding the optimum combination of herbicides and cropping systems is necessary to minimize yield losses when using black medic as a self-regenerating cover crop.

The present study also indicates that an open canopy will result in significant softening of the black medic seed so that when rain eventually comes, black medic will rapidly germinate and form a dense under-story in the main crop. The result will be increased competition with the main crop and a large seed input into the soil seed bank which will emerge year after year. Timing of post-emergent herbicides will also affect the level of competition between black medic and the main crop. Based on the North Dakota case study (Hagen 2001), early applications of post-emergent herbicides resulted in less suppression of the black medic. An early herbicide application gave time for another flush of black medic to emerge from the soil seed bank or for the existing stand to recover from the herbicide which resulted in significant crop competition. As well, continued use of herbicides to which black medic is partially resistant, may also result in black medic becoming a major weed. In the present study, black medic showed some tolerance to MCPA amine and glyphosate. A black medic herbicide tolerance study at the University of Manitoba in 2003 indicated similar results, especially when reduced herbicide doses were used and when black medic had more than 5 true leaves.

Controlling black medic competition with the main crop requires sound management decisions. If producers understand the environmental conditions that favour recruitment, adopt management practices that decrease the competitive ability of black medic, and select an appropriate herbicide program that effectively controls black medic, the benefits of black medic will be realized while reducing the risk of black medic

competition. The same multi-faceted approach promoted for general weed control also applies to black medic.

## **5.6 Recommendations for Future Research**

Research into the use of self-regenerating legume cover crops for continuous grain cropping systems in the Canadian prairies is just beginning. Additional research priorities are as follows.

**1. Ideal black medic ecotype:** The search for the ideal black medic ecotype will continue. Desired characteristics include low competition with the main crop, synchronized resource use, weed suppression, excellent N fixation, and prolific seed production. Selecting for these traits can be done by allowing the cropping system to naturally select for the ideal ecotype and by introducing new accessions of black medic from analogous climatic zones. Growth characteristics of the black medic developed in North Dakota should be compared to the original populations of black medic from Montana to determine the impact of agronomic management on black medic recruitment and growth characteristics.

**2. Black medic farm survey:** Many producers from the Canadian prairies are currently using black medic as a self-regenerating cover crop in their cropping system. Other producers have complained that black medic is becoming a weed. Surveying the experiences of these farmers during the growing season will be valuable in gathering more information on the limitations and benefits of black medic as a self-regenerating cover crop.

**3. Black medic softening patterns:** The present study began to examine some of the factors that influence the softening of black medic seed. Temperature and moisture were highlighted as the most significant factors in dormancy loss. However, more information is needed on the range of temperature and moisture conditions that cause dormancy loss. This information includes: (a) the effect of moisture on both stages of the two-step softening process of black medic, (b) the fluctuation range and duration of temperature treatments on the second stage of the two-step softening process, and (c) the interaction between temperature and moisture on black medic seed softening. In addition, field experiments focusing on the effect of different tillage systems and tillage depth on softening will be helpful to further understand black medic recruitment ecology.

**4. Quantifying the benefits of black medic:** The benefits of including black medic as a self-regenerating cover crop must be quantified to properly evaluate the cropping system. These benefits include the nitrate addition to the soil, plant disease reduction, weed suppression, and water use efficiency. This data from the existing long term trials needs to be gathered and analyzed.

## 6.0 CONCLUSIONS

The following points summarize some of the conclusions resulting from the work of this thesis.

- Temperature and moisture conditions are the primary factors that control dormancy loss and germination of black medic.
- Black medic dormancy loss is the result of a two-stage softening process.
- Fluctuating temperatures with a minimum of 5 °C are required to complete the second stage of dormancy loss during early season black medic recruitment in a temperate climate.
- The delay or inhibition of black medic recruitment at low and high temperatures reduces the risk of freezing or desiccation of seedlings.
- Greater black medic recruitment in flax versus oats may be due to differences in soil temperature or increased residue from the previous crop which improves soil VWC.
- Black medic recruitment is usually greater under ZT versus CT. The primary mechanism for this difference is seed redistribution in the soil profile. Black medic seed in a ZT system is usually concentrated in the upper 2 cm where recruitment is more likely to occur.
- Nitrogen fertilizer does not significantly affect black medic recruitment, although a trend towards higher recruitment levels under low N treatments was observed.

- Black medic recruitment within a crop is affected by broadleaf herbicides, site characteristics, genetic characteristics, and the size of the soil seed bank.

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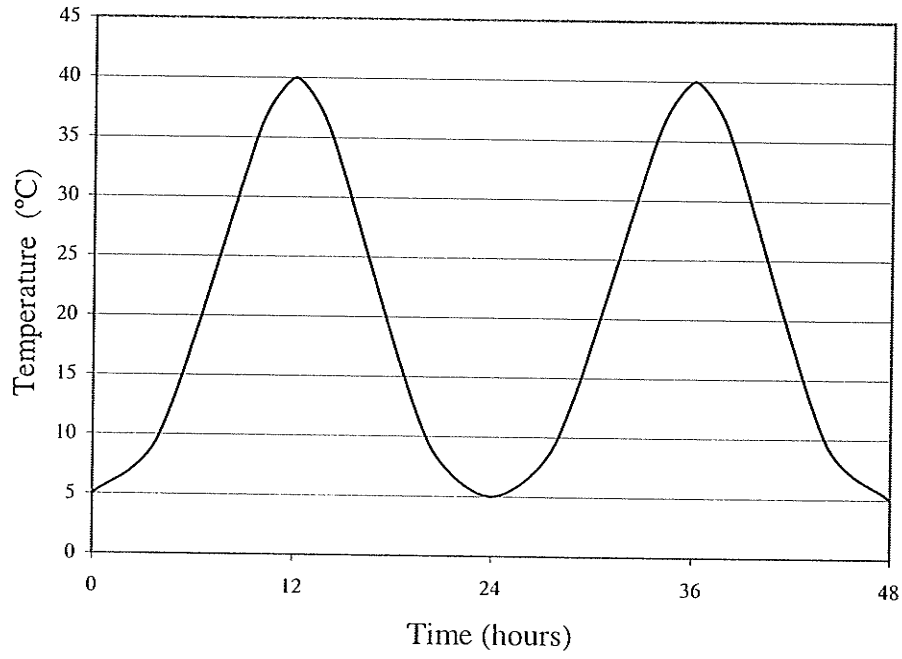
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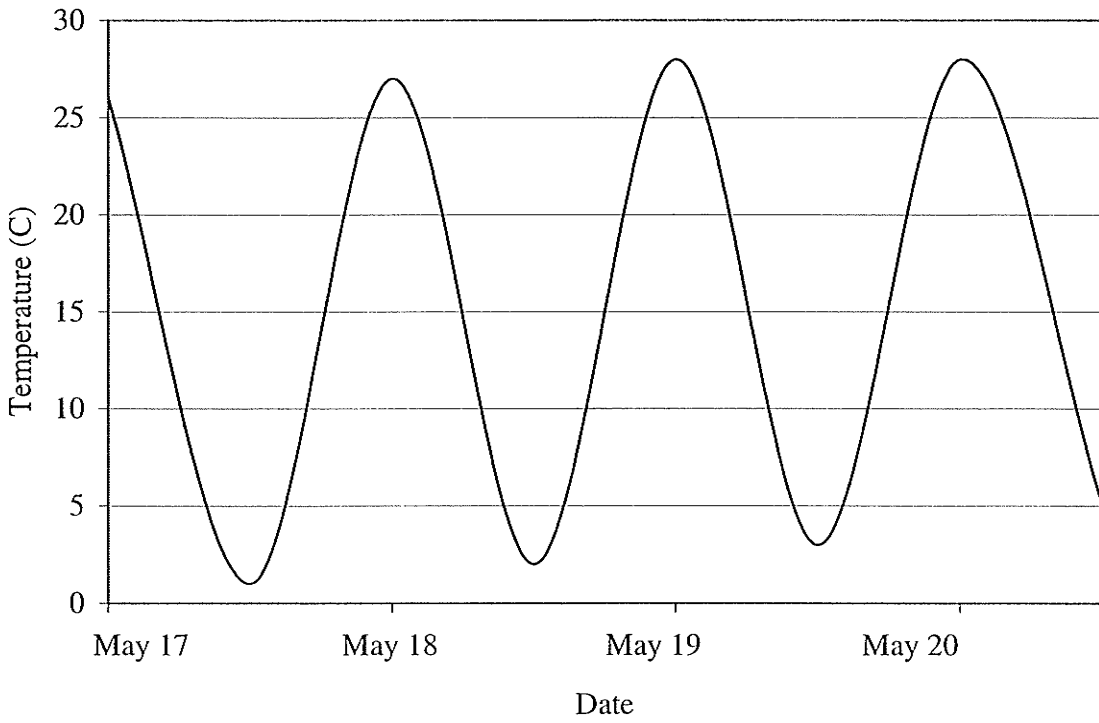
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## APPENDIX A

Diurnal temperature fluctuation (5/40 °C)  
used in laboratory experiments



**APPENDIX B**  
**Soil temperature fluctuations at Carman**  
**from May 17 to May 20, 2002**

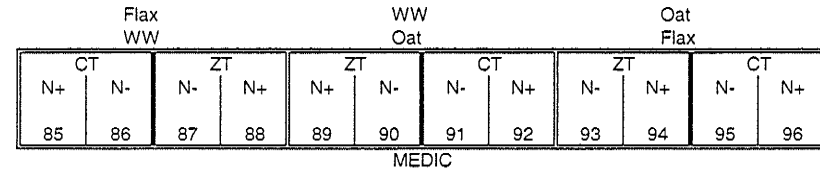
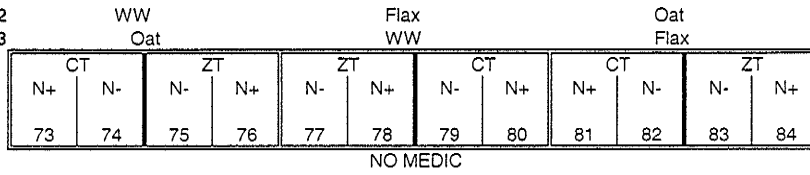


## Appendix C

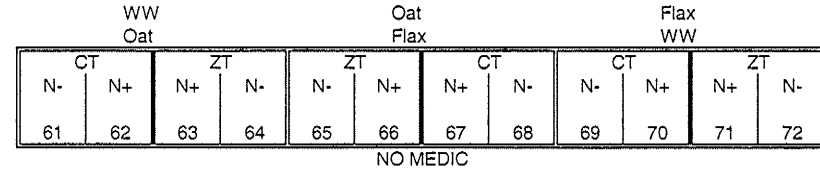
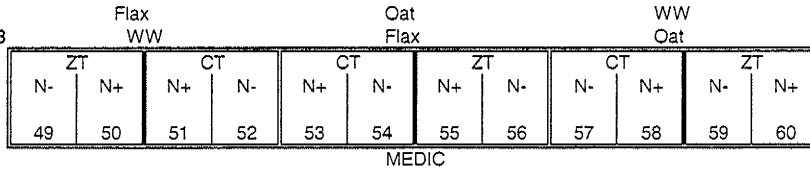
### Black Medic Plot Plan at Carman in 2002 and 2003

N ←-----

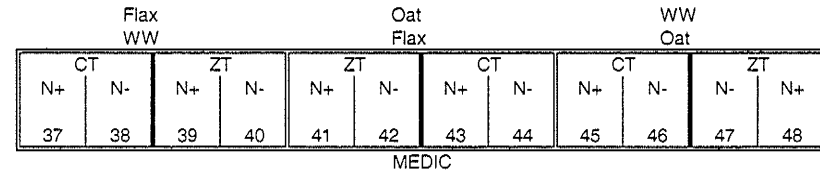
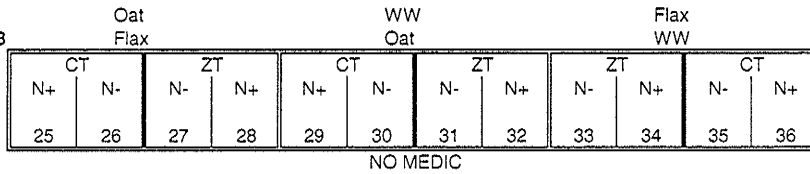
2002  
2003



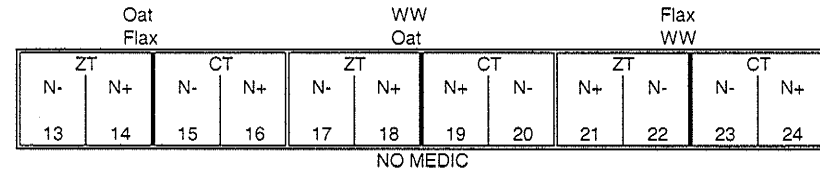
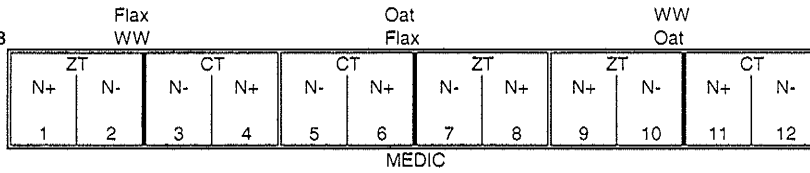
03



03



03



Main plot 60 x 8 m  
1. medic  
2. no medic

subplot 20 x 8 m  
1. Flax  
2. winter wheat  
3. Oat

subsubplot 10 x 8 m  
1. conventional tillage  
2. zero tillage

sssubplot 5 x 8 m  
1. n+  
2. n-

**Appendix D**  
**Flax and oat yields at Winnipeg in 2003 with and without black medic**  
**as a self-regenerating cover crop.**

Black Medic	Flax (low N)	Flax (high N)	Oat (low N)	Oat (high N)
	----- kg ha <sup>-1</sup> -----			
Present	650 a †	1053 b	1382 b	2591 b
Absent	537 b	1442 a	1561 a	3160 a

† Values followed by a different letter within a column are significantly different a P<0.05.