## INSECTS OF ALFALFA IN MANITOBA WITH PARTICULAR REFERENCE TO Lygus spp., Adelphocoris lineolatus (Hemiptera: Miridae) AND Acyrthosiphon pisum (Homoptera: Aphididae) AND THEIR NATURAL ENEMIES

A thesis

Submitted to the

## Faculty of Graduate Studies

of

The University of Manitoba

by

Md. Jashim Uddin

In Partial Fulfillment of the Requirements

for the Degree of

## DOCTOR OF PHILOSOPHY

Department of Entomology

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Winnipeg, Manitoba, Canada

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## Insects of Alfalfa in Manitoba with Particular Reference to Lygus spp., Adelphocoris lineolatus (Hemiptera: Miridae) and Acyrthosiphon pisum (Homoptera: Aphididae) and their Natural Enemies

BY

Md. Jashim Uddin

A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of

Manitoba in partial fulfillment of the requirement of the degree

Of

**Doctor of Philosophy** 

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#### Abstract

# Md. Jashim Uddin. University of Manitoba, 2005. Insects of alfalfa in Manitoba with particular reference to Lygus spp., Adelphocoris lineolatus (Hemiptera: Miridae) and Acyrthosiphon pisum (Homoptera: Aphididae) and their natural enemies.

## Supervisor: Dr. N.J. Holliday

The study was conducted from 1999 to 2001 to determine the most suitable method to be used for sampling insect pests on alfalfa, to determine the seasonal occurrence of common insect pests and natural enemies, to assess the roles of common natural enemies on insect pests, and to find out ways to increase the benefits from insect natural enemies in relation to the production practices of alfalfa in Manitoba. Among different sampling methods, sweep-net sampling was found to be the most consistent and reliable method of sampling most insect taxa, although beat tray sampling provided better estimates for Lygus spp. nymphs. It was found in the present study that Lygus spp. Hahn, Adelphocoris lineolatus (Goeze) and Acyrthosiphon pisum (Harris) were the most prevalent insect pests. Carabids, chrysopids, coccinellids, Nabis spp., Orius spp., spiders and opilionids were the most commonly occurring predaceous taxa. Circumstantial evidence from the field study and evidence from the laboratory study indicate that coccinellids play important roles in population growth of the above pests on alfalfa. Field and laboratory studies also provided evidence that carabids, coccinellids, chrysopids and nabids alone or together as a guild can suppress these pest populations on alfalfa. Parasitoids appeared to be important, as they killed a considerable percentage of Lygus spp. and A. pisum, but not of A. lineolatus. Aphidius ervi Haliday was the most prevalent aphid parasitoid.

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No control measures were required in hay fields, as swathing hay suppressed pest populations below damaging levels, partly through causing *Lygus* spp., and sometimes *A*. *lineolatus*, to move into adjacent seed fields. Conversely, insect pests were more numerous in seed fields, although these fields usually received chemical insecticides as pest control measures. Whether populations of natural enemies can be enhanced in seed alfalfa fields was examined, and it was found that nepetalactone, which is an aphid sex pheromone component, can be used to enhance natural enemy populations, and the benefits thereof, in seed fields.

Successful rearing of *Chrysoperla carnea* (Stephens) and *Chrysopa oculata* Say larvae by using *Megachile rotundata* (Fabricius) (leafcutting bee) prepupae as the chrysopid larval diet expanded the use of leafcutting bees and provided a basis for inexpensive culture of chrysopids. The potential of insect pests and their natural enemies for management in connection with the current alfalfa production practices are discussed.

## Dedication

To the ever deprived, yet greatest pastors: the farmers of Bangladesh;

to the eternal prophesy of a great philosopher: my mother, Mahmuda Begum;

and

to the great sacrifices of my beloved father, Late Abdul Jalil Hawlader, who was eager to

see the completion of this thesis.

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#### ACKNOWLEDGEMENTS

I express my sincere appreciation to my supervisor, Dr. N.J. Holliday, in the Department of Entomology, for the advice, encouragement, support and mentorship he offered throughout the study, and for critical review of the manuscripts. Other members on the advisory committee, Dr. P.A. MacKay, Dr. R.W. Currie and Dr. K.M. Wittenberg, provided invaluable advice throughout the study, and critical review of the manuscripts, which are highly appreciated.

Thanks are due to Dr. L.M. Dosdall for serving as the external examiner of this thesis. I am grateful to Dr. L. Donald, Dr. T.D. Galloway, G. Huebner, R. Lafreniére, and Dr. R.E. Roughley for greatly helping in numerous ways during the study. Without the great support and assistance from S. Graves, Dr. J. Hardie, Dr. T. Hooper, Dr. W. Powell and Dr. L.J. Wadhams in Rothamsted Experimental Station, UK, this study would not have been completed. Generous taxonomic assistantship provided by Dr. S.J. Brooks, Dr. Y. Bousquet, Dr. H. Goulet, Dr. K.S. Pike, and D. Wade is much appreciated.

Tremendous contributions were made to this study by the alfalfa growers: D. Smith, S. Rozznick, V. Ryssel, J. Skinner, B. Simundson, G. Johnson, H. Johnson and K. Fridfinnson. Thank you so much.

Continuous and enthusiastic technical support from L. Babey and D. Holder enabled completion of the research and this thesis, thank you so much. Thanks are also extended to B. Barnadette, R. Boone, P. Calvin, L. Capar, R. Carleton, J. Hartwig, T. Hoeschen, L. Hoppe and M. Lowdon, who helped in both the field and laboratory.

The study would not have been possible without the financial support from the Canadian Commonwealth Scholarship and Fellowship Program, the Agri-Food Research

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and Development Initiative, the Faculty of Graduate Studies, and the Faculty of Agricultural and Food Sciences, Graduate Students' Association, Students' Union, University of Manitoba, and The Entomological Society of Manitoba. Thanks are also extended to D. Cyre, for her administrative cooperation on matters regarding the Commonwealth Scholarship.

All the graduate students in the Department of Entomology, especially M. Alperyn, A. Hawkins-Bowman, K.S. Hemachandra, N. Lauro, A. Mostafa, R. Underwood, H. White and C. Wytrykush, were sources of encouragement at the University.

Thanks are due to the University of Manitoba for providing excellent facilities during the study. I am grateful to the authority of the University of Khulna for granting me the study leave required for this study. The patience and support from students, and my colleagues at the University of Khulna are always recognized.

My parents have always been patient and understanding while I am away for the study. Thank you for the sacrifice you made for me, and the support and encouragement you always extended whenever I needed it. My siblings and family have been very considerate and I am grateful for their continuous support.

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#### CHAPTER 1

#### Introduction

Alfalfa, *Medicago sativa* (L.), is a perennial legume and is one of the most important forage crops grown in Canada (Harper *et al.* 1990). Alfalfa is widely grown in the Prairie Provinces (Goplen *et al.* 1987; Harper *et al.* 1990; Schaber *et al.* 1990b; Schaber and Entz 1991). Because alfalfa is a perennial plant with a dense canopy and complex crown structure, it offers relatively persistent and varied niches for insects in fields (Brown and Fick 1986; Summers 1998). As a result, diverse assemblages of insect pests and their natural enemies develop and establish in alfalfa fields (Pimentel and Wheeler 1973*b*; Harper 1988; Schaber and Entz 1991). A number of these insect pest species cause economic damage to alfalfa crops, particularly in the case of alfalfa grown for seed, which is a much more valuable crop than hay.

Insect pests, which damage Canadian alfalfa, include mainly the lygus bug, *Lygus* spp., the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae) and the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) (Lilly and Hobbs 1962; Harper 1978; Goplen *et al.* 1987; Murrell 1987; Schaber and Entz 1988; Soroka 1991; Soroka and Murrell 1993). In addition, damage caused by the alfalfa weevil, *Hypera postica* (Gyllenhal) (Schaber and Entz 1988; Schaber *et al.* 1990*b*) and the superb plant bug. *Adelphocoris superbus* (Uhl.) (Lilly and Hobbs 1962), has been reported from Alberta. Murrell (1987), however, did not often find these latter insects in alfalfa fields in Saskatchewan. In Manitoba, although prior to the study reported here no thorough studies have been done, *Lygus* spp., *A. lineolatus*, and *A. pisum* have been reported to infest alfalfa crops (Timlick *et al.* 1993; Gerber and Wise 1995).

Pest control by cultural, biological or chemical methods is available for alfalfa (Bolton and Peck 1946; Lilly and Hobbs 1962; Craig 1973; Harper 1978, 1988; Harper et al. 1990; Schaber 1992), though farmers rely heavily on pesticides. Debach and Rosen (1991) reviewed the effects of chemical insecticides and concluded that dependence upon chemical insecticide application is not a sustainable strategy, as chemical insecticides disrupt the natural ecological balance and escalate the resurgence of pest problems. In addition, indiscriminate use of insecticides results in development of pesticide resistant pest genotypes (Debach and Rosen 1991; Snodgrass and Elzen 1995; Snodgrass 1996). Hence, there is interest in holistic schemes to reduce the reliance on chemical insecticides and increase the use of insect natural enemies (Schaber and Richards 1979; Davis 1985) as a part of a sustainable crop production system. In Manitoba, such a scheme is lacking. To develop a holistic pest management scheme, knowledge of the important insect pests and their natural enemies, and their interactions in relation to production practices is required. The present study is designed to gather the required information and the general objectives of this thesis are

- to assemble information on what insects are frequently present in alfalfa fields,
- to examine the seasonal occurrence of more common insect pests and their natural enemies in relation to production practices in alfalfa fields,
- to investigate the roles of natural enemies on important insect pests in alfalfa fields,
- to find ways to increase the benefits from insect natural enemies.

## **Thesis Organization**

The thesis is divided into four main sections: Introduction, Literature Review, Research, and General Discussion. The research section presents results of the thesis research in six different sub-sections (parts), each in the style of a scientific paper. The general discussion relates the important findings from the six research papers, and provides direction for future research and conclusions with respect to insect pests in alfalfa fields of Manitoba.

## CHAPTER 2

#### Literature Review

## Origin and importance of alfalfa

Alfalfa, Medicago sativa Linnaeus (Leguminosae), is a popular and important forage crop grown in Canada. Goplen et al. (1987) reviewed the history of alfalfa, and the following is a summary. The crop probably originated in Asia with present day Iran as the center of origin, and was cultivated in Persia and Turkey more than 3300 years ago. In North America, the crop was first cultivated in Georgia in 1736, but due to poor performance in cold weather, the crop was not popular until a relatively cold-hardy strain "Chilean clover" was introduced from Chile into California in 1850, Economic cultivation was not possible in the northern United States and Canada until a cold-hardier. variegated strain was introduced from Germany into Minnesota in 1857. After several successive winters in Minnesota, a very cold-hardy strain was selected. Although alfalfa was first introduced to Canada from France in 1871, the commonly grown Canadian alfalfa originated from the hardy Minnesotan strain. Professors J. Bracken and I.E. Kirk at the University of Saskatchewan made selections from the Minnesotan strains and distributed breeder seeds of the cold-hardy strain "666" in 1926. Since then various coldhardy, pest- and disease-resistant cultivars have been developed and distributed.

As fresh or processed feed, alfalfa provides superior feed for livestock including cattle, swine, poultry, sheep and horses (Conard and Klopfenstein 1988; Summers 1998). Alfalfa feed is easily digestible, high in protein, vitamins and minerals, and low in fiber (Barnes and Gordon 1972; Conard and Klopfenstein 1988; van Keuren and Matches 1988). Humans also consume alfalfa in the form of sprouts (Summers 1998). The crop

also improves soil structure and water conditions, reduces salt accumulation, fixes atmospheric nitrogen into soil and increases soil fertility (Summers 1998).

## Alfalfa production systems

Goplen *et al.* (1987) reviewed the production of alfalfa in the Canadian Prairies, and the following is a summary. Alfalfa is grown either as livestock feed or for seed production. Differences in stand establishment between the two systems are that alfalfa for feed is grown at a high plant density either as a pure stand or as a mixed alfalfa-grass stand, whereas seed alfalfa is grown as a pure stand at relatively low density. Once established, alfalfa for feed is harvested or grazed at least twice annually, insecticide applications are uncommon and pollinators are not introduced to the crop. In contrast, successful seed production depends on introduced pollinators, insecticides are usually applied, and harvest is once a season.

## **Cultivation for livestock feed**

Deep, fertile, well-drained land of any soil type can be used for alfalfa cultivation. Based on soil testing, the field is fertilized and lime-treated, and the seedbed is prepared to be moist, finely pulverized, level and firm to the seeding depth. Seeds are freshly inoculated with the bacterium *Rhizobium meliloti* Dangeard, sown 1–2 cm deep in the soil, and pressed (Caddel *et al.* 2000). Seeds can be sown in spring, late summer or early fall, but in areas like the Prairies where winter is harsh, spring sowing is recommended (Tesar and Jacobs 1972; Goplen *et al.* 1987). Seeding rate varies depending on locality, soil type and availability of moisture. In Prairie Canada, broadcasting or line sowing of seeds at 1–7 kg/ha is recommended (Goplen *et al.* 1987). A higher seeding rate is

recommended for irrigated alfalfa. If grown as a mixed stand for feed, alfalfa seed is mixed with seed of clover, timothy, or other grasses. Weeds are controlled at the 1–3-leaf stage of the crop or sometimes during late fall of the establishment year. Fertilizers are applied based on soil testing. Irrigation is usually applied at the time of flower initiation in arid and semi arid regions. Insects and diseases are seldom controlled (Goplen *et al.* 1987).

Alfalfa hay crop is usually mown twice in a growing season (Harper *et al.* 1990). If the crop is to provide supplementary protein to animals, cutting or grazing at the early flowering stage, before 10% of plants flower, is recommended, as the protein content and its digestibility decrease thereafter (Smith 1972; Goplen *et al.* 1987). If the crop is used for roughage, it is mown or grazed later than 10% of plants blooming. The subsequent cutting or grazing usually occurs 35 to 42 days after the first harvest. Timing of the second hay cutting influences stand persistence. The critical fall harvest period, when no cutting is recommended, is usually 4–6 weeks before the first killing frost (Goplen *et al.* 1987). After cutting, the crop is cured for hay or silage or processed for dehydrated products such as cubes, pellets, and leaf protein concentrates (Goplen *et al.* 1987).

## Cultivation for seed production

Unlike that grown for livestock feed, alfalfa for seed production often requires pest and disease control, and adequate pollination. The seed field is not harvested in the first year of stand establishment. In subsequent years, the field is managed for seed: alfalfa is a cross-pollinated crop and to increase pollination, shelters containing leafcutting bee, *Megachile rotundata* (Fabricius) (Hymenoptera: Megachillidae), are introduced into the field when the plants start blooming, and remain until late summer. Alfalfa seed growers frequently are also bee producers. Richards (1989) has explained in detail about shelter placement, and introduction, care and management of alfalfa leafcutting bees for better pollination and increased bee cocoon production, simultaneously. When seeds are mature in September, the crop is swathed or combined (Goplen *et al.* 1987).

## Insect problems in Canadian alfalfa

Alfalfa is very palatable not only to livestock but also to insects (Goplen et al. 1987). The occurrence of insect pests in alfalfa fields has been examined both in Canada and in the United States. Pimentel and Wheeler (1973b) found about 600 species of arthropods, whereas Harper (1988) found over 400 species of insects and mites in alfalfa fields in New York and Alberta, respectively. Despite the presence of a large spectrum of insect species, only four or five species appear to have the potential to cause economic damage to the crop in a particular region (Schaber and Entz 1991; Summers 1998). Some species have limited regional distribution, whereas others occur widely. For example, the alfalfa weevil, Hypera postica (Gyllenhal), which has been a serious pest of alfalfa in the lower Great Lakes region of eastern North America (Harcourt et al. 1984), also attacks the alfalfa crop in Ontario, Alberta and Saskatchewan, but the insect has not been a problem in Manitoba (Goplen et al. 1987; Schaber 1992). The blue green alfalfa aphid, Acyrthosiphon kondoi Shinji (Stern et al. 1980) and spotted alfalfa aphid, Therioaphis maculata (Buckton), have been serious pests of alfalfa in the United States (Angalet 1970). The insects also attack alfalfa in Western Canada (Harper and Lilly 1966; Soroka 1991), although their damage to the crop in the Canadian Prairie Provinces is infrequent

(Goplen *et al.* 1987; Soroka 1991). The western lygus bug, *Lygus hesperus* Knight, is prevalent throughout the Rocky Mountain and Pacific States (Beards and Strong 1966), although, the species does not occur in the Canadian Prairie Provinces (Kelton 1975). For simplicity, the present review focuses on the most commonly occurring and economically damaging insect pests of alfalfa crops in North America, particularly in the Canadian Prairies. In the Canadian Prairies, these insects include *Lygus* spp., *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), and *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) (Lilly and Hobbs 1962; Schaber and Entz 1988; Schaber *et al.* 1990*b*; Soroka 1991; Murrell 1987; Schwartz and Foottit 1992*a*; Soroka and Murrell 1993; Gerber and Wise 1995).

## Lygus bugs

Kelton (1955, 1975, 1980) reported that 21 of the 31 North American *Lygus* spp. occur in the Canadian Prairies. Of these only nine were collected from field crops in the Prairies: *L. borealis* (Kelton), *L. elisus* Van Duzee, *L. lineolaris* (Palisot de Beauvois), *L. plagiatus* Uhler, *L. rubrosignatus* Knight, *L. rufidorsus* (Kelton), *L. shulli* (Knight), *L. solidaginis* (Kelton), and *L. unctuosus* (Kelton) (Schwartz and Foottit 1992*b*). Salt (1945) reported that in Alberta, *L. hesperus* infest alfalfa crops, however, the report appeared to be based on misidentification, as *L. hesperus* does not occur in Alberta (Kelton 1975). Several of the above *Lygus* species infest alfalfa, though *L. lineolaris*, *L. borealis* and *L. elisus* are most common (Craig 1983; Murrell 1987; Schaber and Entz 1988; Butts and Lamb 1991; Soroka 1991; Schwartz and Foottit 1992*a*, 1992*b*; Timlick *et al.* 1993; Gerber and Wise 1995; Braun *et al.* 2001). The relative abundance of these insects varies both temporally and spatially (Butts and Lamb 1991; Leferink 1991; Schwartz and Foottit

1992*a*; Timlick *et al.* 1993). In general, *L. lineolaris* is the predominant *Lygus* bug in North America, and is the only species with an economic impact on seed alfalfa in eastern North America (Schwartz and Foottit 1992*a*). *Lygus borealis* is prevalent in parts of the Canadian Prairies (Butts and Lamb 1991; Schwartz and Foottit 1992*b*), whereas *L. elisus* and *L. hesperus* are prevalent western and eastern North America, respectively (Day 1987; Schwartz and Foottit 1992*a*). In Manitoban alfalfa fields, *L. lineolaris*, *L. borealis* and *L. elisus* are predominant in the eastern, north and western, and southern agricultural regions of the province, respectively (Timlick *et al.* 1993). These *Lygus* spp. are Holarctic in distribution (Kelton 1975), and they feed on about 400 plant species (Young 1986).

## Biology

The biology of *Lygus* species has been well studied. Most *Lygus* spp. are similar in both biology and behavior, and are generally referred to as lygus bugs. *Lygus* spp. generally overwinter as adults (Kelton 1975), emerge in the spring, then feed on and oviposit in host plants during spring and summer. At 20°C, eggs develop and hatch to nymphs in less than two weeks; development through five nymphal instars into adults takes about 24 days more. Growth and development rates increase with increasing temperature (Khattat and Stewart 1977). Ridgway and Gyrisco (1960) reported the duration of development of each instar of *L. lineolaris*: at 20°C instars 1–5 develop in 6–8, 3–6, 4–8, 5–7, and 7–10 days, respectively.

The number of generations of *Lygus* bug per year is directly influenced by the accumulated degree-days above 10°C (Champlain and Butler 1967), although other

climatic conditions and host plants also influence the number of generations (Kelton 1975). *Lygus lineolaris* has one generation in northern Alberta (Butts and Lamb 1991) and northern Saskatchewan (Craig 1983), two generations in southern Saskatchewan, southern Manitoba (Timlick *et al.* 1993; Gerber and Wise 1995) and eastern Ontario (Painter 1929), three generations in southern Quebec (Stewart and Khoury 1976), and four or more generations in the southern United States (Day 1987). In Western Canada, *Lygus* species are generally univoltine north of 53°30' N (Craig and Loan 1987), and bivoltine south of 50°N (Schwartz and Foottit 1992*a*).

## Seasonal occurrence

The seasonal distribution of *Lygus* bugs is known from Manitoba (Gerber and Wise 1995), Saskatchewan (Craig 1963; Murrell 1987; Braun *et al.* 2001), and Alberta (Butts and Lamb 1991). Generally, a small number of overwintered *Lygus* adults invade the field and start feeding on young plants as soon as alfalfa seeds germinate or crowns sprout in the spring (Craig 1973; Soroka 1991; Gerber and Wise 1995). The mated females then lay eggs in the plant stems. The eggs hatch and the first generation nymphs appear in the second half of May, and peak during the first half of June (Gerber and Wise 1995). The nymphs pass through five instars to reach adulthood in about a month (Craig 1973; Khattat and Stewart 1977; Soroka 1991). Adults in the first generation appear during mid- to late-June, and peak during early- to mid-July. The first generation adults quickly disappear within two weeks of the peak, and the second generation nymphs appear during late July or early August, and peak during mid- to late-August (Craig 1983;

Murrell 1987; Gerber and Wise 1995; Braun *et al.* 2001). A more or less similar trend has been observed in Minnesota (Jensen 1986). Generally, *Lygus* bugs are more numerous in the second generation than in the first generation (Craig 1983; Murrell 1987; Braun *et al.* 2001). This is partly due to population build up through reproduction. However, migration from other hosts that have senesced and are unsuitable compared with the late maturing alfalfa plants also contribute to late season populations (Murrell 1987).

## Adelphocoris lineolatus

Jensen (1986) reported that Goeze first described the alfalfa plant bug as *Cimex lineolatus* in 1778. The insect was placed under at least seven genera before Reuter (1896, cited in Hughes 1943) called it *A. lineolatus* (Goeze). The insect was Palaearctic, but today it occurs in Europe, Asia, Africa, and North America (Hughes 1943). In North America, the insect was probably introduced as eggs on plant parts in imported alfalfa seeds (Knight 1930). In this continent, the insect was first collected at Cape Breton Island, Nova Scotia in 1917 (Knight 1930). Since then, the insect has dispersed gradually across the continent.

In Manitoba, following the first record in southern districts in 1941 (Hughes 1943), *A. lineolatus* spread westward 48 to 64 km a year, and became abundant in the Interlake and southern regions by 1947 (Bird and Mitchener 1948). In Saskatchewan, the insect was recorded in 1947, and by 1958, the insect had spread westward through the northern agricultural area of Saskatchewan to the vicinity of the Saskatchewan-Alberta border (Craig 1963). By 1964, the insect had spread throughout the entire agricultural area of Saskatchewan, and by 1969, the insect had penetrated deep into northern and

southern Alberta (Craig 1971). Today, *A. lineolatus* occurs throughout Canada, and is one of the most damaging insects to alfalfa (Goplen *et al.* 1987; Schaber 1992).

Unlike Lygus bugs, the host range of A. lineolatus is narrow. The main host plants of A. lineolatus are two cultivated legumes, alfalfa and red clover, Trifolium pratense L. (Craig 1963). The insect has also been found on sweet clover, Melilotus officinalis (L.), wormwood, Artemisia sp., pennycress, Thlaspi arvense L., and goldenrod Solidago spp., near alfalfa fields, and hence Craig (1963) suggested these occurrences were incidental. The bug also infests potatoes, Solanum tuberosum L., alsike, Trifolium hybridum L., buckwheat, Fagopyrum esculentum Moench (Hughes 1943), and soybeans, Glycine max (L.) (Poston and Pedigo 1975). In the laboratory, the insects can be reared on various green plants such as green beans, Phaseolus vulgaris L., or lettuce, Lactuca sativa L., but females do not oviposit on them (Craig 1963).

## Biology

The biology of *A. lineolatus* is well known. The insect overwinters as eggs in the plant stem (Craig 1963), and hatches in spring. The incubation period is 11 and 20 days at 28.6 and 18.6°C, respectively (Hughes 1943). Nymphs then develop through five instars to reach adulthood. At a mean temperature of 26.3°C, instars 1–5 develop in 4–7, 1–8, 2–5, 2–4, and 4–6 days, respectively. Complete nymphal development at this temperature takes an average of 18.47 days. Development time from egg to adult takes 33.5 days at 25.7° and 46.0 days at 17.3°C (Hughes 1943).

The insect may have one or two generations depending on the location and climatic conditions. The species is largely univoltine north of 51°N, with less than 5% of

the eggs hatching in the same year they are laid. However, if the winter is mild and spring is early, two generations can occur (Craig 1963). South of 51°N, the insect is primarily bivoltine (Craig and Loan 1984*a*).

## Seasonal occurrence

The seasonal distribution of *A. lineolatus* differs from that of *Lygus* bugs. In the Canadian Prairies, eggs start hatching and nymphs usually appear during mid- to late May (Craig 1963; Murrell 1987; Soroka 1991). The early (1<sup>st</sup>-3<sup>rd</sup>) instar nymphs attain peak populations in late June (Craig 1963; Murrell 1987). The late (4<sup>th</sup> and 5<sup>th</sup>) instars appear around mid-June and attain peak populations in late June to early July (Craig 1963; Murrell 1987). However, Soroka and Murrell (1993) noted peak occurrence of early and late instars around mid-August; in their samples both early and late instar nymphs reached peak levels at the same time. Murrell (1987) first found adults during mid-June to early July, with peak occurrence during mid-July to early August. In the northern agricultural area of Saskatchewan, Craig (1963) first found adults after mid-June, numbers of which peaked at the beginning of July.

Hughes (1943) studied *A. lineolatus* in alfalfa fields in Minnesota. In southern Minnesota, nymphs appear around mid-May and adults first occur around mid-June. First instar nymphs in the second generation were observed around mid-July, and adults at the end of July. Hughes found various stages of the first and second generations to overlap, and the pooled number of these nymphs and adults peaked in early August. In northern Minnesota, there are differences in dates of occurrence: various stages in both generations occur about two weeks later (Hughes 1943).

# Feeding of Lygus spp. and A. lineolatus (mirids)

Taxonomically *Lygus* spp. and *A. lineolatus* are closely related: they both belong to Miridae, and they cause similar types of impact (Hughes 1943; Goplen *et al.* 1987; Hanna *et al.* 1987). *Lygus* bugs follow a characteristic pattern of attraction to hosts and acceptance (Hatfield *et al.* 1983). Visual and olfactory cues are involved in the initial orientation to hosts (Ave *et al.* 1978; Prokopy *et al.* 1979). Adult *L. lineolaris* are more attracted to white and yellow than to black, red, green or orange rectangles (Prokopy *et al.* 1979). Clear plexiglass is equally or more attractive than pigmented rectangles approximating the spectral reflectance pattern of buds, blossoms, foliages, or bark (Prokopy *et al.* 1979). Prokopy and Owens (1978) found no differential attraction of *Lygus* bugs to models of the color and shapes of plant structures such as buds, blossoms, leaves, twigs or stems. Observing more *L. lineolaris* adults to visit white and yellow colours than to other colours, Prokopy *et al.* (1979) concluded that visual cues may elicit responses of *Lygus* bug adults, though the exact roles of color or shape in host selection of *Lygus* bugs remain unclear.

Olfactory cues are important to flight orientation and host location by mirids. In an electroantenogram study, Chinta *et al.* (1994) found both males and females of *L. lineolaris* responded to different green leaf volatile compounds; the greatest response was to nonanal, a plant volatile identified from cotton buds (Hedin *et al.* 1976). Chinta *et al.* (1994) suggested that both males and females of the bug can detect low concentrations of host plant volatiles which they use in locating food sources or oviposition sites from a distance. Ave *et al.* (1978) found that *Lygus* adults lose feeding preference for frego bract over normal cotton squares when the rostral tip sensilla or antennae are removed. This

suggests that hosts may be recognized, at least from a short distance, through olfactory cues perceived by rostral and antennal chemosensillae. Once an individual arrives on a host plant, simultaneous rostral and antennal tapping follows locomotion showing that both rostral chemo- and mechanosensillae provide sensory inputs at this point (Ave *et al.* 1978). On contact with host plants, mirid individuals exhibit various patterns of feeding behaviour. For example, in *Lygus* bugs the locomotion is first arrested for a while (Ave *et al.* 1978). The insect then moves around tapping the plant with its antennae. Its motion is arrested again, after which it taps the plant with its rostrum. Then it probes by injecting its stylets into plant tissues (Hatfield *et al.* 1983) and starts feeding upon acceptance.

Nutritional requirements influence mirid numbers, distribution and feeding activities. Daily water loss of a *Lygus* individual is at least 50% of its body weight, and hence the bug must feed to maintain water balance (Cohen 1982). Many mirids regulate their activities so that water loss is reduced. For example, Butler (1972), and Muller and Stern (1973) found peak activity of *Lygus* spp. at 5 am and 8 pm in alfalfa and safflower fields. Wilson *et al.* (1984) found *L. hesperus* more often in the middle rather than in the apical portion of cotton plants, and 75% of individuals were on the lower surface of the leaf. Lower temperatures (Fye and Bonham 1971) and wind speed (Edwards and Wratten 1980) at these locations contributed to lower evaporation rates, and reduced requirements for dietary water.

The quantity of available food also influences mirid distribution within a plant. *Lygus* numbers are positively correlated with distribution of fruiting structures within the plant (Wilson *et al.* 1984). Fye (1972) found that 85% of green cotton bolls and 97% of cotton squares are located in the top 60 cm of the plant, the area where 70–100% of all

insects including *Lygus* bugs were located. Snodgrass (1998) found that 75% of *L. lineolaris* adults and nymphs were concentrated within 6 nodes below the mainstem terminals of cotton plants, probably because of the presence of squares, buds and bolls the preferred feeding sites.

Mirids have piercing and sucking mouthparts that penetrate through host plant tissues (Tingey and Pillemer 1977). They feed by a lacerate and flush method without secretion of a stylet sheath (Miles 1972). During feeding, a small amount of saliva is secreted repeatedly from the rostrum to the feeding site, and occasionally the bug tastes the host by sucking up the juice (Miles 1972). Once the feeding site is accepted, the bug inserts, withdraws partially and reinserts the stylets several times, at the same time releasing saliva from the stylets. Once the laceration is complete, the liquefied plant material is ingested. This whole process takes up to 10 minutes for *L. hesperus* (Strong 1970). An individual can consume a considerable amount of food quickly. For example, a *L. hesperus* adult can ingest the liquefied contents of an alfalfa bud in only 23 seconds (Strong 1970).

The salivary composition of mirids, particularly of *Lygus* bugs, has been determined (Miles 1967; Strong and Kruitwagen 1968; Hori 1974). Polygalacturonase (pectinase) is a salivary component that dissolves the middle lamella of cells, and thus aids penetration and maceration of plant tissues. Amylases and proteases usually aid external digestion of starch and proteins (Tingey and Pillemer 1977). In addition, some non-enzymatic compounds are present and probably originate from the food. For example, Miles and Liyod (1967) found dietary amino acids in mirid saliva. Hori (1974) isolated a growth-promoting compound that is analogous to indole-3-acetic acid (IAA).

Miles and Llyod (1967) suggested that some hemipterans synthesize IAA in the salivary glands; however, Strong (1970) and Hori (1974) found no evidence of this in *L. rugulipennis*, *L. hesperus* or *L. disponsi* Linnavuori.

#### Injury and damage caused by mirids

Injury caused by mirids, particularly *Lygus* bugs, falls into five categories: localized wilting and tissue necrosis, morphological deformation of fruit and seed, abscission of fruiting bodies, altered vegetative growth, and tissue malformation. These symptoms are found in a wide variety of crops, however, according to Tingey and Pillemer (1977) only three categories of injury are commonly found in alfalfa; altered vegetative and reproductive growth, abscission of fruiting bodies, and morphological deformation of fruit and seeds.

Both nymphs and adults suck cell sap from plant tissues (Strong 1970; Craig 1973; Tingey and Pillemer 1977). This withdrawal of fluid, the chemicals injected during feeding, and mechanical injuries due to feeding and oviposition cause wilting, discoloration and drying of tissues, a symptom called blasting (Smith and Michelbacher 1946; Leferink 1991). Localized wilting with necrosis was not mentioned for alfalfa in Tingey and Pillemer's review (1977). Hughes (1943) suggested that mechanical injuries resulting from oviposition and feeding generally do little harm to the plant, except that the wound could serve as a gateway to pathogenic infection. Again their feeding on the early growth stage of plants usually does little harm, though a severe infestation can cause the plant to grow tall and slender, and such plants often fail to bloom.

Alteration of vegetative and reproductive growth is a broad category, which includes both positive and negative effects on plant parts, such as increased tillering and stunted growth. For alfalfa, mirid feeding may reduce plant growth, or delay plant regrowth after hay cutting, a sign of injury that is not often noticed. In a number of field cage experiments, *Lygus* spp. (Hughes 1943; O'Neal and Peterson 1971; Newton and Hill 1970) and *A. lineolatus* (Hughes 1943) were found to reduce plant height. Tissue malformation, in which leaves punctured by mirid feeding become stunted or swollen and folded around the injured area, has been reported for various crops including cotton, sugar beet and poplar, but not for alfalfa (Tingey and Pillemer 1977).

As the crop season progresses, the bugs feed on and blast buds (Hughes 1943; Tingey and Pillemer 1977; Goplen *et al.* 1987). They also feed on the ovaries and other parts of flowers causing flower abscission or stripping. It is not known how much cellular content must be withdrawn, what amount of salivary chemicals is required to be injected, and how long the insect has to feed on a particular part before it collapses. In a cage study, compared with the control treatment, Hughes (1943) found that allowing one *A. lineolatus* bug to feed on 5 flowers for 24 hours was sufficient to cause about 50% more flowers to collapse and drop. When one bug was allowed to feed on 10 flowers for 72 hours, the dropping was even greater, presumably because of prolonged feeding activities of the insect. Hughes (1943) also noted a considerable portion of flowers dropped even in a mirid-free condition. Hence, he concluded that flower mortality involves several factors of which the mirid-feeding injury is foremost.

After seedpod appearance, the insects attack and feed on seedpods and seeds, which causes distortion, shrinkage, and darkening of the young fruit/seedpods.

Eventually this results in nonviable seeds, and causes dropping of young seedpods (Hughes 1943; Smith and Michelbacher 1946; Tingey and Pillemer 1977; Walstrom 1983; Soroka 1991).

Lygus spp. (Craig 1963; App and Manglitz 1972; McDonald and Harper 1978; Syrett and Penman 1980; Harper and Kaldy 1982; Craig and Loan 1984b) and A. *lineolatus* (Hughes 1943; Craig 1971; Murrell 1987; Soroka and Murrell 1993) cause significant economic damage to alfalfa, especially to the seed crop. Bolton and Peck (1946) found on average 22% of seeds were damaged due to Lygus bugs, but the loss was sometimes as high as 51% in individual fields in Saskatchewan. McMahon and Arrand (1955) report that depending on their densities, Lygus spp. can reduce alfalfa seed production by 15–100%. Hughes (1943) found that when young alfalfa seedpods are infested by A. lineolatus at a ratio of one bug to five pods for 120 h, pod mortality was 94.6%. Hughes did not indicate seed losses, which would be higher. Soroka and Murrell (1993) found significant yield reductions at densities of two or more A. lineolatus bugs on two stems in a sleeve cage. However, they found seed production was not affected when they allowed one bug to feed on two alfalfa stems during late July and onward. In their open field trial, even though not significantly different, control plots produced 30% less seeds than plots that received early season insecticide treatment. Craig (1961) found that seed yield from untreated plots was 30% less than that from insecticide treated plots.

*Lygus* spp. (Jensen 1986) and *A. lineolatus* (Smith and Ellis 1983) infestations can affect the quality and quantity of alfalfa hay. In a cage study on hay alfalfa after the second cut of the season, Smith and Ellis (1983) found *Lygus* bugs did not affect the degree of chlorosis, plant height or leaf area per plant, but did significantly reduce the dry

matter and protein content of the plants. However, earlier in the season, an alfalfa that had been cut only once previously in the season, the insect affected the degree of chlorosis, plant height and dry matter but not the protein content. These results suggest that the effect of the feeding is variable and is dependent on the time of the season and crop state. Mirids seldom cause economic damage to Canadian alfalfa grown for hay. This difference is due to the differences, compared to seed alfalfa, in crop economics, harvesting strategies and seasonal availability of the crop to pests. Hay fields are harvested at least twice a season, which usually suppresses insects in the Canadian Prairies (Harper *et al.* 1990). In South Dakota, Walstrom (1983) found that population densities of 2.44 *Lygus* spp. and 0.86 *A. lineolatus* per sweep caused significant yield loss to the first crop hay alfalfa. O'Neal and Peterson (1971) found yield and dry matter to be inconsistently reduced when plants of 1 x 0.6 m area of land were caged with 80 *Lygus*/cage.

## Acyrthosiphon pisum

Believed to be a Palaearctic-Oriental insect, *A. pisum* occurs worldwide (Mackauer 1971). In North America, the insect was probably introduced from Europe on infested clover and peas, and was first recorded in Kansas in 1877 (Harper *et al.* 1978). In Canada, the insect was first recorded in Ottawa in 1898 (Johnson 1900). By 1899, the insect was established as a pest in both the United States and Canada (Harper *et al.* 1978). In Canadian agricultural areas, the insect now occurs from coast to coast (Mackauer 1971). In Manitoba, the insect has been recorded as far north as Churchill (58°47') (Robinson 1979).

# Biology

The pea aphid, A. pisum, is holocyclic and reproduces sexually in late summer or autumn in Canada. The insect overwinters as a diploid egg on alfalfa, clover and perennial wild legumes (Harper 1975). The fundatrix or stem mother emerges from the egg in the spring, and parthenogenetically produces a female generation on overwintering hosts. These females parthenogenetically produce wingless and winged female progeny. Winged progeny are produced in response to overcrowding, declining host quality (Kennedy and Forsbrooke 1972), or presence of natural enemies (Sloggett and Weisser 1999) and the production is influenced by maternal age (MacKay and Lamb 1979) and genetic make up of the populations (Lamb and MacKay 1979). The alate aphids migrate to other hosts, thus ensuring a wide distribution (Cooke 1963). In the summer, many overlapping generations, mostly of apterous females, are produced parthenogenetically. In the autumn, with reduced photoperiod and declining temperatures (Lamb and Pointing 1972), parthenogenic females produce a sexual generation comprising oviparae or sexual females and males. These sexuals mate and the females oviposit on the winter host, thus completing the life cycle (Lees 1961).

Field populations of *A. pisum* are generally descendents of many fundatrices, and are therefore composed of many clones. Members within a clone are genetically identical (Blackman 1979), but members within a population usually differ (Lamb and MacKay 1983). The six main *A. pisum* morphs- fundatrix, apterous or alate viviparae, apterous oviparae, and apterous or alate males vary in appearance, notably there is variation in wings, body shape and size. For example, an alate averages 4.5 x 1.5 mm, whereas an

aptera is about 5.0 x 1.6 mm in size (Folson 1909). Davis (1915) has given a complete description of *A. pisum* morphs in North America.

Acyrthosiphon pisum develops through four nymphal stages to reach adulthood (Folson 1909; Davis 1915), and the development is influenced by temperature (Hutchinson and Hogg 1984). Temperature thresholds for each instar vary among locations, populations, and clones within populations (Lamb *et al.* 1987). Lamb *et al.* (1987) compiled a list of the minimum developmental threshold of *A. pisum* in different localities in North America, which indicates that the range may vary from 2.6–5.6°C. Similarly, the maximum developmental threshold probably varies from 24–30°C (Siddiqui *et al.* 1973).

The developmental time from birth to adulthood and the pre-reproductive period, and fecundity vary with temperature, clone, morph and host plant. For example, in the laboratory at 19–20°C, a newborn reaches adulthood in 6.2 days on faba bean (Frazer 1972), 7.6 days on alfalfa (Campbell and Mackauer 1975), and 8.3 days on peas (Hutchinson and Hogg 1984). In field conditions of British Columbia, the mean prereproductive period is 12.3 days (Campbell and Mackauer 1977). In Wisconsin, time to the first reproduction of the apterous *A. pisum* on alfalfa was 12.5 days for spring and 7.5 days for summer (Hutchinson and Hogg 1984).

## Seasonal occurrence

Craig (1973), Murrell (1987) and Soroka (1991) reported the seasonal occurrence of *A. pisum* on alfalfa in western Canada, and the following is a summary. The insect occurs in alfalfa fields throughout the growing season in Canada. The insect first appears

in fields at the beginning of spring. Because of their rapid growth and high reproductive capability, *A. pisum* populations rise rapidly. The populations attain peak levels during late July to late August, after which the numbers decline precipitously due mostly to increased natural enemies, reduced quantity and quality of food supply, photoperiods and temperature. In Minnesota, Radcliffe *et al.* (1976) found aphid population peaks during late August, with a sharp decline at the beginning of September.

# Feeding

Flight initiation of an A. pisum individual takes place in response to the short wavelength radiation of the sky, whereas flight termination results from long wavelength radiation reflected from the ground and an optomotor reaction provoked by objects looming up along the flight path (Kennedy et al. 1961). Because of aphid's low flight speed, airflow determines their flight direction, altitude, and the distance traveled (Dixon 1971). While in the air, their initial selection of host plant is influenced by foliage colour. Cartier (1963) found that yellow-coloured foliage positively influences the alighting response of migrant A. pisum, and that migrants and colonies are least abundant on pea cultivars with deep green foliage. However, the landing and settling of aphids on host plants appear to be based on trial and error: host selection is a matter of leaving or staying (Kennedy and Forsbrooke 1972). If unsatisfied, aphids will leave and land indiscriminately elsewhere. This suggests that the higher numbers on preferred hosts are due to fewer leaving, but not more landing. Non-hosts are often rejected after a brief probe within cell walls rather than probing that reaches the usual feeding site-the phloem sieve tubes (Kennedy and Forsbrooke 1972).

Preferred hosts are usually morphologically suitable, and they contain less or no deterring secondary plant metabolites, but are nutritionally superior, especially in amino acid concentration and balance (Auclair 1976). Alfalfa plants contain saponins, which have been suggested to influence *A. pisum* colonization (Horber *et al.* 1974). Conversely, Bournoville (Unpublished, cited in Febvay *et al.* 1988) found no relationship between saponin concentration and *A. pisum* performance.

Aphids usually respond negatively to gravity. Kennedy (1958) reported that *A. pisum* prefers feeding and colonizing the plant terminal down to about the fourth internode from the plant tip. *Acyrthosiphon pisum* populations tend to differ in host preference, and legumes that are the most common in an area are most preferred (Dudley and Bronson 1952). The primary hosts of *A. pisum* in Canada are alfalfa and clover (Beirne 1972). Cooke (1963) reported peas and alfalfa are the main hosts in the Pacific Northwest.

With piercing and sucking mouthparts, *A. pisum* feed on plant phloem. By extension and retraction of stylets, they pierce through an intercellular path to reach the phloem. A salivary sheath of gelling material is formed from outer to inner tissue (Klingauf 1987). However, feeding from non-vascular tissues is also reported (McLean and Kinsey 1967). Liquid saliva is often secreted during feeding. Time required to penetrate and reach the phloem is from five minutes up to hours depending on insect stage, plant parts, ages, and growth stages (Nault and Gyrisco 1966). After penetration, ingestion of fluid takes place by plant fluid pressure augmented by capillary action; there is a decrease in sap surface tension due to salivary composition. Active sucking through the pharyngeal pump is also involved in the feeding process (Klingauf 1987). If feeding

becomes blocked due to accumulation of larger particles, aphids propel the particles away from the sheath opening by regurgitation, stylet moving, or by lengthening the sheath or forming a branch sheath (Klingauf 1987).

# Injury and damage

Acyrthosiphon pisum individuals withdraw sap from leaves, stems, buds, flowers and pods (Gyrisco 1958), and affect plant growth directly by removal of photosynthetic products, and indirectly by reduction of production due to losses of tissues. Barlow and Messmer (1982) found an 18% reduction of the relative growth rate of new tissue when a pea plant was infested for 10 days by 50 *A. pisum* individuals. The feeding by aphids can deform plants: apical meristems can be shriveled and malformed; heavy infestation can cause plant parts to wilt, yellow and drop leading to stunted, brittle and spindly growth of the plant. Sometimes the plant tops can die (Shade and Kitch 1983; Soroka 1991). The honeydew secreted by aphids can promote fungal growth and attract *Lygus* bugs (Lindquist and Sorensen 1970). Acyrthosiphon pisum can also affect the plant's nitrogen fixation (Sirur and Barlow 1984), and transmit diseases including *Verticillium* wilt, a very destructive disease to alfalfa (Goplen *et al.* 1987).

Acyrthosiphon pisum infestation can also reduce hay quality (Gyrisco 1958; Harper and Lilly 1966; Kindler *et al.* 1971; Harper and Kaldy 1982). Harper and Lilly (1966), and Kindler *et al.* (1971) reported reduction of dry matter, yield, percent carotene, percent protein, and percent digestibility. Harper and Freyman (1979) reported increased winter damage due to feeding by *A. pisum.* Franklin (1953) reported that infestation of 200–400 *A. pisum* individuals per sweep for about 10 days and only 13–75 per sweep for

the next seven days reduced hay production by over 4 tons/ha. Conversely, Hobbs *et al.* (1961) did not find such an effect, and concluded that unless there is a late infestation from which alfalfa plants cannot recover before haying, or unless the variety is susceptible to aphids and aphid-borne diseases, alfalfa plants can recover from aphid feeding when plants receive adequate water.

There are little quantitative data on damage to seed production. Although *A. pisum* is a potentially damaging pest, the widespread use of resistant cultivars from the 1960s eliminated significant aphid damage in the United States (Neuenschwander *et al.* 1975). Aphid resistant alfalfa cultivars are also common in Canada (Petrowski 1999). Hence, *A. pisum* alone seldom causes much damage, but, under favorable conditions, a localized heavy population build up may cause severe damage (Craig 1973). This damage is higher on irrigated alfalfa for hay, which has lush growth that creates a microenvironment favorable for population build up of *A. pisum* (Craig 1973; Soroka 1991).

The amount of damage varies with plant growth stage, initial density, population growth, and duration of aphid infestation (Barlow and Messmer 1982). According to Maiteki and Lamb (1985), pea crops can recover from pre-bloom feeding by *A. pisum*. No such information is known from alfalfa. *Acyrthosiphon pisum* causes millions of dollars of damage annually in the United States (Shade and Kitch 1983). In the 1960s, aphid infestation caused alfalfa production losses of \$60 million a year, more than nine million dollars in Kansas alone in 1968 (Harper *et al.* 1978).

#### Insect pest control

Humans have been struggling to control alfalfa pests since they began its cultivation (Summers 1998). Despite the fact that several methods including cultural, chemical, and biological control are available, the chemical method is the most popular method of controlling pests of alfalfa crops (Broadbent *et al.* 2002). Since *Lygus* spp., *A. lineolatus*, and *A. pisum* infest alfalfa fields simultaneously, control measures, particularly insecticide applications, often are intended to affect most inhabiting pest species simultaneously. Hence, the control methods for the three groups of insects are reviewed together below.

## **Cultural control**

Before the development of synthetic insecticides, cultural methods were commonly used for control of alfalfa pests in North America (Smith and Michelbacher 1946). During that time, interest focused on burning, adjustment of harvesting method, and resistant varieties (Blanchard 1933; Carlson 1940; Hughes 1943; Bolton and Peck 1946; Smith and Michelbacher 1946; Craig 1973; Soroka 1991; Summers 1998). In the United States, additional methods including flaming, flooding, disking and harrowing, early harvesting, and adjustment of planting date have been reported, but were not adopted because they were inadequate or yields were affected or both (Schaber and Entz 1988).

Burning involves destruction of plant remnants and green growth by fire to achieve control of insects (Blanchard 1933). Burning can be done in early or late spring or in fall. In places where winter is harsh and comes early, early spring burning is

encouraged as late burning can cause delay of seed setting and maturity, and increase the risk of frost damage (Bolton and Peck 1946). Carlson (1940), Hughes (1943), and Tippens (1964) in the United States, and Bolton and Peck (1946), Craig (1973), and Schaber and Entz (1988) in Canada indicated that spring burning of alfalfa stubble reduces populations of Lygus and Adelphocoris spp., H. postica, and A. pisum. Conversely, Lilly and Hobbs (1962) found a significant increase of A. pisum numbers, although numbers of A. superbus were reduced in spring-burnt plots in Alberta. They also found destruction of most predaceous insects, though Nabis ferus (L.) (Hemiptera: Nabidae), Chrysopa harrisii Hagen (Neuroptera: Chrysopidae), Coccinella transversoguttata quinquenotata Kby and Hippodamia spp. (Coleoptera: Coccinellidae) overcompensated in the later part of the season. Schaber and Entz (1988) found mixed effects on predators: spring burning had no effect in one field, whereas in another field both coccinellids and spiders were reduced in the year of burning, but coccinellid numbers increased in the following year. Schaber and Entz (1991) in an 8-year study in Alberta found burning in autumn significantly reduced H. postica populations but had little or no effect on aphids. Burning was also found to reduce disease incidence, stimulate plant growth, and increase seed yield (Bolton and Peck 1946; Lilly and Hobbs 1962; Tippens 1964), possibly due to the increased availability of nutrients following burning (Lilly and Hobbs 1962; Dormaar and Schaber 1985). Various factors including the insect species, age and growth of plants, dew, degree of damage, weed growth, wind, temperature and humidity influence the effectiveness of burning (Schaber and Entz 1988, 1991). Several operational problems stand in the way of widespread adoption of burning.

as it is very difficult to get a good and uniform burn, also, in dry and windy weather, burning may be hazardous and is often legally prohibited.

Adjustment of the harvest method has been reported to control pests in the forage crop in both Europe (Starý 1970) and North America (Blanchard 1934). In North America, options available include swathing or direct combining (Harper et al. 1990), border- (Summers 1976) or strip-cutting (van den Bosch and Stern 1969) or solid-cutting (Summers 1976). Harper et al. (1990) found that when the mown hay is immediately removed from the field, pest populations are effectively controlled for 3-4 weeks. They also noted rapid recovery of predator populations within 2 weeks after fields were combined. The trend was present with both the first and second cut, and reasons for such recovery of predators seems unclear. Summers (1976) recommended border-cutting, in which a narrow strip is left uncut. He found significantly more natural enemies in the border-cut field compared with the solid-cut field. In that study, he also found that hay quality or grade was unaffected in the border-cut plots. Godfrey and Leigh (1994) found a significantly lower number of L. hesperus in a strip-cut plot, and van den Bosch and Stern (1969) reported that strip- or band-cutting protects natural enemies by providing a refugium for increased natural suppression of insect pests. However, the effects of modifying harvesting strategies may depend on the availability of alternative habitats. For example, in arid areas, where alternative habitats for herbivores or natural enemies are lacking, the effects of refuge creation are likely to be greater than where alternative habitats abound. Because of the associated extra work, adjustment to the operating system, and some technical and operational problems, modifications in harvesting were not popular (Summers 1976, 1998; Harper et al. 1990). Although, there are benefits in

adjusting time of hay cutting (Hagan 1918, cited in Summers 1998), in areas with a harsh and early winter, delay in the second-cut can affect cold-tolerance (Goplen *et al.* 1987). On the other hand, hay contains maximum protein if cut at the bud stage (Goplen *et al.* 1987). Therefore, adjustment of cutting schedule may decrease hay quality or quantity.

Use of resistant cultivars is popular in North America. There are a few alfalfa cultivars resistant to Lygus bugs, but the level of resistance is not sufficient to protect the crop effectively (Tingey and Pillemer 1977; Summers 1998). The greatest success of resistance has been against aphids including A. pisum (Summers 1998). Blanchard and Dudley (1934) first found alfalfa strains resistant to A. pisum, and since then aphidresistance has been investigated frequently (Pimentel and Wheeler 1973a). Since the 1970s many aphid resistant cultivars have been developed (Manglitz and Radcliffe 1988; Summers 1998), and used (Goplen et al. 1987; Summers 1998), and the mechanisms for resistance involve antixenosis, antibiosis or tolerance (Sorensen et al. 1988). Petrowski (1999) lists about 50 such cultivars. Radcliffe et al. (1976) in a study on five alfalfa cultivars found one-sixth as many A. pisum on cultivar "Team" than on "Ranger". Similarly, "Kansas" supports fewer aphids than "Vernal" (Pimentel and Wheeler 1973a). These resistant varieties may also experience reduced Lygus bug damage. Butler (1968) found increased survival of L. hesperus nymphs in the presence of A. pisum or T. maculata. Lindquist and Sorensen (1970) found that the survival of L. lineolaris increased in presence of A. pisum or T. maculata, and that the density of Lygus bugs is positively correlated with that of T. maculata. Despite the fact that Lygus bugs are mainly phytophagous insects, Lindquist and Sorensen (1970) and Wheeler (1977) observed that Lygus bugs feed on aphids. Lindquist and Sorensen (1970) proposed that the honey dew

secreted by aphids probably serve as an attractant, feeding stimulant or food source for *Lygus* bugs. *Acyrthosiphon pisum* is a cool season pest, and temperatures <15°C reduce plant resistance (Summers 1998). As a result, after an extended period of cool temperatures, there may be an outbreak of the pest even on resistant cultivars.

## Chemical control

Chemical insecticides are frequently integral components of insect pest management in alfalfa (Broadbent *et al.* 2002). Before the discovery of synthetic insecticides, inorganic insecticides such as arsenate, sulfur and gypsum dusts, calcium cyanide, and some botanical compounds including pyrethrin dusts and nicotine were used (Sorensen 1939). Dudley *et al.* (1936) obtained nearly 100% kill of *A. pisum* by spraying rotenone-containing derris. The effectiveness of botanical insecticides depends upon the condition of sunlight, physical and chemical nature of the compound, and moisture conditions in fields (Harper 1956). Frequent pest increases following inorganic insecticide application have been noted (Wilson and Davis 1952).

After Hills' (1944) report that DDT dust was very effective, interest was concentrated on DDT in North America (Smith and Michelbacher 1946). During the 1950s and 1960s, synthetic organic insecticides were the preferred materials (Summers 1998), and various organochlorine insecticides including DDT, TDE, lindane, aldrin and toxaphene were commonly used (Jewett *et al.* 1958). Following the use of organochlorine insecticides, several organophosphate, carbamate, and synthetic pyrethroid insecticides have been used. Today, few of them are registered for control of *Lygus* spp. in Canada (Broadbent *et al.* 2002; Anonymous 2003). In Manitoba, azinophos-methyl, cyhalothrin-

lambda, dimethoate, deltamethrin, malathion, naled, and trichlorfon are recommended for alfalfa pest control (Anonymous 2003).

There are some thresholds proposed for control of mirids and *A. pisum* on seed alfalfa. Soroka (1991) suggested to control mirids when there are five bugs per 180° sweep of a sweep-net at bud formation and flowering stage of alfalfa plants. Schaber (1992) recommended to control mirids when eight *Lygus* bugs or four 4<sup>th</sup> and 5<sup>th</sup> instar nymphs and adults of *Adelphocoris lineolatus* bugs appear in a 180° sweep. The recommended threshold for control of *Lygus* spp. and *A. lineolatus* late in the season is 12–16 mirids per 180° sweep (Harris 1992, cited in Soroka and Murrell 1993).

A threshold of 100–200 aphids per 180° sweep is recommended to control *A*. *pisum* in dry land production, though under irrigation, more than 1400 individuals per sweep did not affect forage yield (Soroka 1991). The threshold seems rather high, particularly for cultivars that are susceptible to diseases and for crops grown in dry areas. This is because drought-stressed alfalfa is most susceptible to injury (Soroka 1991). Cuperus *et al.* (1982) determined an economic threshold of 58–70 aphids per sweep for hay alfalfa in Minnesota.

Seed growers usually apply insecticides twice a season: a clean-up application regardless of insect numbers and stages before pollinator leafcutting bees are introduced into alfalfa fields in June, followed by a second treatment often in mid to late August regardless of insect numbers and stages, summer condition and seed maturity stage (Murrell 1987). This indicates that thresholds are seldom considered in pest managementdecision making. The use of insecticides is of concern: insecticides often affect the pollinators, and are unsafe to humans and natural biological control agents. In addition,

pest insects may develop resistance to the insecticides, as reported for *Lygus* bugs in the United States (Snodgrass and Elzen 1995; Snodgrass 1996). Therefore, biological control appears to be an important alternative (Broadbent *et al.* 2002).

#### **Biological control**

Alfalfa fields in North America abound in natural enemies including predators, parasitoids and pathogens (Pimentel and Wheeler 1973*b*; Wheeler 1977; Harper 1988; Summers 1998). These beneficial organisms play important roles in natural pest suppression (van den Bosch *et al.* 1956; Neuenschwander *et al.* 1975; Frazer *et al.* 1981*a*, 1981*b*). Hagen and van den Bosch (1968) reviewed the impacts of predators, parasitoids and pathogens on aphid populations in general. However, there is no such comprehensive report for *Lygus* spp. and *A. lineolatus*.

There is evidence from field studies that predaceous insects can reduce *Lygus* (Godfrey and Leigh 1994) and aphid populations (Radcliffe *et al.* 1976; Frazer *et al.* 1981*a*, 1981*b*) in North America. Several species of coccinellids (ladybird beetles) are active in alfalfa fields, and they consume soft-bodied insects, especially aphids (Lilly and Hobbs 1962; Wheeler 1977; Neuenschwander *et al.* 1975; Frazer *et al.* 1981*a*, 1981*b*). Neuenschwander *et al.* (1975) showed that during its lifetime a coccinellid adult and larva can destroy 5625 and 1295 aphid individuals, respectively. Frazer *et al.* (1981*a*, 1981*b*) observed that coccinellids alone control the early season population of *A. pisum*, while a guild of predators, including coccinellids, controls the population in the later part of the season in Vancouver and Alberta. Lilly and Hobbs (1962) found a rapid decline of *A. pisum* population in response to upsurges of coccinellids, along with nabids (damsel bugs) and chrysopids (green lacewings), in a burnt alfalfa plot. In Oklahoma, Fenton

(1959) found coccinellids, along with chrysopids and aphidiids (aphid parasitoids) to limit the early season aphid population development. Quayogode and Davis (1981) reported control of *H. postica* by coccinellids when *A. pisum* were absent.

Chrysopids, predominantly *Chrysoperla carnea* (Stephens) and *Chrysopa oculata* Say, are generalist predators, and are active in alfalfa fields in North America (Neuenschwander *et al.* 1975; Harper 1988). Chrysopids consume aphids and other softbodied insects (Whitcomb and Bell 1964; Neuenschwander *et al.* 1975; Wheeler 1977). Wheeler (1977) observed that chrysopid larvae consume *H. postica* larvae, *A. lineolatus* nymphs, *A. pisum* and some other insects. Neuenschwander *et al.* (1975) documented that during its lifetime a *Chysopa* larva can consume 3668 aphid individuals, and reported that chrysopids regulate aphid populations in the absence of coccinellids.

Nabids are predators of several insect pests including *Lygus* spp. (van den Bosch and Hagen 1966, cited in Godfrey and Leigh 1994; Clancy and Pierce 1966; Wheeler 1977), *A. lineolatus* (Wheeler 1977), and *A. pisum* (Taylor 1949; Lilly and Hobbs 1962; Neuenschwander *et al.* 1975; Wheeler 1977). In laboratory studies, nabid adults and nymphs readily attacked *Lygus* eggs and nymphs but not adults. Nabids also consume eggs and larvae of the alfalfa caterpillar, *Colias eurytheme* (Boisduval), and western yellow-striped armyworm, *Spodoptera praefica* (Grote), and together with other natural enemies, maintain those insects below economic threshold levels in the United States (Benedict and Cothran 1980).

Despite anthocorids (minute pirate bugs), pentatomids (stinkbugs), syrphids (syrphid flies), carabids (ground beetles), and spiders being regarded as important predator groups, there are few reports of effects of their predation in alfalfa fields. Frazer

*et al.* (1981*b*) concluded that anthocorids contribute to natural suppression of *A. pisum* numbers during the later part of the season. Pentatomids consume *A. pisum* and *H. postica* larvae (Wheeler 1977). Syrphid larvae are also important predators of *A. pisum* (Fluke 1929; Neuenschwander *et al.* 1975). Neuenschwander *et al.* (1975) showed that a syrphid fly larva may eat more than 1100 aphids during its lifetime, and hence contribute greatly toward natural regulation of aphids.

Some of the above predaceous taxa are non-native. For example, *Coccinella septempunctata* L. (coccinellids) and *Pterostichus melanarius* Illiger (carabids) were introduced from Europe. It has been reported that these predatory species have affected native communities of coccinellids (Turnock *et al.* 2003) and carabids (Niemelä and Spence 1991), respectively in western Canada, but there is little information about the impact of such effects on predation and aphid populations. As introduction of these species was not directly intended for controlling pests on alfalfa crops, aspects associated with predator mediated classical biological control are not discussed further.

A considerable amount of work has been done on insect suppression by parasitoids in alfalfa fields in North America. Broadbent *et al.* (2002) reviewed and compiled a list of parasitoids of *Lygus* bugs in North America, and the review indicates that several native parasitoids attack *Lygus* eggs, nymphs and adults. In Canada, little is known about the extent of *Lygus* egg damage caused by parasitoids. However, four indigenous species of nymphal parasitoids, including *Peristenus pallipes* (Curtis), are active (Broadbent *et al.* 2002) and they reduce *Lygus* bug populations (Loan and Craig 1976; Craig and Loan 1987), although ineffectively since they cause low levels of parasitism in the field (Broadbent *et al.* 2002). Braun *et al.* (2001) found an average of 9–

28% of *Lygus* bug nymphs were killed by the native parasitoid, *P. pallipes*, in Saskatchewan. A higher parasitoid-inflicted mortality of mirids is desired. But the native parasitoids do not parasitize *Lygus* and *A. lineolatus* bugs sufficiently, probably because most plants that are damaged by these two bugs are not native to North America, or possibly due to their preference for other mirids. Hence, attempts are being made to establish European parasitoids (Day 1996).

In Europe, several species of nymphal parasitoids suppress populations of *Lygus* bugs (Day 1987). To increase the control by parasitoids, the European parasitoids, *P. digoneutis* and *P. stygicus* were introduced in alfalfa fields of western Canada during 1978–81, but they did not appear to establish (Craig and Loan 1984*b*). From 1978 to 2000, European *Peristenus* spp. including *P. digoneutis* were released several times in Western Canada, but no recovery attempts were made (Broadbent *et al.* 2002). However, it is now believed that *P. digoneutis* may have established in western Canada (Soroka and Carl 2002).

In the United States, Sorensen (1939) reported that 47% of eggs and 18% of nymphs of *Lygus* spp. were killed by parasitoids in alfalfa fields in Utah. Clancy and Pierce (1966) found that up to 11% *Lygus* nymphs were parasitized by *Leiophron uniformis* Gahan in California. To increase the control by parasitoids, native and European parasitoids were released in alfalfa fields in several localities. In 1964, an attempt was made to relocate and establish the native North American parasitoid *P. pallipes* in California (Clancy and Pierce 1966). In 1965, over 2200 *Lygus* nymphs collected from the released sites were dissected, and three nymphs were found to host the parasitoid. However, in 1966, 450 *Lygus* nymphs were dissected, and no parasitoids were

found. The results indicated that successful establishment was doubtful (Clancy 1968). Being considered superior over indigenous parasitoids in the sense that they parasitize plant bugs in various habitats (Day 1996), European parasitoids, *P. adelphocoridis*, *P. conradi*, *P. digoneutis*, *P. rubricollis*, and *P. stygicus*, have been introduced for establishment (Carl 1979; Craig and Loan 1984*a*, 1984*b*; Day *et al.* 1990), though only *P. conradi* and *P. digoneutis* are now established (Day *et al.* 1990, 1992). In New Jersey, *P. digoneutis*, became the dominant parasitoid within nine years of the first release in 1979 (Day 1996), and contributes the most to *Lygus* bug mortality (Day *et al.* 1990; Day 1996): from 1989–1994, *P. digoneutis* contributed 88% of all parasitism of *Lygus* nymphs; the remaining 12% was caused by native parasitoids (Day 1996). The species prefers *Lygus* bugs, and the parasitization rate was 3.6 times higher in *Lygus* spp. than in *A. lineolatus* (Day 1996).

Reports on the level of parasitization of *A. lineolatus* are fewer than for *Lygus* spp. In Ontario, 40–60% of nymphs of *A. lineolatus* were parasitized (Loan 1965), but only 2–3% were parasitized in Saskatchewan during the1960s (Craig 1963). To increase parasitism, in addition to releases stated previously for *Lygus* spp., European parasitoids including *P. adelphocoridis*, were released in Saskatchewan during the 1980s. Apparently, the attempt was unsuccessful, as no recovery has been made of the released species of parasitoids (Soroka and Carl 2002).

In the United States, Day *et al.* (1992) found 8% of nymphs of *A. lineolatus* were killed by native parasitoids in New Jersey during 1986–1990. To increase parasitism, European parasitoids were released in the United States (Day 1987). In 1984, *P. adelphocoridis* was released in New Jersey, although the species recovered from the

release area during 1985–1989 was *P. conradi*, which suggests that *P. conradi* was possibly released unknowingly with *P. adelphocoridis* (Day *et al.* 1992). The establishment resulted in three-fold increase of parasitism of *A. lineolatus* nymphs in New Jersey during 1985–1989 (Day *et al.* 1992). Since its establishment, *P. conradi* is spreading northeastward along the eastern seaboard of the United States (Day *et al.* 1992), and has spread into Quebec (Broadbent *et al.* 1999). The other established parasitoid, *P. digoneutis*, prefers *Lygus* spp. to *A. lineolatus*, and hence contributes little toward suppression of the latter insect (Day 1996).

A significant proportion of *A. pisum* is killed by parasitoids in North America. Several native parasitoid species are active (Mackauer and Finlayson 1967). To reinforce parasitic suppression, exotic parasitoids have been released in Canada and in the United States since the 1950s (Mackauer and Finlayson 1967). In Manitoba, *A. smithi* was released during 1980s and became established (Wylie *et al.* 2005), though *A. ervi* Haliday is now predominant (Matheson 1988; Chapter 3.2).

In the United States, various *Aphidius* spp. were introduced in 1958 and 1959, however, only *A. smithi* became established. Within ten years of release in Idaho and New Jersey, *A. smithi* became widespread in North and Central America (Angalet and Fuester 1977). *Aphidius ervi ervi* Haliday was introduced from Europe and released several times between 1959 and 1968 in Arizona, California, Delaware, Idaho, Maine, New Jersey, Oregon and Washington (Mackauer and Finlayson 1967; Angalet and Fuester 1977). This species became established and spread widely displacing *A. smithi*, and causing up to 80% of all the parasitism of *A. pisum* (Neuenschwander *et al.* 1975). Today, four introduced species, *A. ervi*, *A. smithi*, *Praon pequodorum* Viereck, and *P.*  *occidentale* Baker, along with the predaceous insects, suppress aphid populations for most of the season in North America (Neuenschwander *et al.* 1975).

Pathogen-inflicted mortality of Lygus and A. lineolatus has not been well studied, so little is known about this aspect. However, there have been reports on pathogenic suppression of A. pisum populations. In Nova Scotia, MacLeod (1955) found over 41% of field-collected A. pisum were killed by a fungus, Empusa (= Entomophthora) aphidis Hoffman. Milner et al. (1980) reported that in the central and northern United States, there is a high degree of A. pisum mortality due to infection by Entomorthhora sphaerosperma Fresenius. Hutchinson and Hogg (1985) report three species causing substantial suppression of A. pisum population in Wisconsin, Erynia neoaphidis (Remaudière and Hennebert), Erynia oxidentalis (Thaxter), and Entomophthora planchoniana Cornu. van den Bosch et al. (1966) noted that under favourable condition pathogens can decrease aphid populations to the point that natural enemies of aphids may starve and their populations are reduced by food shortage. An indigenous fungal pathogen Zoophthora phytonomi (Arthur) regulates H. postica populations substantially throughout much of its range including eastern Canada (Harcourt and Guppy 1991; Kingsley et al. 1993). There is little information about attempts to introduce pathogenic agents.

# Discussion

Alfalfa is grown mainly for livestock feed and seed production. Although crops of alfalfa are attacked by a plethora of insect pests, *Lygus* spp., *A. lineolatus* and *A. pisum* appear to be the most common and primarily damaging insect pests in the Canadian Prairies. The biology of these insects is mostly known. Although aspects associated with

the host finding and feeding chemistry of *Lygus* spp. and *A. pisum* have been substantially studied, little is known about *A. lineolatus* in these regards. Taxonomically *Lygus* spp. and *A. lineolatus* are closely related in that they both represent the Family Miridae (Hemiptera). As a result, it has been assumed that *Lygus* spp. and *A. lineolatus* might exhibit similarity in host finding and feeding behaviour. However, there are some differences between *Lygus* spp. and *A. lineolatus*. For example, *Lygus* spp. feed on a wide variety of host plants, and the insects overwinter as adults under leaf litter, whereas the host range for *A. lineolatus* is limited, and the insect overwinters as eggs in plant stems. Therefore, there might exist some differences between the two taxa in biological aspects like host finding and feeding chemistry.

In the Canadian Prairies, studies on the occurrence and seasonal distribution of insects on alfalfa crops have been conducted mainly in Alberta and Saskatchewan. There is some variation in the insect spectrum among different provinces. Timlick *et al.* (1993), after an extensive survey, concluded that the occurrence and seasonal distribution of insect pests and their natural enemies can vary across geographical locations and among crop types. Although, Timlick *et al.* (1993), and Gerber and Wise (1995) studied *Lygus* bugs in alfalfa fields in Manitoba, detailed information on the occurrence and seasonal distribution of alfalfa fields in Manitoba is lacking. The present study will be designed to gather this information.

Many farmers in Manitoba grow fields of alfalfa for hay in close proximity to seed alfalfa stands. In the Canadian Prairies, the hay crop is swathed usually twice in a crop-growing season (Goplen *et al.* 1987; Harper *et al.* 1990), and the seed crop is combined once in the fall (Goplen *et al.* 1987). The hay cutting reduces insect

populations by affecting their survival (Harper et al. 1990) and inducing their migration into adjoining seed fields (Stern et al. 1964; Summers 1976; Schaber et al. 1990b). Little is known about what proportion of the insect population survives and moves to neighbouring seed fields, and what is the impact of such movement on overall pest populations in seed fields, it is assumed that pest pressures are less intense in hay fields than in seed fields (Harper et al. 1990). However, this may not necessarily be the case always. Migration of mirids depends upon their life stages, the condition and growth stage of host plants (Hughes 1943; Khattat and Stewart 1980; Schaber et al. 1990b), and the presence or absence of physical barriers (Schaber et al. 1990b). In late August when most crops are harvested in the Prairies, plants that have regrown in hay fields after being swathed are still tender, succulent and more attractive than those in seed fields. There has been lack of comparative studies of pest insects and their natural enemies in hay and seed fields of alfalfa. There is also a need to assess the impact of hay-cutting on overall arthropod populations in alfalfa fields. Such studies will provide insight into improved production practices of alfalfa. The present study will examine the effect of hay-cutting and compare pest populations in hay and seed fields.

Although different control methods including cultural, chemical and biological control are available, current management practices tend to ignore biological control aspects. Notable success in controlling diseases and *A. pisum* populations has been achieved through using resistant cultivars of alfalfa. However, this aspect has been less explored for the other primary pests like *Lygus* spp. and *A. lineolatus*. Although found promising (Schaber and Entz 1991, 1994), the use of cultural controls like burning alfalfa stubble are less popular, probably because of operational problems and safety concerns;

dry and windy conditions sometimes make it risky to burn stubble in field conditions. Burning removes soil-cover and may increase the probability of soil erosion.

It appears that alfalfa seed growers in Canada rely heavily on chemical insecticides; such a practice is likely to have significant negative consequences including disruption of natural enemy populations and development of insecticide resistant pest populations (Debach and Rosen 1991; Summers 1998). Insecticide resistant *Lygus* populations have been frequently found in the United States (Xu and Brindley 1993; Snodgrass and Elzen 1995; Snodgrass 1996), although similar reports from Canada are infrequent. Despite the fact that *Lygus* spp., *A. lineolatus* and *A. pisum* co-occur in alfalfa fields and are exposed simultaneously to the insecticides, resistance problems have been frequently reported for *Lygus* spp. only. This is primarily because those studies focused on *Lygus* populations in cotton fields, one of the most popular crops in the United States and a crop not usually attacked by *A. lineolatus*. Detailed investigation is required in order to assess the insecticide resistance situation of insect pests frequently occurring in alfalfa fields in the Canadian Prairies.

The extent of mortality caused by natural biocontrol agents is important, and has implications in pest management decision-making processes. Although little is known about the suppression of insect pests caused by pathogens and nematodes, reports on pest suppression caused by predators and parasitoids are available. For predators, interest has been focused primarily on coccinellids, chrysopids and nabids. Predators are frequently generalists, and many of them – for example, coccinellids, chrysopids and nabids, which are common in alfalfa fields in the Canadian Prairies, consume *Lygus* spp., *A. lineolatus*, and *A. pisum*. Despite reports that coccinellids along with other natural enemies

successfully suppress *A. pisum* populations on alfalfa in Alberta (Frazer *et al.* 1981*b*), there is little documentation of what effects these predators have on populations of the other insect pests, particularly of *Lygus* spp. and *A. lineolatus* in Canadian alfalfa fields. In addition, although alfalfa fields have been reported to be rich in predaceous carabids (Loss and Allen 1983; Barney *et al.* 1983; Barney and Pass 1986) and arachnids (spiders and harvestmen), information on these taxa is lacking from alfalfa fields in Canada. Information on their occurrence in relation to current context of alfalfa fields and the effects of these predaceous taxa on pest populations will contribute toward improved pest management in fields of alfalfa. The present study will examine populations of carabids and arachnids, and explore what roles the commonly occurring insect natural enemies have on pest populations including *Lygus* spp. and *A. lineolatus*.

Parasitoids are an integral component of alfalfa fields in North America. Their presence and effects on insect pests including *Lygus* spp., *A. lineolatus* and *A. pisum* in alfalfa fields have been studied elsewhere in the Canadian Prairies and in the United States. Introductions of the parasitoids of *A. pisum*, *Lygus* spp. and *A. pisum* have been made in both Canada and United States in order to increase the natural mortality of these pests. This review provides circumstantial evidence that those introduced parasitoids have been spreading to regions where they were not originally released (Day 1996; Broadbent *et al.* 1999). These parasitoids along with the native parasitoids kill high proportions of their hosts in the United States (Neuenschwander *et al.* 1975; Day *et al.* 1990; Day 1996). Although, information on the post-release establishment of mirid parasitoids in Canada is lacking, there has been substantial mortality caused by parasitoids in western Canada (Braun *et al.* 2001; Broadbent *et al.* 2002). Although

information on pea aphid parasitism is available (Matheson 1988; Deneka 1992), little is known about mirid parasitism in alfalfa fields of Manitoba. The present study will examine parasitism of these insect pests in fields of alfalfa in Manitoba.

Adherence to threshold levels reduces the chances and extent of the negative effects of insecticide use and increases the benefits from insect natural enemies. This review indicates that growers in the Canadian Prairies use nominal economic thresholds, and the benefits of insect natural enemies are usually ignored in pest control decisionmaking processes. In addition, alfalfa growers seldom deploy any measures to increase natural enemy activities. Natural enemy activities are usually increased through releasing or attracting them in fields (Debach and Rosen 1991). Due to problem in retaining natural enemies in fields to where they are released (Frazer 1988; Zhu et al. 1999), compounds attractive to insect natural enemies could be used to successfully attract and retain them in crop fields (Zhu et al. 1999). Recently, interests in using semiochemicals, particularly the sex pheromone compounds, are increasing in the field of pest management in field crops. Although pheromones alone are of limited potential in suppressing pest populations in a crop field, they can be effectively used along with other methods in an integrated pest management program. This is because the pheromone compound can be used in such a way that the deleterious effects of chemical insecticides can be off-set (Howse 1998). There have been reports that A. pisum sex pheromone compounds attract chrysopids (Boo et al. 1998) and aphid parasitoids (Hardie et al. 1991, 1994). Therefore, it is desirable that potential of these compounds in pest management be examined in the context of alfalfa fields. The present study will assess whether aphid semiochemical compounds can be used to enhance natural enemy activities in fields of alfalfa.

Clearly, alfalfa fields are simultaneously visited by a number of insect pests and their natural enemies, and these taxa are interacting with each other. Also noted in this review is that although a plethora of strategies are available along with the biological pest suppression, pest management strategies on alfalfa are dominated by the frequent use of synthetic insecticides. An insecticide-dependent pest management strategy usually does not take into account the interactions between insect pests and their natural enemies. Knowledge of pest occurrence, understanding of pest population dynamics, establishment of pest control economics along with development and popularization of nonchemical alternatives are required for effective and better management of pest populations on alfalfa. The present study will gather information on pest insects and other information that will form the basis of improved pest management in fields of alfalfa. CHAPTER 3.1

Comparison of five sampling techniques for estimating populations of Lygus spp.,

Adelphocoris lineolatus (Hemiptera: Miridae) and Acyrthosiphon pisum

(Homoptera: Aphididae) in seed alfalfa fields

## Abstract

Five methods of insect sampling, sweep-net, vacuum, whole plant bag, beat tray and fumigation cage sampling, were evaluated for estimating abundance of lygus bugs, *Lygus* spp. Hahn, alfalfa plant bug, *Adelphocoris lineolatus* (Goeze), and pea aphid, *Acyrthosiphon pisum* (Harris) on alfalfa plants in Manitoba. Regressions of estimates of insect numbers from one sampling method on numbers from others allowed the conversion of estimates from one method to corresponding estimates from the others. Examination of estimates and precision in relation to time of season indicated that sweepnet sampling was the most reliable method of sampling insect pests on alfalfa. For all three insect taxa, sweep-net sampling provided consistent precision of estimates over most of the season. However, beat tray sampling was more efficient for sampling *Lygus* spp. nymphs, suggesting that in situations where juveniles of *Lygus* spp. are of critical interest, beat tray sampling could effectively replace sweep-net sampling for monitoring the pest population.

# Introduction

For economic pest management programs in crops, the decision on whether to apply pest control measures requires information on insect pest abundance. For effective decision making, precise and accurate estimates of insect abundance are needed (Pedigo 1999). Precision indicates the closeness of sample estimates to their means, whereas accuracy indicates the deviations of the sample estimates from the population parameter that is being estimated (Sokal and Rohlf 1995; Fowler and Witter 1982). The accuracy of an estimate can be determined only if unbiased estimates or those with known bias are available (Southwood 1978), however, this is seldom possible in real field situations (Fowler and Witter 1982). For decision making, a precise and consistently biased method may be more satisfactory than an accurate but imprecise technique (Cochran 1977). Precision and accuracy are related to sampling cost; in particular, costs tend to increase with increasing precision (Ruesink 1980).

Pest mirids, mainly lygus bugs, *Lygus* spp. Hahn and alfalfa plant bug, *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), and the pea aphid, *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) are common insect pests that often cause economic damage to alfalfa crops in North America (Goplen *et al.* 1987; Schaber 1992; Summers 1998 and references therein). Various sampling methods are available for monitoring pest mirids and aphids in crop fields (Schotzko and O'Keeffe 1986, and references therein). The ideal method is one that maximizes precision while minimizing sampling time and cost (Gomez and Gomez 1984). Because they tend to be easier and less laborious, relative sampling methods that provide population estimates without reference to unit of habitat (Southwood 1978) are commonly used (Schotzko and

O'Keeffe 1986). Sweep-net sampling is a relative sampling method, and is the most frequently used tool for estimating insects on crops including alfalfa (Fleischer *et al.* 1985; Schotzko and O'Keeffe 1986, and references therein; Murrell 1987; Schaber 1992; Snodgrass 1993; Soroka and Murrell 1993; Braun *et al.* 2001).

The accuracy of insect population estimates from sweep-net sampling is influenced by many factors. For example, estimation of populations of *Acyrthosiphon pisum* (Harris), *Empoasca fabae* (Harris), and *Philaenus spumarius* (L.) in alfalfa fields is influenced by the time of day and by the person sampling, but sweep-net estimates of the former two insect species are most affected by plant height (Saugstad *et al.* 1967). Insect distribution, density and activity, plant height and density were found to influence sweepnet estimates on cotton (Ellington *et al.* 1984). Schotzko and O'Keeffe (1986) found that in lentil fields, sampling in the afternoon provides more accurate estimates of *Lygus hesperus* (Kelton) than in the morning. Nevertheless, sweep-net sampling is less time consuming than alternatives and can detect insect presence even at low densities. However, it does not provide absolute estimates of insect densities (Maiteki *et al.* 1986).

Beat tray sampling involves striking the plant or a part of it a prescribed number of times with a stick while placing a tray underneath to collect the dislodged insects (Pedigo 1999). This method is often used for sampling arthropods in different field and horticultural crop fields (Southwood 1978; Horton and Lewis 1997, and references therein). In alfalfa fields, the shake-bucket method, a knockdown method in which a bucket replaces the tray, is frequently used to monitor larvae of alfalfa weevil, *Hypera postica* (Gyllenhal) (Hoff *et al.* 2002). Although more tedious and slower than sweep-net sampling (Hoff *et al.* 2002), the beat tray method is more useful when plants are not tall

enough for effective sweeping, or when plants are tangled together. However, the method can be inefficient for smaller arthropods that are often overlooked, or for the active insects that can escape before being noticed (Southwood 1978), and therefore, can be misleading for assessing the age distribution of a population (Poe 1980). However, the use of a smaller beat tray may reduce the frequency of smaller arthropods being overlooked.

Kogan and Pitre (1980) described the fumigation cage method of sampling, in which boards or hard sheets are placed around plant bases, and a wooden, metallic or plastic can is dropped so that the plant to be sampled is caged. Then plants inside the cage are fumigated with insecticides and shaken well to dislodge insects that are clinging to them. After removing the cage, insects that have fallen on the board or sheet are counted. The fumigation cage method estimates the number of individuals per unit habitat, and the effectiveness of the method is less influenced by environmental, human and other biological factors (Kogan and Pitre 1980). Fumigation cage sampling has been used for estimating pest populations on soybean (Kogan and Pitre 1980), although, it has seldom been used on alfalfa.

In the vacuum sampling method, arthropods are caught by operating a suction machine while placing the suction hose over plants or parts thereof to be sampled. Arthropods that are accumulated in the removable collection net located inside the suction hose are counted (Pedigo 1999). Vacuum sampling has been used for estimating insect populations in field crops. The most commonly used vacuum sampler is the D-Vac, which has been frequently used for estimating plant bugs in various field crops including alfalfa (Pruess *et al.* 1977; Schotzko and O'Keeffe 1986; Šedivý and Kocourek

1988). The method is suitable for small insects that are not induced to jump or fly by the noise of the suction machine (Kogan and Pitre 1980). In lentil fields, D–Vac samples provided better estimates of *L. hesperus* adults and nymphs than did sweep-net sampling (Schotzko and O'Keeffe 1986). However, initial and operational costs are high and maintenance problems frequent for vacuum sampling devices (Kogan and Pitre 1980).

Whole plant bag sampling involves random selection of plants to be sampled and the counting of arthropods on the entire plant. In this method, a cylindrical bag is usually lowered over the selected plant and folded flat on the ground so that the bag surrounds the base of the plant. After allowing a period of time to pass in order to minimize the effect of disturbance, at sampling the plant and arthropods on it are collected by pulling the bag rapidly over the plant and closing both ends. Then the sample is processed and arthropods inside the bag are counted (Byerly et al. 1978). Whole plant bag sampling has been used for estimating pest populations on crop plants including cotton (Byerly et al. 1978; Fleischer et al. 1985) and potato (Senanayake and Holliday 1988). The method is too complex for use in routine scouting of arthropods in field crops (Senanayake and Holliday 1988). However, Byerly et al. (1978) found that the whole plant bag sampling method was a more reliable method than suction, sweep-net and visual methods for estimating active arthropod populations on cotton. Senanayake and Holliday (1988) found the method to be more suitable than visual sampling for potato flea beetles. The method, however, has been seldom used for estimating arthropod populations on alfalfa.

It appears that various sampling methods are available for estimating arthropod populations on the alfalfa crop, although the sweep-net is commonly used. The effectiveness of sweep-net sampling has not been compared with that of alternative

methods in alfalfa fields. The present study compares the characteristics of sweep-net sampling with those of vacuum, whole plant bag, beat tray and fumigation cage methods of sampling to determine the most effective practical method for estimating populations of pest mirids and aphids on alfalfa plants.

## **Materials and Methods**

The study was conducted in one commercial alfalfa field managed for seed production in each of the three years during 1999 to 2001. The study fields were near Teulon, Arborg, and Riverton, Manitoba in 1999, 2000 and 2001, respectively. In 1999, the methods of sampling examined were sweep-net sampling, whole plant bag sampling and vacuum sampling. In 2000, fumigation cage and beat tray sampling replaced the whole plant bag and vacuum methods of sampling. In 2001, the fumigation cage method of sampling was discontinued.

Starting from about the beginning of June and continuing to about the end of August, samples were taken at weekly, at two- or three-week and at two- to five-week intervals in 1999, 2000 and 2001, respectively. Samples were collected between about 1100 and 1500 h CDST. On each sampling occasion, six to 12 samples were collected from each field by each method of sampling. Sampling sites in a field were marked, and these sites were separated from each other by at least 50 m.

For sweep-net samples, a 38 cm diameter sweep-net with a 90 cm long handle was used. During sweeping, the crop was swept at arm's length 30 or 50 times through an arc of 180°, which constituted a sample. Fifty sweeps constituted a sample when the number of insects was low during June; later in the season a sample consisted of 30 sweeps. During sweeping, the bag frame went through a horizontal plane, with the top of

the frame at the level of the plant tops. The same plants were not sampled in two successive weeks. Each sample was transferred to a 33 x 25 cm polyethylene Ziploc<sup>®</sup> bag containing a cotton-wool wad soaked with ethyl acetate to kill the insects and prevent predation and reproduction. The samples were transferred to the laboratory, where Lygus spp., A. lineolatus and A. pisum were counted. Although Lygus complex in alfalfa fields of Manitoba comprise L. lineolaris, Lygus borealis and L. elisus (Schwartz and Foottit 1982b; Timlick et al. 1993; Mostafa, pers. comm.), their numbers were not recorded separately because, nymphs are not possible to identify to species and most of the adults in samples were Lygus lineolaris. Lygus spp. and A. lineolatus nymphs were recorded in categories: first- to third-instar nymphs were recorded as younger, whereas the fourth and fifth instars were recorded as older nymphs. When A. pisum numbers were high, they were indirectly estimated by weighing. Two representative sub-samples, each of 10 mg, were randomly taken from the sample and the average number of individuals in a subsample was determined. The total number of individuals in a sample was then calculated as follows:

## Weight of all aphids (mg) x average number of aphids in a sub-sample (10 mg) 10 mg

For the whole plant bag sample, a plant was selected near every swept site. Two to three weeks before taking the sample, a 80 cm long transparent cylindrical tube (35 cm diameter) of polyethylene was lowered over the selected plant. The lower end of the tube was tied around the plant base, and the bag was folded flat on the ground so that it surrounded the plant base. During sampling, the open end of the tube was rapidly pulled up over the plant and closed at the top to form a bag. The plant was then cut at ground level. In the laboratory, samples were kept at 5°C for at least 24 h to pacify the insects.

*Lygus* spp., *A. lineolatus*, and *A. pisum* in samples were sorted out, and recorded as before.

For vacuum samples, a reverse-blow BR 400 backpack airbroom (single cylinder two-stroke air-cooled engine, Stihl<sup>®</sup>), which can displace a maximum of 13.5 m<sup>3</sup>/min of air, was used. Samples were taken within 2 m of each sweeping site. While operating the machine at full power, insects were vacuumed by placing the 8.5 cm (dia) suction-hose over plants and quickly lowering the nozzle until it reached the bottom of the plant's canopy. Each nozzle placement, covering approximately one alfalfa plant, constituted a sample. The sample was collected in a cone shaped netting-bag fitted inside the suction-hose, and the bag was emptied into a Ziploc<sup>®</sup> bag. The samples were processed in the same way as for sweep-net samples.

For beat tray samples, a 32 x 27 x 5 cm (long x wide x deep) white plastic tray was used. A sampling spot within 2 m of each sweeping site was chosen; the tray was placed under the plant canopy, and plants over the tray were beaten vigorously – but without damaging them – by means of a stick. Numbers of *Lygus* spp., *A. lineolatus* and *A. pisum* individuals were recorded. In 2000, *Lygus* spp. and *A. lineolatus* were recorded together as mirids, but in 2001, nymphs and adults of each taxon were recorded separately.

The method described by Kogan and Pitre (1980) was modified for fumigation cage sampling. A 40 x 30 cm (deep x diameter) plastic bucket (Pro-Western Plastic Ltd.) was used. Within 2 m of each sweeping site, the bucket was placed upside down over alfalfa plants that had not previously been disturbed, and a white coloured, rigid plastic board, about 1 cm thick, was pushed in between the ground and the bucket mouth. During

the push, the plastic board usually bent plant stems that were in its way to the far wall of the bucket. Through a 4-cm hole in the sidewall of the bucket, synergized pyrethrins (allethrin 0.176%, tetramethrin 0.09%, piperonyl butoxide 1.25%) were sprayed from an aerosol spray canister, so that the trapped insects were knocked down on the plastic board. One minute after the spray was made, one side of the bucket was slightly lifted and a hand was inserted through it to shake off any insects clinging on the plants inside. Then the bucket was removed and the numbers of insects on the plastic board were counted. Only insects in the three groups of interest were recorded. The number of insects was very low in most instances, and therefore, *Lygus* spp. and *A. lineolatus* were recorded together as mirids; *A. pisum* were recorded separately.

To compare sampling methods, General Linear Modeling (Systat 2002) was performed, and Tukey's test was used to compare mean estimates from different sampling methods in 1999 and 2000. Data collected in sweep-net samples were converted to equivalents of a single sweep. Weekly mean catches per sample-type per field were considered for the analysis. Area covered in a sample was not measured, and hence it was not possible to establish absolute estimates and compare the methods accordingly. Variance:mean ratio is a measure that indicates insect dispersion (Sokal and Rohlf 1995), and was analyzed in order to compare biases among different sampling methods in this regard. Coefficient of variation (CV = 100\*standard deviation/mean), which compares the magnitude of variation among means (Sokal and Rohlf 1995), and relative variation (RV = 100\*standard error/mean), which indicates the degree of error in making estimates (Pedigo 1999), were calculated for each sample type and date, as measures of precision. Adults of *Lygus* spp. and *A. lineolatus* were often missing in samples taken by using

vacuum and whole plant bag methods. In this case, calculation of individual CVs and RVs was impossible. Numbers of different life stages of *Lygus* spp. and *A. lineolatus* in samples were often small. Therefore, their pooled numbers regardless of taxon and life-stage were used for determination of CVs and RVs.

Regressions of insect numbers for different sampling methods were used to obtain calibration coefficients, allowing prediction of estimates from one sample method to another. Data paired by sampling sites within the field and dates were used for regression lines, and lines were fitted separately for each year. Lines were forced through the origin, 0, when intercepts were not significantly different from zero. Seasonal changes in regression coefficients were examined only for overall significant relationships. Analysis of covariance was performed to compare between regressions calculated separately for June, July and August. Intercepts were always included in this analysis. Unless otherwise specified, a significance level of  $P \le 0.05$  was used for experiment-wise comparisons.

## Results

Among the three sampling methods examined in 1999, sweep-net samples contained most catches of mirids (Table 3.1.1) comprising *Lygus* spp. and *Adelphocoris lineolatus*. For *Lygus* bugs, catches of adults, and adults and nymphs together (total) differed significantly among the sampling methods. However, there were no significant differences among sample types when estimating *Lygus* nymphs. Similar trends were found for *A. lineolatus*: sampling method affected estimates of adults and total *A. lineolatus*, but not estimates of *A. lineolatus* nymphs. For estimates of adults and total of both genera of mirids, sweep-net estimates were significantly greater than those from the other methods (Table 3.1.1), and vacuum sampling provided the lowest catches of *Lygus* 

and *A. lineolatus* adults (Table 3.1.1). Catches of *A. pisum* did not differ significantly among sampling methods, although numbers per sweep were considerably greater than those per sample collected by other methods (Table 3.1.1).

In 2000, estimates of mirids differed among sampling methods; the fumigation cage method of sampling appeared inefficient for estimating mirids compared with sweep-net and beat tray. The numbers of mirids per sample unit from the latter two methods of sampling were not significantly different (Table 3.1.1). Estimates of *A. pisum* numbers did not differ among the sampling methods, although the fumigation cage provided considerably lower estimates than did sweep-net and beat-tray methods.

In 2001, sweep-net and beat tray estimates of total mirids were again not significantly different (Table 3.1.1). The result was similar for nymphs, adults and nymphs plus adults (total) of *Lygus* spp. and *A. lineolatus*. However, sweep-net samples tended to have numerically more *A. lineolatus* nymphs, adults and nymphs plus adults, whereas, beat tray estimates tended to have greater estimates of total *Lygus* nymphs (Table 3.1.1). Beat tray sampling appeared to be more effective for young *Lygus* nymphs: the number of adults in beat tray samples was 44% of that in a sweep, but for young nymphs, it was 465% ( $F_{1,16} = 12.40$ ; P < 0.01) and for older nymphs it was 154% ( $F_{1,16} = 0.66$ ; ns). For *A. lineolatus*, the percentages were: 42, 55 and 55, respectively, which is fairly constant. From this, it can be concluded that for *Lygus* nymphs, particularly young nymphs, beat tray sampling is relatively more efficient than for *Lygus* adults or for *Adelphocoris lineolatus* (Table 3.1.1). Sweep-net and beat tray sampling did not differ in catches of *A. pisum*, however, a beat tray sample provided twice the average number in a single sweep (Table 3.1.1).

There was a difference in the variance:mean ratio among sampling methods: in 1999, this was significant for mirids ( $F_{2,24} = 9.33$ ; P < 0.01), and *A. pisum* ( $F_{2,24} = 4.86$ ; P < 0.01). Of the methods examined in 1999, the variance:mean ratio was often below 1.0 in sweep-net samples, and above 1.0 in whole plant bag and vacuum samples (Fig. 3.1.1). Deviations to this trend were at the beginning of September, and during mid-July to the beginning of August, when the ratio in sweep-net samples was above 1.0 for mirids (Fig. 3.1.1 a) and *A. pisum* (Fig. 3.1.1 b), respectively.

In 2000, the sampling methods significantly differed in the variance:mean ratio for mirids ( $F_{2,15} = 4.16$ ; P < 0.05) and *A. pisum* ( $F_{2,15} = 5.51$ ; P < 0.05). As in 1999, the variance:mean ratio was usually below 1.0 for mirids in sweep-net samples. Except in July, the ratio was usually below 1.0 in fumigation cage samples, however, for mirids in beat tray samples the ratio was frequently above 1.0 (Fig. 3.1.2 a). For *A. pisum*, the variance:mean ratio was usually below 1.0, whereas in beat tray and fumigation cage samples, the ratio was usually greater than 1.0 (Fig. 3.1.2 b).

In 2001, the variance:mean ratio significantly differed for mirids ( $F_{1,16} = 23.03$ ; P < 0.01) and A. pisum ( $F_{1,16} = 18.17$ ; P < 0.01) among sampling methods. The ratio was usually below 1.0 in sweep-net samples, and frequently above 1.0 in beat tray samples (Fig. 3.1.3 a, b).

There was variation in coefficients of variation (CVs) among sampling methods. In 1999, this variation was highly significant for mirids ( $F_{2,24} = 20.69$ ; P < 0.01), and significant for *A. pisum* ( $F_{2,24} = 4.21$ ; P < 0.05). Of the methods examined in 1999, sweep-net sampling provided the lowest and most consistent percent coefficients of variation (seasonal means for mirids:  $58.58 \pm 11.98\%$ , *A. pisum*:  $57.92 \pm 12.03\%$ ).

However, in September, the precision of sweep-net sampling declined greatly to become comparable to that of the vacuum sampling method (Fig. 3.1.4). Coefficients of variation for both vacuum (means for mirids:  $171.88 \pm 16.70\%$ , *A. pisum*:  $110.32 \pm 23.91\%$ ) and the whole plant bag sampling (means for mirids:  $171.66 \pm 28.07\%$ , *A. pisum*:  $112.56 \pm 21.31\%$ ) methods were relatively high and variable. There were instances where no individuals appeared in samples collected by vacuum and the whole plant bag sampling methods, and therefore, no CVs could be calculated (Fig. 3.1.4). Although, vacuum and the whole plant bag sampling provided similar levels of mean CVs, the latter appeared to be the most inconsistent method (Fig. 3.1.4). There was no significant correlation of the seasonal variation in CVs for different methods except for the vacuum and the whole plant bag samples, for which the correlation was nearly significant for mirids (Table 3.1.2).

In 2000, the variation of CVs among sampling methods was significant for mirids  $(F_{2.15} = 10.89; P < 0.01)$  and *A. pisum*  $(F_{2,15} = 9.19; P < 0.01)$ . As in 1999, it appeared that sweep-net sampling was the most precise method, as it had the lowest and most consistent coefficient of variation for both mirids (seasonal mean  $51.26 \pm 10.63\%$ ) and *A. pisum* (mean  $73.23 \pm 19.96\%$ ) (Fig. 3.1.5). Beat tray sampling (means for mirids:  $91.34 \pm 27.11\%$ , *A. pisum*:  $153.34 \pm 27.58\%$ ) tended to be more precise than fumigation cage sampling (means for mirids:  $132.00 \pm 28.71\%$ , *A. pisum*:  $172.99 \pm 26.31\%$ ). There was significant correlation or nearly significant correlation of the seasonal variation in CVs for the three different methods, except for sweep net and fumigation cage sampling (Table 3.1.2).

In 2001, the variation of CVs between the sampling methods was almost significant for estimating mirids ( $F_{1,16} = 12.39$ ;  $P \approx 0.06$ ), but not for *A. pisum* ( $F_{1,16} = 1.93$ ; P < 0.22). As in 2000, the coefficient of variation tended to be lower and less variable in sweep-net samples (means for mirids: 29.53 ± 5.31%, *A. pisum*: 80.74 ± 22.17%) than in beat tray samples (means for mirids: 97.17 ± 32.19%, *A. pisum*: 129.46 ± 31.56%) (Fig. 3.1.6). Sweep-net sampling appeared almost consistently precise for mirids throughout the season, although, for *A. pisum*, the precision tended to decline as the season progressed. In beat tray samples, precision tended to increase with the progress of the season (Fig. 3.1.6). There was significant correlation of the seasonal variation in CVs for sweep-net and beat tray samples estimating mirids, although the relationship was not significant in the case of *A. pisum* (Table 3.1.2).

In terms of relative variation, sweep-net sampling was the most precise and consistent method for estimating mirids and *A. pisum*. For mirids, sweep-net samples provided relative variation values of about 25% in 77% (range 57–100%) cases of sampling, and in 67% (range 57–82%) cases for *A. pisum*. The relative variances were about 10% in 39% (range 14–67%) cases of sweeping for mirids, and in 24% (range 14–44%) cases for *A. pisum*. Beat tray samples provided relative variation values of about 25% in 62% (range 57–67%) occasions of sampling for mirids, and in 25% (range 22–28%) cases for *A. pisum*. Beat tray seldom provided relative variations around 10% for any taxa. Vacuum samples never, and whole plant bag and fumigation cage samples seldom, provided relative variation near 25%.

The linear regression equations relating estimates from different sampling methods are given in Tables 3.1.3–3.1.5. In 1999, there was a significant linear

relationship between A. pisum numbers in the different sampling methods examined, but not between mirid numbers in different sampling methods (Table 3.1.3). Most A. pisum individuals occurred in late July, and the relationships in numbers between different sampling methods were inconsistent during most of the season. The intercepts were significant in all the relationships examined, suggesting that sweep-net sampling was more efficient in collecting insects at low densities compared with the whole plant bag and vacuum sampling. Similarly, vacuum sampling was more efficient than the whole plant bag sampling at low pest populations (Table 3.1.3). Analysis of covariance showed a significant difference in intercepts and slopes for the regression lines obtained for A. *pisum* in different months except for the slope for the relationship between vacuum and whole plant bug samples {sweep-net and whole plant bag samples:  $F_{3,80} = 13.75$  (P < 0.01) and 3.14 (P < 0.05); vacuum and whole plant bag samples:  $F_{3,80} = 9.26$  (P < 0.01) and 0.39 (ns); sweep-net and vacuum:  $F_{3,80} = 2.78$  (P < 0.05) and 3.08 (P < 0.05) for intercepts and slopes, respectively}, although the significance disappeared when data from the month of July were excluded.

In 2000, there was a significant linear relationship between sweep-net and beat tray samples estimating mirids and *A. pisum* numbers, although in August the relationship was not significant for mirids (Table 3.1.4). Analysis of covariance showed significant difference in intercepts ( $F_{2,66} = 13.47$ ; P < 0.01) but not in slopes ( $F_{2,66} = 1.36$ ; ns) for the regression lines obtained for mirids in different months: the intercept in August differed significantly from those in June or July, although intercepts for lines in the latter two months did not differ. For *A. pisum*, however, there was no difference in intercepts ( $F_{2,66} = 0.31$ ; ns) for the regression lines obtained in different

months. There was no significant relationship between sweep-net and fumigation cage samples estimating mirids or *A. pisum* numbers (Table 3.1.4). For mirids, beat tray sampling was significantly correlated with fumigation cage sampling, although the relationship disappeared after June (Table 3.1.4). In this case, intercepts differed significantly ( $F_{2,66} = 11.90$ ; P < 0.01), although slopes did not differ ( $F_{2,66} = 1.34$ ; ns) among different months. For *A. pisum*, however, no significant relationship between beat tray sampling and fumigation cage sampling existed (Table 3.1.4), and intercepts ( $F_{2,66} = 0.58$ ; ns) and slopes ( $F_{2,66} = 1.78$ ; ns) did not differ either.

In 2001, the relationships for mirid and *A. pisum* numbers between sweep-net sampling and beat tray sampling was similar to those in 2000, except that for mirids, the results were opposite in the months of July and August (Table 3.1.5). Analysis of covariance showed significant differences in intercepts ( $F_{2,66} = 9.70$ ; P < 0.01) and slopes ( $F_{2.66} = 14.47$ ; P < 0.01) for the regression lines obtained for mirids in August compared to those in June or July, although those for lines in the latter two months did not differ. For *A. pisum*, however, there was a significant difference in slopes ( $F_{2,66} = 3.81$ ; ns) but not in intercepts ( $F_{2,66} = 1.19$ ; ns) for the regression lines obtained in different months.

## Discussion

In addition to the sampling technique itself, several factors including the efficiency and experience of samplers; density, structure, growth stages and height of plants; distribution, density, life stages, and behaviour of organisms being sampled; time of the season and of the day; and temperature, radiation and moisture may influence the variability and efficiency of sampling (Southwood 1978; Ellington *et al.* 1984; Frazer and Raworth 1985). The distribution of different life stages of an insect species, which can

vary temporally and spatially (Wilson et al. 1984; Schotzko and O'Keeffe 1989b, 1989c; Snodgrass 1998), may have implications for sampling efficiency. It is likely that variability among samples of an insect that is uniformly distributed will be less pronounced than that of an insect with aggregated distribution. In alfalfa, colonies of A. pisum start from individuals that parthenogenetically reproduce and produce non-winged offspring. As a result, they tend to be aggregated in distribution, although crowding provokes development of winged individuals that disperse to other plants (Soroka 1991). This may result in a larger proportion of plants having colonies, and so a reduction in the level of aggregation is likely. Schotzko and O'Keeffe (1989b; 1989c) monitored Lygus hesperus by using a sweep-net, and found that the distribution of Lygus adults and nymphs varied as the growing season of cotton progressed and pest population density changed. At the beginning adults were aggregated, in the middle they were aggregated at low densities, random to uniform at high densities, and at the end of the season, they were clumped. In contrast, nymphs were uniform to random until late in the season, when increase of their number resulted in them being clumped. These changes may be due to changes in the insect's biological aggregation, or due to changes in the sampling efficiency of sweep-net sampling with changes in plant structure as the plants grow, or both. Early in the season, plant bugs feed on vegetative structures. As soon as flowers and fruits appear, the bugs feed preferentially on these reproductive parts (Hughes 1943). Adults and nymphs of Lygus bugs also differ in distribution within the host plant. Snodgrass (1998) reported that adults of L. lineolaris prefer vegetative and meristematic structures, whereas nymphs prefer fruiting structures, although more nymphs than adults tend to visit the lower halves of cotton plants. Wilson et al. (1984) found that nymphs of

*L. hesperus* are distributed on cotton plants mostly within the fifth through seventh nodes from the terminal. Such seasonal and spatial differences and changes of insect distributions are likely to affect sampling efficiency on a season-long basis, a trend that was also reflected in seasonal trends of CVs (Figs. 3.4–3.6) and RVs achieved by using different sampling methods in the present study.

Generally, Lygus spp., and A. lineolatus occur simultaneously in alfalfa fields, and therefore, pest control decisions for alfalfa are often based on pooled numbers of these two insect pests (Murrell 1987; Schaber 1992). Both sweep-net and beat tray methods provided similar estimates of mirids. It was not possible to standardize the area and volume covered by a beat tray sample, although the area of the tray used was known. This is because, tangled alfalfa plants often formed a mat of foliage representing parts of several neighboring plants. However, beat tray sampling was more effective than sweepnet for estimating A. pisum and nymphs, particularly the young ones, of Lygus; this is not surprising. Although active on shoot terminals, A. pisum are perhaps less able to cling on the plant in the face of the sudden jarring of the beat than they are during the sweeping motion of the net. For Lygus bugs, as nymphs are more active in the lower portion and fruiting structures of plants (Snodgrass 1998). Alfalfa fruiting bodies are coiled in shape (Goplen et al. 1987). Therefore, shaking or beating plants is more likely to dislodge nymphs than is sweeping (Snodgrass 1998). Adults of Lygus bugs are most active on leaves and meristematic terminals (Schotzko and O'Keeffe 1989b; Snodgrass 1998). Sweep-net sampling usually samples upper plant parts (Šedivý and Kocourek 1988), and so is a more efficient sampling tool for adults (Snodgrass 1998). Sweep-net sampling appeared to be equally as efficient as beat tray sampling for older nymphs, which more

frequently visit the leaves and the upper terminals of plants than the young ones (Wise and Lamb 1998). In previous studies, sweep-net sampling has been found to be inefficient for sampling *Lygus* nymphs on different crop plants (Byerly *et al.* 1978; Ellington *et al.* 1984; Fleischer *et al.* 1985; Schotzko and O'Keeffe 1986; Butts and Lamb 1991; Snodgrass 1993). Fleischer *et al.* (1985) found that sweep-net sampling collected only 10% of nymphs present on cotton plants. Wise and Lamb (1998) concluded that sweep-net samples underestimate true densities, because they do not collect all plant bugs in the sampled area. Byerly *et al.* (1978) proposed that the sweepnet would be more efficient when plants are small and the canopy is open. However, as the canopy closes and plants become taller, the sweep-net would tend to sample only the upper parts of plants and become less efficient.

Pruess *et al.* (1977) reported that in alfalfa fields, vacuum samples can contain insects suctioned from outside the sampling area. The superiority of D–Vac over sweepnet has been previously reported for sampling different insects (Schotzko and O'Keeffe 1989*a*; Šedivý and Kocourek 1988). By using a D–Vac, Šedivý and Kocourek (1988) captured twice as many insects as in the sweep-net from alfalfa fields. However, in the present study, vacuum sampling consistently gave lower counts of all three groups of insects. This is possibly because, a vacuum sample taken in the present study covered a considerably smaller area and volume of plant canopy compared with a D–Vac sample in reported studies, and also compared with a sweep-net sample in the present study. In addition, the vacuum sampler probably differed in suction capacity and speed from a D– Vac, and this would influence sampling performance (Southwood 1978). However, it

would appear that the vacuum sampler does a tolerable job of sampling aphids (Table 3.1.1).

Byerly et al. (1978) compared the whole plant bag sampling with suction, sweepnet and visual sampling of insects on cotton plants, and concluded that the whole plant bag sampling provided the best estimates of active arthropods. Senanayake and Holliday (1988) compared whole plant bag with sweep-net and visual sampling, and also found a similar result for potato flea beetles on potatoes. The whole plant bag method also estimated Colorado potato beetle more efficiently than did visual sampling, although for sampling potato leafhoppers, the whole plant bag method appeared to be inferior to sweep-net method (Senanayake and Holliday 1988). However, in the present study, whole plant bag sampling appeared to be ineffective compared with sweep-net sampling. The reason for the different result is unclear, however, plant characteristics in addition to the nature of the pest species are likely to have contributed, at least partly, to the result. Cotton plants are usually erect with less complex canopy structure. Potato plants are usually evenly spaced in wider rows, and they are often trailing when large, although plants of the cultivar "Norland" used by Senanayake and Holliday in 1988 are relatively small and compact (Senanayake and Holliday 1989), and are unlikely to entangle, as do alfalfa plants, which have complex canopy structure. Alfalfa plants in the studied fields were unevenly spaced, often tangled with each other, and plant shoots often spread beyond the perimeter of the polyethylene tube. These characteristics complicated unrolling the polyethylene tube over the plant without loss of active insects like Lygus spp. and A. lineolatus. The apparently greater relative efficiency of the whole plant bag sampling for mirid nymphs and A. pisum (Table 3.1.1) provides evidence in support of

this. In addition, Senanayake and Holliday (1988) buried the tube under soil during the two-week pre-sampling period. They also used a draw-string bag, which allowed them to pull the tube tight around the base of the plant while sampling. In addition, they used fabric bags, which were expensive, but perhaps had less optical qualities, and permitted more free movement of insects up and down the plants, than did the polyethylene tubes that were tied around the stems at the time of deployment in the present study.

The fumigation cage method of sampling also appeared to be ineffective for sampling alfalfa pests. Resistance from alfalfa plants made it very difficult to push the plastic board through in between the mouth of the bucket and the ground. It is likely that the disturbance while pushing the plastic board elicited escape responses in the insects, particularly in mirid nymphs and *A. pisum*, which probably fell down on the ground and did not appear in the samples.

For pest management programs in crop fields, obtaining relative variation near 25% is usually satisfactory, although for population and ecological research greater precision with relative variation near 10% is required (Pedigo 1999). In the present study, such a level of precision (25–10%) was obtained in most cases only in sweep-net samples. The result is also reflected in the coefficient of variation. The coefficients of variation were frequently fairly high for beat tray, fumigation cage, vacuum and the whole plant bag sampling compared with sweep-net, although, between beat tray and sweep-net samples, the difference tended to decline as the season progressed. In the early part of the season when insect populations were low, these sampling methods failed to give non-zero values in most samples, whereas insects were always present in sweep-net samples. Compared with sweep-net, the other sampling methods covered smaller areas:

due to failure to integrate over insect aggregations, a sample from a smaller area is likely to be intrinsically more variable than a sample that covers a larger area.

Variance:mean ratio is a measure that indicates dispersion of insects (Sokal and Rohlf 1995): a variance:mean ratio of <1, 1 or >1 indicates an uniform, random or aggregated distribution, respectively, of insects being sampled. However, variance:mean ratios can be used to compare biases among different sampling methods (Sokal and Rolf 1995); biases in different sampling methods may lead to different conclusions about insect dispersion. In this study, variance:mean ratios were not consistent in most methods of sampling for mirids and *A. pisum* over the season. Changes in insect dispersion, which has been described previously, may have partly contributed to this result. However, the lack of significant correlation of variance:mean ratios among different sampling methods suggest that sampling techniques themselves contributed more to the result than did insect dispersion. Increasing the number of samples and maximizing the precision of sampling techniques may reduce such biases.

CVs are conventionally used as measures of comparing variabilities among sampling techniques (Ruesink 1980; Sokal and Rohlf 1995). Although they increase with means, CVs can be reduced by increasing the number of samples and using standardized sampling techniques (Karandinos 1976). However, unless the insects are uniformly distributed, the variation in insect numbers across different sampling units contributes to the CV (Senanayake and Holliday 1988). Therefore, increasing sampling precision and the number of samples cannot reduce CVs to 0, but can greatly reduce the component associated with sampling error (Senanayake and Holliday 1988).

There was a lack of significant correlations of the seasonal variation in CVs for different sampling methods, as found in most cases except for the beat tray and the sweep-net for sampling mirids. This suggests that the sampling methods themselves were major contributors to the seasonal variability. Changes in insect distribution patterns may also have contributed to the variability of the sweep-net and beat tray samples. The objective in the present study was, however, to compare sampling methods, and sweepnet appeared to be the most precise method of sampling, a result that agrees with Schotzko and O'Keeffe (1986), who estimated L. hesperus on lentils. A sweep-net sample of 30-50 sweeps covered many plants from a large area. Thus, it is likely that sweep-net sampling collected insect individuals even if the population was low and aggregated in local patches. Such aggregations are typical of many insects (Southwood 1978) including Lygus spp. (Schotzko and O'Keeffe 1986) and various species of aphids (Lowe 1971). In such cases, samples from small areas would exhibit increased variability and reduced precision, and precision would increase with increases in number of samples, and area sampled (Southwood 1978; Šedivý and Kocourek 1988).

The study provides calibration equations for different sampling methods for mirids and *A. pisum*, although none of them from 1999 seems to be reliable due to smaller coefficients of determination ( $r^2$  values in Table 3.1.3). However, the equations relating beat tray and sweep-net samples were reliable for pooled mirids and *A. pisum*, although the relationships were unreliable when mirid taxa were considered separately (Tables 3.1.4 and 3.1.5). Both taxa of mirids occur simultaneously in fields and pest management decisions are usually based on their combined numbers (Murrell 1987; Schaber 1992; Soroka and Murrell 1993). Therefore, lines calibrated for mirids and *A.* 

*pisum* can be used to convert insect densities obtained in one sampling method into another. The regression lines calculated based on season-long data do not preclude the influence of seasonal bias. The study provides evidence that the efficiency of sampling can vary across the season. There were also differences in lines fitted for mirids in June and August, the months in which pests in Manitoban seed alfalfa fields are usually controlled. Therefore, caution should be exercised in selecting the appropriate equations, particularly when the seasonal bias may be high. However, many factors can affect sweep-net accuracy (Southwood 1978; Ellington *et al.* 1984; Frazer and Raworth 1985), and so a regression equation that incorporates abiotic and biotic conditions is usually more satisfactory (Ruesink 1980). Although they produce better fits to existing data, such models usually add complexity to the whole process, and they often have no better predictive power than simple models.

The choice of a sampling method should maximize precision, while minimizing the sampling time and cost (Gomez and Gomez 1984). The sweep-net sampled the largest unit area and volume and provided numerically larger estimates with better precision of insect populations in most cases compared with the other methods. However, CVs provided by sweep-net were sometimes greater. Perhaps this represents the results of changes in aggregation effects, which is indicated by the variance:mean ratios. This may be reduced if more samples are pooled together to form a unit (Šedivý and Kocourek 1988). The greater precision of estimates provided by the sweep-net sampling is important for monitoring insect populations. Based on the area covered, sweep-net sampling is a quick, easy and cheap method of sampling (Schotzko and O'Keeffe 1989*a*); and these are characteristics preferred by growers, who are busy during the cropping

season. Although sweep-net sampling of plant bugs and aphids, use of the method for studies of the population dynamics remains questionable, as the reliability of the method appeared to be sensitive to life stage of insects.

.,	Methods and	Number	Lygus spp.		Adelphocoris lineolatus			Mirids	Acyrthosiphon	
Years	statistics	per	Adults	Nymphs	Total	Adults	Nymphs	Total	(Total)	pisum
1999	Sweep-net Vacuum WPBS	Sweep Sample Sample	$0.7 \pm 0.2^{a}$ $0.1 \pm 0^{b}$ $0.1 \pm 0.1^{b}$	$0.4 \pm 0.2$ $0.2 \pm 0$ $0.4 \pm 0.1$	$1.2 \pm 0.4^{a}$ $0.2 \pm 0^{c}$ $0.5 \pm 0.2^{b}$	$0.3 \pm 0.1^{a}$ 0 <sup>b</sup> $0.1 \pm 0^{a}$	$0.5 \pm 0.2$ $0.1 \pm 0$ $0.1 \pm 0$	$\begin{array}{c} 0.8 \pm 0.3 \\ 0.1 \pm 0 \\ 0.1 \pm 0 \\ \end{array}^{b}$	$1.9 \pm 0.6^{a}$ $0.4 \pm 0.1^{b}$ $0.6 \pm 0.2^{b}$	$4.7 \pm 2.0 \\ 1.9 \pm 0.5 \\ 2.5 \pm 0.7$
	$F_{2,30}$ statistics		8.18 (0.01)	1.18 (ns)	4.35 (0.05)	4.28 (0.05)	2.76 (ns)	4.65 (0.05)	4.65 (0.05)	0.25 (ns)
2000 *	Sweep-net Beat tray Fumigation cage	Sweep Sample Sample							$3.4 \pm 1.0^{a}$ $3.5 \pm 0.9^{a}$ $0.5 \pm 0.1^{b}$	$1.9 \pm 0.7$ $2.1 \pm 0.7$ $0.7 \pm 0.3$
	$F_{2,19}$ statistics								5.77 (0.05)	1.64 (ns)
2001	Sweep-net Beat tray	Sweep Sample	$0.8 \pm 0.4$ $0.3 \pm 0.1$	$0.6 \pm 0.2$ $1.2 \pm 0.3$	$1.3 \pm 0.5$ $1.5 \pm 0.4$	$0.5 \pm 0.2$ $0.2 \pm 0.1$	$1.6 \pm 0.5$ $0.9 \pm 0.3$	$2.1 \pm 0.5$ $1.1 \pm 0.3$	$3.4 \pm 0.8$ $2.6 \pm 0.5$	$1.8 \pm 0.8$ $3.6 \pm 1.7$
	$F_{1,16}$ statistics		0.95 (ns)	2.70 (ns)	0.37 (ns)	1.29 (ns)	0.78 (ns)	1.68 (ns)	0.30 (ns)	1.44 (ns)

Table 3.1.1. Number (seasonal mean ± SE) of various insects and stages collected in different methods of sampling in alfalfa fields in Manitoba, 1999-2001.

WPBS = the whole plant bag sampling. \*No records of different life stages were kept in beat tray and fumigation cage samples in 2000. Means within columns marked by the same letter are not significantly different (Tukey's test at  $P \le 0.05$ ).

Values in parentheses indicate the levels of significance; ns = not significant.

	-		S	5	
Years	Taxa	Related sampling methods	r	n	P <
		Sweep-net and vacuum	0.29	10	ns
	Mirids	Sweep-net and whole plant bag	0	10	ns
		Vacuum and whole plant bag	0.63	9	0.08
1999		Sweep-net and vacuum	0.28	10	ns
	Acyrthosiphon pisum	Sweep-net and whole plant bag	0.48	10	ns
		Vacuum and whole plant bag	0.54	9	ns
2000		Sweep-net and beat tray	0.97	7	0.01
	Mirids	Sweep-net and fumigation cage	0.72	6	ns
		Beat tray and fumigation cage	0.69	6	ns
		Sweep-net and beat tray	0.80	7	0.05
	Acyrthosiphon pisum	Sweep-net and fumigation cage	0.62	6	ns
		Beat tray and fumigation cage	0.95	6	0.01
2001	Mirids	Sweep-net and beat tray	0.83	9	0.01
	Acyrthosiphon pisum	Sweep-net and beat tray	0.06	9	ns

Table 3.1.2. Correlation of seasonal variation in CVs for different methods of sampling pests in seed alfalfa fields in Manitoba, 1999–2001.

Mirids comprise Lygus spp. and Adelphocoris lineolatus.

n refers to the number of observations (pairs) on which the correlations were based. ns = not significant.

Estimates		Period of			Statistics		
Types	As a function of	Тахоп	season	Regression equations	F (P)	df	$r^2$
Sweep-net	WPBS	Mirids	Entire	Y = 1.71 + 0.34X	1.69 (ns)	1,86	0.02
Vacuum	WPBS	Mirids	Entire	Y = 0.34 + 0.02X	0.05 (ns)	1,86	0
Sweep-net	Vacuum	Mirids	Entire	Y = 1.88 + 0.12X	0.09 (ns)	1,86	0
Sweep-net	WPBS	Acyrthosiphon pisum	Entire	Y = 3.36 + 0.52X	5.46 (0.05)	1,86	0.06
Sweep-net	WPBS	Acyrthosiphon pisum	June	Y = 0.90 + 0.04X	0.25 (ns)	1,22	0.01
*Sweep-net	WPBS	Acyrthosiphon pisum	July	Y = 11.51 - 0.37X	5.46 (0.05)	1,22	0.06
Sweep-net	WPBS	Acyrthosiphon pisum	August	Y = 1.29 + 0.88X	8.43 (0.01)	1,30	0.22
Sweep-net	WPBS	Acyrthosiphon pisum	September	Y = 0.17X	13.46 (0.01)	1,6	
Vacuum	WPBS	Acyrthosiphon pisum	Entire	Y = 1.48 + 0.18X	5.31 (0.05)	1,86	0.06
Vacuum	WPBS	Acyrthosiphon pisum	June	Y = 1.21 + 0.04X	0.06 (ns)	1,22	0
Vacuum	WPBS	Acyrthosiphon pisum	July	Y = 3.78 + 0.06X	0.12 (ns)	1,22	0
Vacuum	WPBS	Acyrthosiphon pisum	August	Y = 0.66 + 0.21X	12.87 (0.01)	1,30	0.30
Vacuum <sup>**</sup>	WPBS	Acyrthosiphon pisum	September	Not possible		1,6	
Sweep-net	Vacuum	Acyrthosiphon pisum	Entire	Y = 1.87 + 1.45X	28.76 (0.01)	1,86	0.25
Sweep-net	Vacuum	Acyrthosiphon pisum	June	Y = 0.83 + 0.11X	1.67 (ns)	1,22	0.07
Sweep-net	Vacuum	Acyrthosiphon pisum	July	Y = 6.56 + 1.00X	4.61 (0.05)	1,22	0.17
Sweep-net	Vacuum	Acyrthosiphon pisum	August	Y = 2.89 + 0.98X	1.22 (ns)	1,30	0.04
Sweep-net	Vacuum <sup>*</sup>	Acyrthosiphon pisum	September	Not possible		1,6	

Table 3.1.3. Regression equations of relative density estimates (Y) as functions of different sampling method estimates of insect pests on alfalfa in Manitoba, 1999.

Mirids comprise Lygus spp. and Adelphocoris lineolatus.

Values in parentheses refer to the level of statistical significance.

ns = nonsignificant relationship. WPBS = the whole plant bag sampling.

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Regression equations September for A. pisum estimates in September are not calibrated due to frequent absence of individuals in samples.

Note that the slope is negative, which is different from the general trend in other relationships.

\*\* No individuals were present in vacuum samples collected on 01 September.

]	Estimates	Period of			Statistics			
Types	As a function of	Taxon	season	Regression equations	F(P)	df	$r^2$	
Sweep-net	Beat tray	Mirids	Entire	Y = 1.30 + 0.63X	54.26 (0.01)	1,70	0.44	
Sweep-net	Beat tray	Mirids	June	Y = 0.57X	73.93 (0.01)	1,10	$0.87^{*}$	
Sweep-net	Beat tray	Mirids	July	Y = 0.72 + 0.47X	16.71 (0.01)	1,22	0.44	
Sweep-net	Beat tray	Mirids	August	Y = 4.65 + 0.21X	2.15 (ns)	1,34	0.06	
Sweep-net	Fumigation cage	Mirids	Entire	Y = 3.34 + 0.60X	1.67 (ns)	1,70	0.02	
Beat tray	Fumigation cage	Mirids	Entire	Y = 3.03 + 1.29X	7.60 (0.01)	1,70	0.10	
*Beat tray	Fumigation cage	Mirids	June	Y = 4.66X	7.93 (0.05)	1,10	0.42	
Beat tray	Fumigation cage	Mirids	July	Y = 0.37 + 0.07X	0.58 (ns)	1,22	0.18	
Beat tray	Fumigation cage	Mirids	August	Y = 5.14 + 0.54X	0.61 (ns)	1,34	0.02	
Sweep-net	Beat tray	Acyrthosiphon pisum	Entire	Y = 0.88 + 0.50X	61.55 (0.01)	1,70	0.47	
Sweep-net	Beat tray	Acyrthosiphon pisum	June	Y = 0.45 + 0.38X	51.54 (0.01)	1,10	0.84	
Sweep-net	Beat tray	Acyrthosiphon pisum	July	Y = 1.47 + 0.49X	16.46 (0.01)	1,22	0.43	
<sup>*</sup> Sweep-net	Beat tray	Acyrthosiphon pisum	August	Y = 0.66X	86.79 (0.01)	1,34	0.50	
Sweep-net	Fumigation cage	Acyrthosiphon pisum	Entire	Y = 1.73 + 0.36X	3.09 (ns)	1,70	0.04	
Beat tray	Fumigation cage	Acyrthosiphon pisum	Entire	Y = 2.04 + 0.26X	0.85; (ns)	1,70	0.01	

Table 3.1.4. Regression equations of relative density estimates (Y) as functions of different sampling method estimates of insect pests on alfalfa in Manitoba, 2000.

Mirids comprise *Lygus* spp. and *Adelphocoris lineolatus*. Values in parentheses refer to the level of statistical significance.

ns = nonsignificant relationship.

Note that, the  $r^2$  represents the proportion of the variance about 0 in a no-intercept regression, and that about the mean in an intercept included regression. Therefore, the value of the  $r^2$  for a no-intercept model is higher than for a regression with intercept.

Estimates		_	Period of		Statistics			
Types	As function of	Taxon	season	Regression equations	F(P)	df	$r^2$	
<b>G</b>	<b>D</b>		<b>F1</b> . •					
Sweep-net	Beat tray	Mirids	Entire	Y = 1.46 + 0.73X	51.54 (0.01)	1,76	0.40	
Sweep-net	Beat tray	Mirids	June	Y = 0.93 + 1.25X	45.46 (0.01)	1,34	0.57	
Sweep-net	Beat tray	Mirids	July	Y = 0.97 + 1.38X	3.12 (ns)	1,10	0.24	
Sweep-net	Beat tray	Mirids	August	Y = 3.89 + 0.28X	5.54 (0.05)	1,28	0.17	
Sweep-net	Beat tray	Lygus adults	Entire	Y = 0.51 + 0.37X	3.30 (ns)	1,76	0.04	
<sup>*</sup> Sweep-net	Beat tray	Lygus nymphs (total)	Entire	Y = 0.39X	41.61 (0.01)	1,76	0.35	
Sweep-net	Beat tray		June	Y = 0.05 + 0.07X	8.45 (0.01)	1,34	0.20	
Sweep-net	Beat tray		July	Y = 0.22 + 0.05X	1.49 (ns)	1,10	0.13	
Sweep-net	Beat tray		August	Y = 0.78 + 0.14X	2.03 (ns)	1,28	0.07	
Sweep-net	Beat tray	Adelphocoris lineolatus adults	Entire	Y = 0.38 + 0.21X	2.33 (ns)	1,76	0.03	
Sweep-net	Beat tray	Adelphocoris lineolatus nymphs (Total)	Entire	Y = 1.24 + 0.53X	5.04 (0.05)	1,76	0.07	
Sweep-net	Beat tray	1	June	Y = 1.15 + 2.33X	26.86 (0.01)	1,34	0.44	
Sweep-net	Beat tray		July	Y = 0.21 + 0.03X	0.57 (ns)	1,10	0.05	
Sweep-net	Beat tray		August	Y = 1.80 - 0.41X	0.56 (ns)	1,28	0.02	
*Sweep-net	Beat tray	Acyrthosiphon pisum	Entire	Y = 0.53X	341.20 (0.01)	1,76	0.82	
Sweep-net	Beat tray	Acyrthosiphon pisum	June	Y = 0.40 + 0.36X	7.94 (0.01)	1,34	0.67	
*Sweep-net	Beat tray	Acyrthosiphon pisum	July	Y = 0.60X	14.25 (0.01)	1,11	0.87	
*Sweep-net	Beat tray	Acyrthosiphon pisum	August	Y = 0.32X	122.34 (0.01)	1,29	0.81	

Table 3.1.5. Regression equations of relative density estimates (Y) as functions of different sampling method estimates of insect pests on alfalfa in Manitoba, 2001.

Mirids comprise *Lygus* spp. and *A. lineolatus*. Values in parentheses refer to the levels of statistical significance. ns = nonsignificant relationship. Young and old nymphs of *Lygus* spp. and *Adelphocoris lineolatus* exhibited similar relationships to corresponding total nymphs, and are not shown separately.

Note that, the  $r^2$  represents the proportion of the variance about 0 in a no-intercept regression, and that about the mean in an intercept included regression. Therefore, the value of the  $r^2$  for a no-intercept model is higher than for a regression with intercept.

Fig. 3.1.1. Variance:mean ratio for weekly sweep-net, vacuum and the whole plant bag sampling (WPBS) estimates of mirids and *Acyrthosiphon pisum* in 1999.Mirids represent *Lygus* spp. and *Adelphocoris lineolatus* pooled together regardless of taxa and life stages.

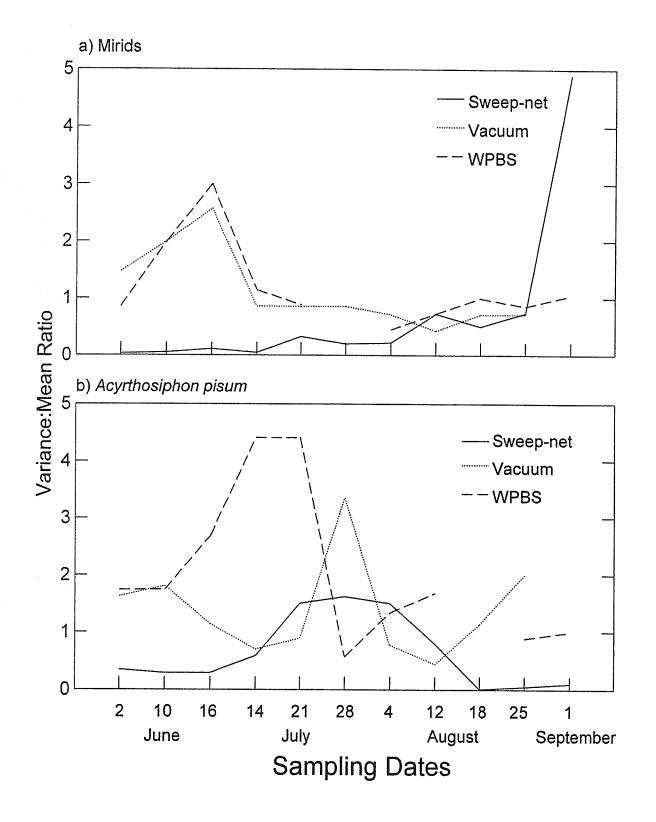


Fig. 3.1.2. Variance:mean ratio for weekly sweep-net, beat tray and fumigation cage sampling estimates of mirids and *Acyrthosiphon pisum* in 2000.Mirids represent *Lygus* spp. and *Adelphocoris lineolatus* pooled together regardless

of taxa and life stages.

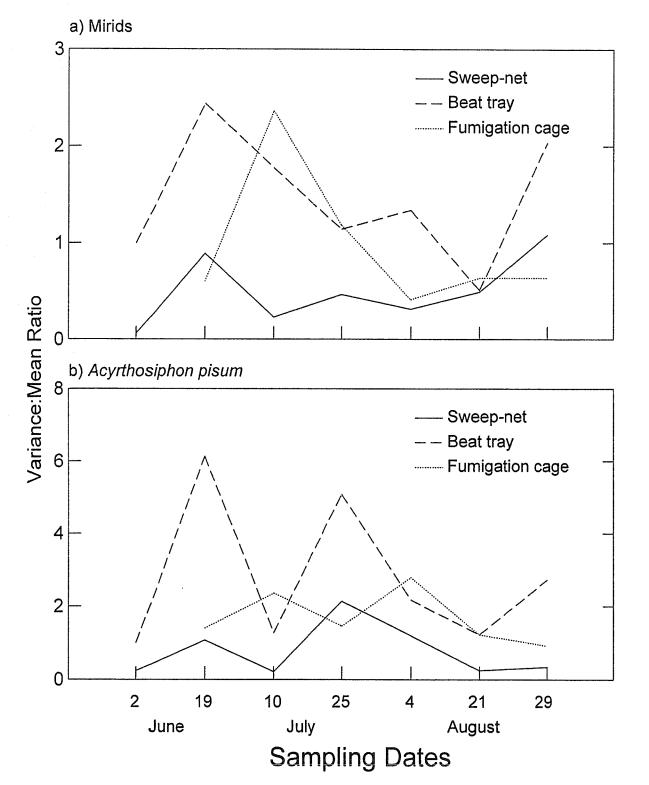


Fig. 3.1.3. Variance:mean ratio for weekly sweep-net and beat tray sampling estimates of mirids and *Acyrthosiphon pisum* in 2001.

Mirids represent *Lygus* spp. and *Adelphocoris lineolatus* pooled together regardless of taxa and life stages.

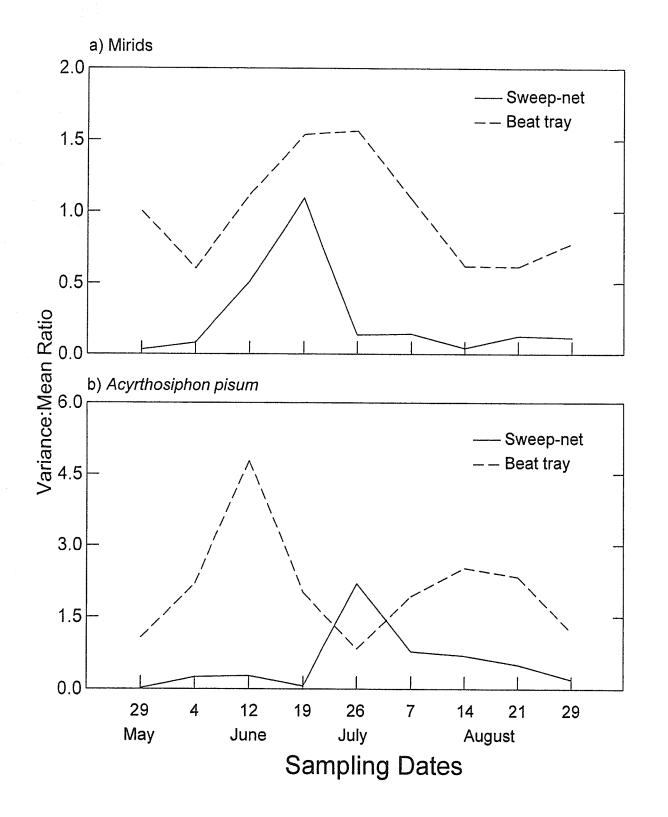


Fig. 3.1.4. Coefficient of variation for weekly sweep-net, vacuum and the whole plant bag sampling (WPBS) estimates of mean density of mirids and *Acyrthosiphon pisum* in 1999.

Mirids represent *Lygus* spp. and *Adelphocoris lineolatus* pooled together regardless of taxa and life stages.

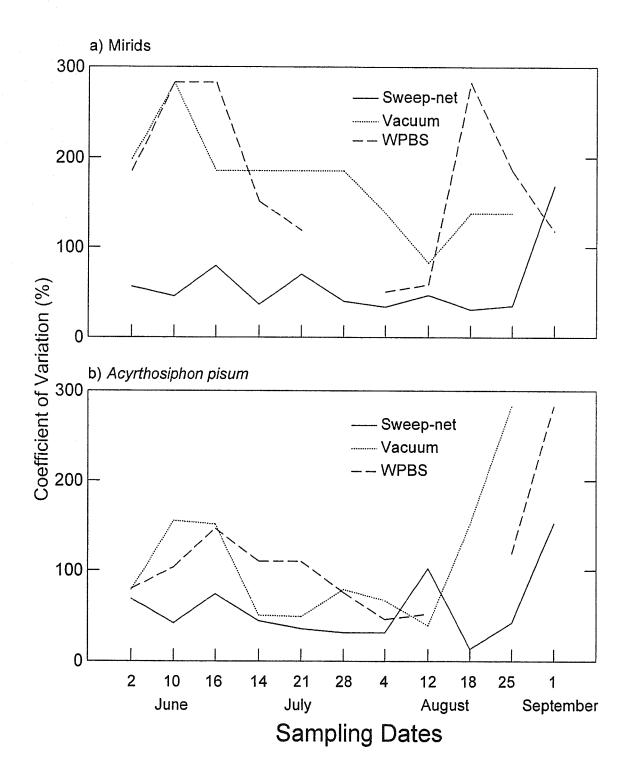
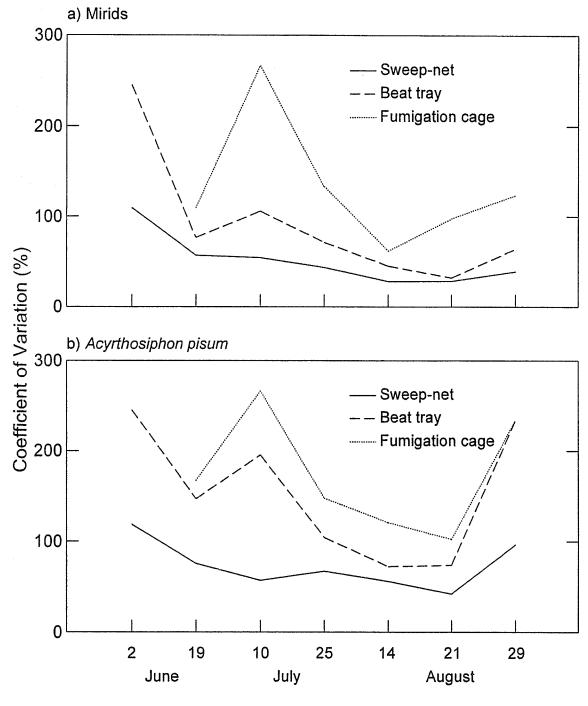


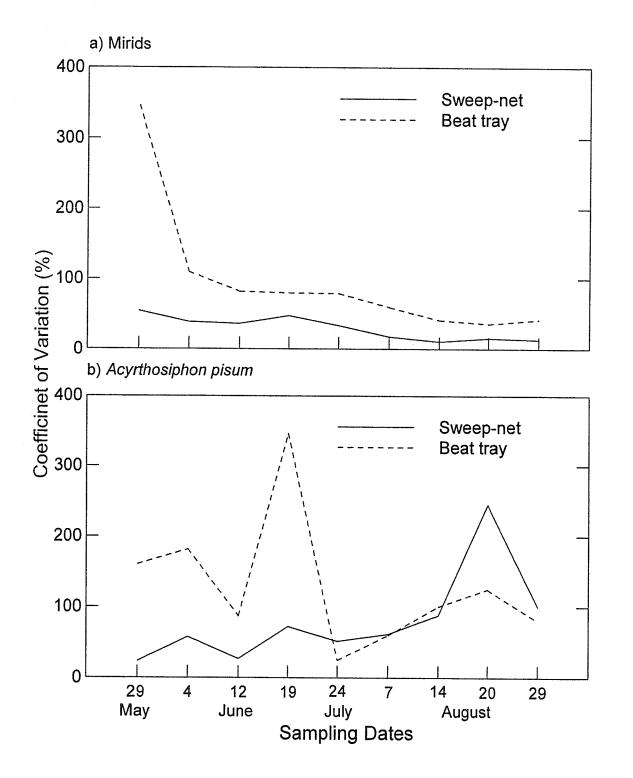
Fig. 3.1.5. Coefficient of variation for weekly sweep-net, beat tray and fumigationcage estimates of mean density of mirids and *Acyrthosiphon pisum* in 2000.Mirids represent *Lygus* spp. and *Adelphocoris lineolatus* pooled together regardlessof taxa and life stages.



Sampling Dates

Fig. 3.1.6. Coefficient of variation for weekly sweep-net and beat tray estimates of mean density of *mirids* and *Acyrthosiphon pisum* in 2001.

Mirids represent *Lygus* spp. and *Adelphocoris lineolatus* pooled together regardless of taxa and life stages.



# CHAPTER 3.2

Insect pests and their natural enemies in relation to production practices in alfalfa fields of Manitoba, with particular reference to *Lygus* spp., *Adelphocoris lineolatus* 

and Acyrthosiphon pisum

## Abstract

The seasonal abundance of common insect pests and their natural enemies was examined in hay and seed fields of alfalfa in Manitoba during the crop growing seasons from 1999 to 2001. Thirteen taxa of insect pests were often found, though only three taxa, namely *Lygus* spp. Hahn, *Adelphocoris lineolatus* (Goeze) and *Acyrthosiphon pisum* (Harris) were frequent and numerous. Nine taxa of predaceous insects were often found, among which carabids, chrysopids, coccinellids, and nabids appeared more frequently and their presence appeared to be associated with the populations of insect pests. Two taxa of arachnids, namely opilionids and spiders, were found, though their importance with respect to predation of insect pests was not clear. It appeared that parasitoids were active, and killed a considerable proportion of *Lygus* spp., but not *A. lineolatus*. There were six parasitic species attacking *A. pisum*, though *Aphidius ervi* was the predominant species. These parasitoids killed *A. pisum* individuals and contributed greatly toward natural suppression of *A. pisum*.

Alfalfa fields for hay production were mown twice in a growing season, and in mown fields insect pests were suppressed to levels not warranting pest control measures. There was evidence that the mowing resulted in movement of *Lygus* spp., and sometimes, *A. lineolatus* into adjacent seed fields. In seed fields, insect pests were more numerous, and often warranted control measures. The seasonal occurrence, population growth and implications of these insect pests and their natural enemies in relation to current production practices are discussed.

### Introduction

Alfalfa occupies about five million hectares of land in Canada (Goplen et al. 1987). About 30,000 hectares of this land is managed for pedigreed seed production. In the three Prairie Provinces, a considerable area of pedigreed seed and common seed is grown (Schaber and Entz 1991). Alfalfa is a perennial legume crop, which has a long productive life (Summers 1998), and seed alfalfa stands can remain productive for eight years in the Prairies (Schaber and Entz 1991). The crop plant has a dense canopy with a complex crown structure, which offers a great variety of habitats and niches (Brown and Fick 1986), and favours development of diversified insect assemblages (Schaber and Entz 1991; Summers 1998). Harper (1988) recorded 437 insect species in alfalfa fields in southern Alberta, whereas Pimentel and Wheeler (1973b) recorded 591 species in alfalfa fields in New York. Some of these arthropods are active on vegetations, some others are under leaf-litter, while some others can be found on both vegetation and litters. Methods used to sample arthropods depend upon where and what taxa to be sampled; sweep-nets are most commonly used to sample arthropods on vegetations, whereas pitfall traps can be used to sample carabid beetles and some other litter-dwelling arthropods (Southwood 1978).

The relative abundance of insects injurious to alfalfa can vary temporally and spatially (Schwartz and Foottit 1992*a*; Timlick *et al.* 1993; Gerber and Wise 1995). In Alberta, insects damaging to alfalfa grown for seed production are *Lygus* spp., *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), *Acyrthosiphon pisum* (Harris) (Homoptera: Aphididae) and *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae), (Lilly and Hobbs 1962; Harper 1978; Schaber and Entz 1988). These species, except *H*. *postica*, also cause major damage to alfalfa grown for seed production in Saskatchewan (Soroka 1991; Soroka and Murrell 1993). In Manitoba, the occurrence of *Lygus* spp. has been frequently reported (Schwartz and Foottit 1992*a*, 1992*b*; Timlick *et al.* 1993; Gerber and Wise 1995). Although, Timlick *et al.* (1993), and Gerber and Wise (1995) studied the seasonal occurrence and number of generations of *Lygus* spp. in alfalfa fields in southern Manitoba, no detailed studies have been carried out to determine the effect of alfalfa production practices on insect pests and their natural enemies in Manitoba.

Despite the lack of scientifically collected information on the relative abundance and importance of insect pests and their natural enemies, Manitoban growers often spray insecticides twice to control insect pests in seed alfalfa fields. With concerns about chemical insecticide use (Debach and Rosen 1991), a holistic scheme that minimizes the use of chemical insecticides and maximizes the benefits from insect natural enemies is desirable (Steffey and Armbrust 1981). In order to develop such a scheme, knowledge about the occurrence, distribution, relative importance, and interaction of insect pests and natural enemies in relation to crop production practices is required. The present study was carried out to gather this information for alfalfa fields in Manitoba.

## **Materials and Methods**

#### Study sites

The study was conducted in commercial alfalfa fields near Dugald and Teulon in 1999, and near Arborg and Riverton in 2000 and 2001. The locations are shown in Fig. 3.2.1. The fields were all managed for commercial production, but varied in age, size, plant density, cultivars, stages, soil-types, topography and ownership. Details of these fields are summarized in Table 3.2.1.

Each year, three alfalfa fields near each of two localities were sampled. The three fields were a hay field, a seed field beside the hay field (hereafter called the adjacent seed field), and a seed field at least 500 m away from any hay fields (hereafter called the non-adjacent seed field). The adjacent seed field shared a common margin with the hay field. The margin between the hay and adjacent seed fields near Dugald (1999) was about 0.5 m wide, and it was occupied by dense grassy vegetation. The space between the hay and adjoining seed field near Arborg (2000 and 2001) was very narrow, approximately 20–25 cm. There were no plants other than the sporadic presence of alfalfa plants in that margin. The margins between the hay and adjacent seed fields in the other localities were more than 1 m wide, with a ditch in between.

In this thesis, the three fields studied in a locality are generally regarded as fieldtypes, the hay and seed fields are regarded as crop-types, and the adjacent and nonadjacent seed fields are regards as seed field-types.

# Sampling

Fields were sampled each week from late May to the end of August or mid-September except in 2000, when sampling in fields near Riverton was not initiated until June 28. Fields within a locality were sampled on the same day between 0900 and 1700 h CDT. The layout for sampling individual fields is given in Fig. 3.2.2. "Edge" and "middle" areas were established within the crop in each field. Edge areas were transects within 8–10 m of the field margin, whereas middle areas were at least 100 m from the margin. In each of the selected edge areas, one (1999) or two (2000 and 2001) sampling sites were located centrally. In the middle area, four (1999, sites in a square) or two (2000

and 2001, sites in a line) sampling sites were selected centrally in each field. Sampling sites within a sampling area were separated from each other by at least 50 m.

In 1999, fields were sampled using sweep net and pitfall traps. The method of sweep net sample collection was as described previously (Chapter 3.1), except that the layout of sampling sites and spots differed. Fifty sweeps constituted a sample when the number of insects was low during May and June, after which 30 sweeps made a sample. As indicated in Fig. 3.2.2, in each of the four edge areas, two sweep-net samples were taken, one from each spot on either side of the sampling site; each sample was taken while walking parallel to the field margin. From middle areas, one sample was taken from a sampling spot near each sampling site. Sweeping spots within a sampling site were separated by at least 50 m, and the same plants were not sampled in two successive weeks. After collecting in Zip-loc<sup>®</sup> bags containing cotton wads soaked with ethyl acetate, samples were transferred to the laboratory, where insects were identified and their numbers were counted. These included Lygus spp. {Lygus bugs were not identified to species, because nymphs of different species are not possible to separate. Although adults are possible to specify, due to limitation of time Lygus adults were not identified to species. However, previous reports indicate that *Lygus* bugs were presumably *L*. lineolaris, L. borealis and L. elisus (Timlick et al. 1993)}, A. lineolatus, A. pisum, cicadellids (leafhoppers), acridids (grasshoppers), coccinellids (ladybird beetles), chrysopids (green lacewings), Orius spp. (anthocorids), Nabis spp. (nabids), pentatomids (stinkbugs), syrphids (hover flies), opilionids (harvestmen), and spiders (Aranae). For Lygus spp., and A. lineolatus, nymphal instars were recorded in groups: first- to third-

instar nymphs were recorded as young, whereas the fourth and fifth instars were recorded as old nymphs. Aphid numbers were estimated as described previously (Chapter 3.1).

Each year, pitfall trap samples were collected from each sampling site in each field. A pitfall trap consisted of a 450 ml plastic beer-cup {11 (deep) x 10 (diameter) cm} one-third filled with saturated salt solution to which liquid detergent was added at the rate of 1–2 drops per 4 liter of solution. Over the trap, a 15 x 15 cm plywood cover was suspended 3–4 cm above the ground surface by nails (Fig. 3.2.3). In 1999, eight pitfall traps were installed in each field on 24 and 25 May in Dugald and Teulon, respectively. In edge areas, one pitfall trap was installed centrally in each of the north, east, south, and west edge areas of each field. In the middle area, four traps were installed (one at each site) in a square in each field. Traps within a sampling area were separated from each other by at least 50 m. Starting from the end of May and continuing until the middle of September, traps were emptied once each week and contents were strained from the solution and preserved in 70% ethyl alcohol. Each time, the salt solution in pitfall traps was changed. In the laboratory, trap contents were sorted and adult carabid beetles were identified to species. Numbers of carabid beetles, acridids, Gryllus, opilionids, staphylinid beetles, elaterid beetles and spiders were recorded.

In 1999, in addition to sweep-netting and pitfall trapping, the level of leafminer infestation was determined following a modified protocol of Harcourt and Binns (1980). Near each sampling site, 20 randomly selected stems were collected, and 25–40 middle leaflets were separated from each sample. These leaflets were examined for the presence of leafmines, and the percentage of mined leaflets was recorded.

In 1999, the infestation level of the seed chalcid, *Bruchophagus roddi* (Gussakovsky), in seed fields was determined following Soroka and Spurr (1998). Accordingly, samples were collected from seed fields, and from each sample, 100 seedpods were separated and the seeds were collected and examined for percentage of chalcid infestation.

In 2000 and 2001, only sweep-net and pitfall trap samples were taken, and the methods of sweeping and pitfall trapping were as described previously. A modified sampling plan (Fig. 3.2.2) was used in which only the middle area, and two edge areas – the adjacent edge parallel to the common margin, and one of the two edges that were perpendicular to the shared margin – were sampled. In the edge areas, four sampling sites, two along each selected edge, were marked. In the middle area, two sampling sites were marked centrally. The sampling sites were separated from each other by at least 50 m. At sweeping, one sample was collected from each of the two sites selected in the selected edges, and two samples were collected from each of the two sites selected in the middle. For sweep net samples, interest was concentrated on the most common taxa, which included *Lygus* spp., and *A. lineolatus*, *A. pisum*, chrysopids, coccinellids, *Nabis* spp., *Orius* spp., syrphid flies and spiders.

Hay-cut and insecticide spray records were obtained from growers, and weather records were obtained from Environment Canada (2003) for 1999 to 2001. Weather records from Dugald (Latitude 49°52' N; longitude 96°49' W) and Riverton (Latitude 50°59' N; longitude 96°59' W) were not available for the study period. Fields near Dugald were approximately 30 km north of Steinbach (Latitude 49°32' N; longitude 96°46' W) and fields at Riverton were about 15 km east of Arborg (Latitude 50°56' N; longitude 97°5' W) weather stations. It is likely that weather conditions in Dugald and Riverton would not differ greatly from those in Steinbach and Arborg, respectively. Therefore, records from Stienbach and Arborg were used for Dugald and Riverton areas, respectively.

In addition, by using a stroke of the sweep-net at every 10 steps and collecting up to five individuals from each sample (stroke), 50 *A. pisum* adults were collected from the hay field near Riverton on 23 and 30 July, and 7 August in 2001, and specimens in each sample were preserved in a 118 ml screw-cap specimen container containing 40–50 ml of 70% ethanol. In the laboratory, 25 adults from each sample were dissected under a microscope and the number of embryos inside each adult was recorded in two groups, late embryos and early embryos. Embryos that had visible appendages and that were easily recognizable as aphids were recorded as late embryos, and those which were recognized by only a pair of reddish eyes and a hyaline body were recorded as early embryos. Also in 2001, random sub-samples of spiders were taken from samples, and the specimens were identified to species. Growth stages of alfalfa plants in seed fields were determined over the season in 2001 following Hall (1996).

# Parasitism

Parasitism was examined and percent parasitism of *Lygus* spp., *A. lineolatus* and *A. pisum* was estimated from field-collected insect samples each week during the period when host insects were sufficiently abundant for adequate collections to be made in a reasonable amount of time.

## Lygus spp. and A. lineolatus

In 1999, depending on their availability, 30–50 third to fifth instar nymphs, and the same number of adults of each of *Lygus* spp., and *A. lineolatus* were collected separately by sweeping each field. Five sweeps were made at a time, and individuals of the two insect groups were collected with forceps from the sweep net. The collected individuals were transferred into 118 ml screw-cap specimen containers containing 30–40 ml of 70% ethyl alcohol, and stored at 5°C until dissection. Percentage parasitism was determined by dissecting 15–20 nymphs and 15–20 adults of each taxon from each field sample, as described by White (2002).

Estimation of percent parasitism of *Lygus* spp. and *A. lineolatus* appeared unsatisfactory in 1999. Therefore, a slightly modified protocol was used in 2000 and 2001: the sweep net-collected specimens were transferred to opaque polyethylene containers (250 ml {10 [diam] x 4.5 [height] cm}honey containers), each containing one or two 10 cm alfalfa shoots and sealed with a perforated lid. Twenty to 30 individuals of the same taxon and stage were collected in each container. Specimens in containers were transferred in a picnic cooler to the laboratory and stored at 5°C. To determine percent parasitism in each sample of nymphs and adults of both taxa, 30–50 live-individuals, instead of the 15–20 used in 1999, were dissected following Braun *et al.* (2001). No parasitoids were found in adults except once in *A. lineolatus*. Therefore, only nymphs were dissected in 2001.

#### <u>Acyrthosiphon pisum</u>

Parasitism was examined and percent parasitism of *A. pisum* was determined by rearing field-collected aphids. In preparation for rearing, nutrient enriched agar medium

was prepared in 60 x 15 mm covered Petri dishes following Milner (1982). After cooling, the agar-filled dishes were stored in a refrigerator. Before using for aphid rearing, a laboratory-raised excised *Vicia fabae* L. leaf was placed upside down in each Petri dish.

Depending on availability, 30–70 *A. pisum* were collected by using a succession of single strokes of a sweep net. After each stroke, aphid individuals were removed from the net with forceps or camelhair brush. Care was taken to avoid injured aphids. The aphids were collected and transferred to the laboratory as explained for *Lygus* spp., and *A. lineolatus* in 2000 and 2001.

In the laboratory, 20–40 aphids from each field were reared each week. Using forceps or a camelhair brush, groups of four to six aphids from a sample were transferred to the previously prepared *V. fabae* leaf in a Petri dish. Petri dishes were covered, placed on a tray and incubated at  $22 \pm 2^{\circ}$ C, 18:6 (L:D) and  $70 \pm 5\%$  relative humidity. Excess aphids were saved in a 5°C room in case they were needed later. Aphids on leaves were examined every second day for eight days, and the number of aphid mummies was recorded. Any aphids that died naturally during the first two days were excluded from calculation of percent parasitism.

#### Data analysis

Numerical records of *Lygus* spp., *A. lineolatus*, *A. pisum*, *Gryllus* spp., carabid beetles, coccinellids, syrphid flies, *Orius* spp., *Nabis* spp., chrysopids, spiders and opilionids were kept in all three years, and hence analyses are concentrated mostly on them. Although there were two hay fields and four seed fields sampled each year, only one hay field and one seed field was chosen to graphically illustrate the seasonal distribution of different taxa in a year. There were occasions when data collection from

some fields was interrupted due to rain or crop production practices. Therefore, fields from which more complete and meaningful seasonal data were available were chosen to present the results graphically. Fields chosen were the hay field near Dugald and the nonadjacent seed field near Teulon (1999), the hay field and the non-adjacent seed field both near Arborg (2000), the hay field and the adjacent seed field both near Riverton (2001).

It was noted that the numbers of young nymphs of *Lygus* spp. and *A. lineolatus* were low. Therefore, young and old nymphs of *Lygus* spp. were pooled for analysis; similarly, pooled data for *A. lineolatus* nymphs were analyzed. For coccinellids, larvae were not recorded separately in 1999, and in other years, their numbers were low compared with adults (means 0.6 and 1.5 per 30 sweeps for larvae and adults, respectively). Analysis of adults and larvae separately produced similar results. Therefore, data for coccinellid adults and larvae were pooled and analyzed. Similarly, chrysopid larvae were not separately recorded in 1999, and in the other two years, numbers of adults caught were low, only 37% of those of larvae. Therefore, chrysopid adults and larvae were also pooled and analyzed. Analyses and results of carabid beetles are reported in a separate paper in this thesis (Chapter 3.3). Records of a few additional taxa were kept only in 1999; they will not be discussed in detail.

Analyses of differences among fields based on mean weekly and mean seasonal catches per sampling area per field produced more or less similar results for *Lygus* spp., and *A. lineolatus*. Hence, results based on mean seasonal catches are presented. Data were transformed using  $\log_{10}(X)$ ,  $\log_{10}(X + 1)$  or  $\arcsin\sqrt{percentage}$  as appropriate to reduce heteroscedasticity, and were analyzed by General Linear Modeling for a nested split plot design using Systat 10.2 (Systat, 2002). The experimental unit used in the

overall analysis was the field, and variance was partitioned into components attributable to field-type (hay, adjacent seed or non-adjacent seed fields), year-location (block) and their interactions. Contrasts between hay and seed fields, and also between adjacent and non-adjacent seed fields were made. Field-area within a field was considered as the subplot, and to examine differences between edge and middle field-areas, analysis was performed accordingly. Data within each year were also analyzed separately.

Analysis of Covariance (ANCOVA) was performed to examine the population growth of the three major pests. Data after the date of the first disturbance (hay-cut or insecticide application) to the date when the corresponding insect reached peak population were considered for the analysis. In the preliminary analysis, cumulative degree-days (CDD) were more significantly related to population size than were calendar days, and hence CDD for the relevant periods for Lygus spp., A. lineolatus and A. pisum were used. The lower developmental threshold of the lygus bug, L. lineolaris, is 10.6°C (Fleischer and Gaylor 1988), but the threshold is unknown for A. lineolatus. Because Lygus spp., and A. lineolatus are closely related, the threshold for Lygus bugs was used to estimate the adjusted CDD (adjusted following Lindsey and Newman 1956) for both taxa. For A. pisum, the lower developmental threshold is 4.7°C (Lamb et al. 1987), which was used for the estimation of adjusted CDD. In this part of the analysis, field-areas were not of interest, and hence to determine important components that explain most of the population growth, the analyses were based on a Randomized Complete Block design. Weekly mean catches of insects from the edge and middle of each field were used for the analysis. The model for the analysis included CDD, field, and their interaction. For population growth, the main effects are of little interest, and CDD x field interaction is a

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measure that represents variation in population growth of the pest. This interaction was partitioned to identify components that were important and exerted considerable influence on population growth. In the partitioning process, parasitism rate was not included in the analysis, as data on parasitism were not available from each field. Similarly, plant growth stage was not considered, as I had only one years data in this regard. A stepwise backward selection multiple regression analysis was performed for identifying important predaceous arthropods that from the interaction of their numbers with CDD appeared to exert a significant influence on the population growth of a particular pest species. Levels of significance of these predaceous arthropods in interaction with CDD were assessed against the residuals of CDD x field. In this analysis, a significant residual effect would mean that some other factors, which were not identified in this study, also have influenced respective pest population growth, and they will not discussed.

Post hay-cut migration of arthropods was examined by comparing their numbers in field-edges and -middles of adjacent and non-adjacent seed fields: insect numbers a week before and after the hay-cut were analyzed. General Linear Model repeated measures analysis was performed. Data related to the first and second hay-cut were analyzed separately, and contrasts were examined to determine a) whether the post-cut increase in the edge area of adjacent seed fields differed from those in all other places, or b) whether the post-cut increase in adjacent seed fields differed from that in non-adjacent seed fields. Previous reports suggest that many arthropods move into adjacent fields, when the hay crop is mown (Rakickas and Watson 1974; Schaber *et al.* 1990*b*).

Therefore, a one-tailed analysis was performed on the a priori assumption that arthropods will move into adjacent seed fields.

Throughout this paper, interactions are not mentioned unless they were significant. An experiment-wise alpha level of 0.05 was used for analyses. However, for the ANCOVA, an alpha level of 0.15 was used as the criterion for inclusion of any component in the model.

#### Results

The study was conducted in alfalfa fields managed for commercial production. These fields varied in physical and biological characteristics. Decisions for mowing of alfalfa for hay, application of insecticides, control of diseases and weeds, and harvesting of seeds were under the discretion of the growers, who owned these fields. There were variations in localities, the time and frequency of disturbances inflicted in the studied fields, which are summarized in Table 3.2.1. Some fields for seed production received insecticide applications twice, whereas others received them once in a crop-growing season. The study was conducted in three years, and therefore variation in weather conditions occurred in these three years, and is summarized in Appendix I. Note that the year 2000 was consistently cooler than the other years, and was relatively dry in July and August. Results associated with different insect pests and their natural enemies as found in this study are discussed.

## Insect pests

A total of 13 taxa of insect pests were commonly found in alfalfa fields (Table 3.2.2). Of them *Lygus* spp., *A. lineolatus* and *A. pisum* occurred regularly and sometimes

in significant numbers. Hence, interest was concentrated on these insect groups. Detailed results for these insects are presented below.

### Lygus spp.

### Seasonal occurrence

The typical patterns of seasonal occurrence of Lygus bugs found in different fields in different years are represented in Fig. 3.2.4. Except for a few days after fields were mown or sprayed with insecticides, Lygus adults were present throughout the study period. In the majority of fields (10 fields) there was a small peak of adult numbers around mid-June (Fig. 3.2.4 e, f). Note that corresponding data from three fields near Riverton (2000) are not available because of a late start of sampling. Because of low numbers, no mid-June peak was detectable in the other five fields. There was a second peak in each of seven fields (three in 1999, one in 2000, and three in 2001) around midto late July (Fig. 3.2.4 a, b, d, e), and sometimes this peak was long-lasting, although in the majority of the fields, such peaks were not detected possibly because of the first haycut or insecticide application. Unless there was a hay-cut or insecticide application at this time, there was a strong adult peak during mid- to late August (Fig. 3.2.4 a-d). Note that both the first and second hay-cut or insecticide application (disturbance) affected adult populations, although unlike the first disturbance, the effect of the second disturbance was not long-lasting as the number of adults rebounded after the second disturbance.

*Lygus* nymphs usually first appeared at the beginning of June, and rose in number until the first hay-cut or insecticide application, which usually interrupted the rise. In six of the 18 fields studied, nymphal numbers rose to a peak around mid-June (Fig. 3.2.4 d). When reduced by the first hay-cut or insecticide application, *Lygus* nymphs began to

increase in numbers following these operations, and attained peak numbers again usually around mid-August (in 13 fields of the 18) (Fig. 3.2.4 a–d), and nymphal numbers at this time were greater than early in the season.

## Parasitism

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No parasitoids were found in *Lygus* bug adults in 1999 and 2000, and hence dissection of adults was discontinued in 2001. In dissections, parasitoids were found each year in the third- to fifth-instar nymphs of *Lygus* spp. In 1999, parasitoids were found in only three instances: in the fourth week of July from a sample near Dugald, and in the second weeks of July and August from two samples near Teulon. Hence, the overall percent parasitism appeared low (Table 3.2.3), though parasitoids were recovered from 20% of nymphs in July samples.

In 2000, the number of *Lygus* nymphs was low until mid-July. During this portion of the season, examination of parasitism was concentrated on specimens from the hay field near Riverton. Parasitoids were first recovered from nymphs in the third week of June, and 15% of those nymphs hosted parasitoids. Parasitism was up to 44% in the fourth week of June (Fig. 3.2.4 c represents the hay field near Arborg). From mid-July to August, parasitoids were recovered from nymphs in every seed field with peak recovery frequently at the end of July or at the beginning of August (Fig. 3.2.4 d). After the peak, parasitoid presence in *Lygus* nymphs declined rapidly, and despite the presence of numerous nymphal hosts (e.g. Fig. 3.2.4 d), no parasitoids were recovered after mid-August.

In 2001, nymphs were found until mid-June, though no parasitoids were recovered. From mid-June to the beginning of July, collection of nymphs was difficult due to hay-cut or insecticide application. From fields near Arborg, only 12 individuals were dissected on 26 July, and two-thirds of them were parasitized (Table 3.2.3). However, specimens from fields near Riverton were dissected weekly from the beginning to the end of July, and parasitoids were recovered on each occasion with peak occurrence at the end of July, after which the percentage of parasitism declined precipitously (Fig. 3.2.4 f).

#### **Population analyses**

**Population variation.** Overall, the total number (nymphs plus adults) of *Lygus* bugs did not differ among field-types, between hay and seed fields, between adjacent and nonadjacent seed fields (Table 3.2.4), or between field-edges and -middles (Table 3.2.5). However, numbers in hay fields averaged 78% of those in seed fields. When data from each year were considered separately, hay fields ( $8.95 \pm 3.0 \text{ per } 30 \text{ sweeps}$ ) had significantly fewer *Lygus* bugs than seed fields ( $22.75 \pm 7.60 \text{ per } 30 \text{ sweeps}$ ) ( $F_{1,10} =$ 21.92; P < 0.05) in 2001; the same trend was evident but not significant in other years. *Lygus* adults did not differ overall or in separate year analyses among field-types, between hay and seed fields, between adjacent and non-adjacent seed fields (Table 3.2.4), or between field-areas (Table 3.2.5). For *Lygus* nymphs analyzed over all years, numbers differed significantly among field-types, and hay fields ( $4.5 \pm 0.8 \text{ per } 30 \text{ sweeps}$ ) had significantly ( $F_{1,10} = 10.16$ ; P < 0.01) fewer *Lygus* nymphs than seed fields ( $7.1 \pm 1.3 \text{ per}$ 30 sweeps); overall, adjacent and non-adjacent seed fields did not differ (Table 3.2.4).

The trend for there to be more nymphs in seed fields than hay fields was significant in 2001 (6.3  $\pm$  3.1, and 10.2  $\pm$  2.9 per 30 sweeps in hay and seed fields, respectively) ( $F_{1,2} =$ 21.34; P < 0.05). A similar but not significant trend was also evident in other years. The old nymphs largely contributed to this pattern, as they exhibited similar overall trends  $(2.2 \pm 0.3, 6.4 \pm 1.5, and 4.4 \pm 0.8 per 30$  sweeps in hay, adjacent seed fields, and nonadjacent seed fields, respectively; for field-types  $F_{2,10} = 5.25$ ; P < 0.05). Old nymphs also differed among field-types ( $F_{2,2} = 30.91$ ; P < 0.01), between hay ( $1.9 \pm 0.9$  per 30 sweeps) and seed fields (4.30 ± 1.4 per 30 sweeps) ( $F_{1,2} = 42.27$ ; P < 0.05), between adjacent (5.6  $\pm$  2.6 per sweep) and non-adjacent (2.98  $\pm$  1.15 per sweep) seed fields ( $F_{1,2}$ = 19.71; P < 0.05) in 1999, and among field-types ( $F_{2,2} = 21.19$ ; P < 0.01) and between hay  $(2.13 \pm 1.08 \text{ per sweep})$  and seed  $(7.78 \pm 2.96 \text{ per sweep})$  fields  $(F_{1,2} = 42.35; P < 1.08 \text{ per sweep})$ 0.05) in 2001. Numbers of young nymphs relative to old ones were very low, and there were no significant differences of the numbers of young nymphs at any level of comparison. Examination of Lygus numbers from mid-July to the end of sampling revealed that there were significantly fewer nymphs ( $F_{1,10} = 17.19$ ; P < 0.01) but not adults in hay fields (nymphs:  $3.3 \pm 0.6$ , adults:  $12.0 \pm 2.2$  per 30 sweeps) than in seed fields (nymphs:  $11.6 \pm 1.7$ , adults:  $18.2 \pm 2.8$  per 30 sweeps). In four of the six fields sampled in the present study, the second hay-cut occurred before or around the time nymphs could rise to peak numbers (Fig. 3.2.4 e).

**Population growth**. The model for ANCOVA included cumulative degree-days (CDD), field, and the interaction. For examining population growth, the interaction effect was of interest, and was further partitioned into components that were identified to be significant

by using a backward stepwise selection multiple regression analysis, as shown in Tables 3.2.6–3.2.8.

For the total number (nymphs plus adults) of Lygus bugs, the model explained 80% of the number during the post-first-disturbance growth. Cumulative degree-days explained 57% of the variability and appeared as the most influential factor (Table 3.2.6). Cumulative degree-day in interaction with field accounted for about 15% of the variation, and the effect was significant (Table 3.2.6). In the stepwise analysis, the interaction of coccinellids with CDD had a significant effect: total Lygus population growth was negatively related with numbers of coccinellids (Table 3.2.6), and they accounted for 8.7% of the variability of total Lygus population growth rate in different fields. When adults and nymphs were considered separately in the stepwise analysis, the relationship was similar, but not significant for adults ( $F_{1,12} = 1.40$ ; ns) and nymphs ( $F_{1,10} = 0.98$ ; ns) of Lygus bugs. Nevertheless, the coccinellids x CDD interaction accounted for 1.9 and 6.1% of the variability of the population of Lygus adults and nymphs, respectively. Acridids also exhibited a non-significantly negative relationship only with Lygus nymphs  $(F_{1,248} = 0.33; \text{ ns})$  and accounted for 2.8% of the variability of the population growth of Lygus nymphs. Carabids, chrysopids, Nabis spp., and spiders appeared to be positively related to the rate of population growth of Lygus bugs (total, and or adults or nymphs), and their effects are shown in Tables 3.2.6–3.2.8.

**Migration after hay-cut**. At the time of the first hay-cut, the number of adult *Lygus* bugs in the week following the cut was higher than in the week before the cut in both regions of both types of seed field. The post-cut increase in numbers of adult *Lygus* bugs at the

edges of the adjacent seed field was significantly greater compared than that at the middles of adjacent and at the edge and middle of non-adjacent seed fields. The post-cut increases in numbers of adult *Lygus* bugs in adjacent seed fields were also greater than in non-adjacent seed fields (Table 3.2.9). After the first hay-cut, the average increase of adult *Lygus* bugs at the edges of the adjacent seed field was 2.1 times the number in the preceding week, equivalent increases were only 0.6 times at the middle of adjacent seed fields, and only 0.4 and 0.5 times at the field- edges and middles, respectively, in non-adjacent seed fields. No such trend was evident for nymphs of *Lygus* bugs (Table 3.2.9). The results provide evidence of *Lygus* adults' migration into adjacent seed fields. After the second hay-cut, the result was different for adult *Lygus* bugs: no evidence for their migration was found (Table 3.2.10).

#### <u>Adelphocoris lineolatus</u>

In the present study, insects in the genus *Adelphocoris* was represented by only *A*. *lineolatus*. Results associated with the insect pests are presented below.

## Seasonal occurrence

Seasonal occurrence of *A. lineolatus* presented in Fig. 3.2.5 is typical of the fields sampled. In 1999 and 2001, *A. lineolatus* nymphs were present in fields when sampling began at the end of May or beginning of June, but in 2000 near Arborg, nymphs first appeared a week later (Fig. 3.2.5 c, d). Although numbers of nymphs were increasing, the first hay-cut or insecticide application eliminated them for weeks. After that, the number of nymphs rose for up to three weeks and reached the peak level during mid- to late August (Fig. 3.2.5 a, b, e, f). In 2000, however, the peak occurred at the end of July (Fig.

3.2.5 c, d). After the peak, the numbers of nymphs declined regardless of whether or not there was a disturbance.

Generally, adults first appeared in seed fields in late June or the beginning of July and their numbers gradually climbed to a peak during mid- to late August (Fig. 3.2.5 b-d, f). A deviation from this trend occurred in the hay and non-adjacent seed fields near Dugald in 1999 and in hay fields in 2001: adults first appeared at the end of June, and rose to peak numbers in mid-July (Fig. 3.2.5 a, e). In August, adult numbers tended to decline after the peak regardless of the hay-cut or insecticide application.

# Parasitism

No *A. lineolatus* adults were found to host parasitoids in 1999 and 2000, and no examination of parasitoids in adults was done in 2001. Unlike *Lygus* bug nymphs, *A. lineolatus* nymphs were not found to host parasitoids, except on 26 June in 1999 when one nymph of the insect (5% of the sample) collected from a seed field near Dugald was found to contain one parasitoid.

# **Population analyses**

**Population variation**. Overall, the number of total *A. lineolatus* did not differ among field-types, between hay and seed fields, between adjacent and non-adjacent seed fields (Table 3.2.4). Overall, numbers of adults and total *A. lineolatus* were significantly greater in field-edges than in middles (Table 3.2.5). The numbers of nymphs did not differ between field-edges and middles; lack of significant differences was also the case with regardless of whether old and young nymphs were considered together (Table 3.2.5) or separately. In individual year analyses, in 2001, there were fewer *A. lineolatus* in hay

fields (21.2 ± 12.0 per 30 sweeps) than in seed fields (42.0 ± 14.2 per 30 sweeps) ( $F_{1,2}$  = 22.06; P < 0.05). *Adelphocoris lineolatus* adults, and old nymphs caused this difference as both groups were significantly fewer in hay fields (8.7 ± 3.9, and 10.0 ± 6.5 adults and old nymphs per 30 sweeps, respectively) than in seed fields (14.2 ± 5.1, and 21.8 ± 9.1 adults and old nymphs per 30 sweeps, respectively) ( $F_{1,2}$  = 21.87; P < 0.05;  $F_{1,2}$  = 52.40; P < 0.05, respectively). The trend was not significant but similar in 2000, but reversed in 1999. No such difference was found for young nymphs in any year.

Population growth. Components in the model for ANCOVA were the same as described for Lygus population growth. Interest was focused on the CDD x field interaction effect, which was partitioned into components that appeared significant in the backward stepwise selection multiple regression analysis, as shown in Tables 3.2.11-3.2.13. The model explained 77% of the total number of A. lineolatus during the post-firstdisturbance growth phase. About 38% of the variation was explained by CDD, which appeared as the most influential factor (Table 3.2.11). CDD x field interaction accounted for 14% of the variability in population growth of A. lineolatus. In the stepwise analysis, the acridids x CDD interaction was marginally significant: total number of acridids was negatively related to population growth of *A. lineolatus* ( $F_{1,12} = 2.10$ ;  $P \approx 0.17$ ). The acridids x CDD interaction accounted for 2.8% of the variability of the population growth of A. lineolatus. The interaction of coccinellids with CDD accounted for 6.2% of the variability of population of nymphs, although this was not significant. Carabids, chrysopids, Nabis spp., opilionids, Orius spp. and spiders were positively correlated with total and or adult or nymphal population growth of A. lineolatus (Tables 3.2.11–3.2.13).

Migration after hay-cut. After the first hay-cut, post-cut increase in numbers of adults at the edges of adjacent seed fields did not significantly differ from those at the middles of adjacent and at the edges and middles of non-adjacent seed fields. The result was similar when the numbers at the edges and middles of adjacent seed fields were compared with those of non-adjacent seed fields. However, in adjacent seed fields, the increase of adults at the edges was 13 times the number in the preceding week, which was far greater than the equivalent increase of 0.9 times at the middles of the adjacent seed fields, and 0.3 and 2.1 times at the edges and middles, respectively of the non-adjacent seed fields (Table 3.2.9). When data obtained from different years and localities were considered separately, it was found that the adjacent seed field near Arborg had a pronounced increase in A. *lineolatus* numbers in edge areas than in middle areas following the first hay-cut, resulting in the relatively large SE value. The results provide evidence of A. lineolatus adult migration into adjacent seed fields in some cases, and overall the effect was probably masked by high SE value. No such differential trend was found for A. lineolatus nymphs. After the second hay-cut, the increase in A. lineolatus adult numbers was similar in edge and middle areas of both fields: the increases varied from 0.2- to 0.5- times (Table 3.2.10). As an evidence for a lack of migration, there were no significantly different changes in post-cut nymphal numbers in edges and middles of adjacent and nonadjacent seed fields.

# <u>Acyrthosiphon pisum</u>

## Seasonal occurrence

The seasonal occurrence of *A. pisum* presented in Fig. 3.2.6 is typical of the fields sampled. Generally, small numbers of *A. pisum* were present in fields when sampling was

initiated. The first hay-cut or insecticide application virtually eliminated the pest from fields. Their number remained low until early July, after which the numbers increased rapidly. Their numbers climbed to a peak in mid-July in 1999 (Fig. 3.2.6 a, b) and usually at the end of July in 2000 and 2001 (Fig. 3.2.6c–f). Frequently within a week after the peak, the numbers declined precipitously regardless of whether or not there was a hay-cut or insecticide application.

Although no numerical records were kept, there were few winged aphids and numerous wingless ones in samples collected during mid-July to early August. Dissection of *A. pisum* adults revealed that during mid-July to early August, there was a trend of reduction in numbers of total embryos. A similar trend was also found for early embryos, but not for late embryos (Fig. 3.2.7). When compared with population trends of predators, it was observed that collapses of *A. pisum* populations usually coincided with population increases of a complex of natural enemies, which included anthocorids, chrysopids, coccinellids and nabids (Fig. 3.2.8).

# Parasitism

Overall, 24–39% of *A. pisum* individuals hosted parasitoids (Table 3.2.3). When reared, *A. pisum* individuals were found to host parasitoids from the beginning of June in 1999 (Fig. 3.2.6 b) and 2001 (Fig. 3.2.6 e, f) and from mid-June in 2000 (Fig. 3.2.6 c, d). The first hay-cut or insecticide application virtually eliminated populations of *A. pisum* in most fields. Fields from which *A. pisum* could be collected and reared during this low occurring period of *A. pisum*, it was found that parasitism still occurred (Fig. 3.2.6 b–f). The peak level of parasitism of *A. pisum* occurred around mid-July in 1999 and 2001 and in late July in 2000, and the peak parasitism frequently coincided with peak levels of host occurrence. After the peak, percent parasitism declined, as did the numbers of the host, and after mid-August it was not possible to examine parasitism because of low numbers of *A. pisum*.

## **Population analyses**

**Population variation**. Overall, the numbers of aphids did not differ among field-types, between hay and seed fields, or between adjacent and non-adjacent seed fields (Table 3.2.4). Although hay fields had 65% more aphids than did seed fields, the result was not significant probably because of high levels of variation among fields of the same type. In 1999, and 2001, the hay fields near Arborg and Riverton, respectively, were heavily infested with *A. pisum*, which probably skewed the result. There were no significant differences between field-edges and -middles (Table 3.2.5). When data for each year were analyzed separately, no different results were found at any level of comparison.

**Population growth**. The components used in the model were the same as stated for *Lygus* spp. The interaction of CDD with field was partitioned into components that appeared significant within it. In the analysis involving CDD, field and their interaction, the model explained 80% of the variation in numbers of *A. pisum* during their post-first-disturbance growth. CDD explains 54% of the variation and appeared as the most influential factor (Table 3.2.14). The CDD and field interaction explained 9% of the variation, and the effect was significant (Table 3.2.14). *Adelphocoris lineolatus* adults and *Lygus* spp. nymphs were positively related with population growth of *A. pisum* (Table 3.2.14).

**Migration after hay-cut**. For *A. pisum*, there were no significant changes of their numbers in edges and middles of both fields following the first hay-cut. After the second hay-cut, aphid numbers declined to more or less similar extents irrespective of seed field-type and areas within the field: numbers in the week following the hay-cut were 67–78% of the numbers in the preceding week at the edges and middles of both fields, suggesting that no significant migration was evident after the cut (Tables 3.2.9 and 3.2.10).

#### Gryllus spp.

Generally, the numbers of field crickets in the genus *Gryllus* shown in the fields in Fig. 3.2.9 were considerably higher than in other fields. In seed fields near Arborg in 2000, they were seldom found. In 2001, their numbers were generally low compared with the other years. *Gryllus* spp. first appeared in pitfall traps usually around mid-July (Fig. 3.2.9 b–e), but sometimes as in the hay fields near Dugald in 1999 (Fig. 3.2.9 a) and near Arborg in 2000 (Fig. 3.2.9 c), they first appeared in late July. After their first appearance, the numbers of *Gryllus* spp. increased to peak levels in late August (Fig. 3.2.9 a–c, e, f) or early September (Fig. 3.2.9 d). The second hay-cut or insecticide application did not usually affect their numbers, except in the non-adjacent seed field near Teulon (Fig. 3.2.9 b) where the insecticide application greatly reduced their number in 1999.

The number of *Gryllus* differed significantly among field-types: hay fields had the most *Gryllus*, 13–14 times the numbers in seed fields ( $F_{1,10} = 9.39$ ; P < 0.05), but the two types of seed fields did not differ from each other (Table 3.2.4). There was no edge effect: catches in field-edges and field-middles did not differ (Table 3.2.5). When data

from each year were analyzed separately, the only significant effect was the crop-type in 2000: *Gryllus* catches in hay fields were greater than in seed fields ( $F_{1,2} = 24.48$ ; P < 0.05). In other years, the trend was similar, though not significant. There was no significant migration evident for *Gryllus* spp. after the hay-cut (Tables 3.2.9 and 3.2.10).

## Other insect pests

In addition to the insects described above, occurrence of a few other insect pests as mentioned in Table 3.2.2 were noted. Numerical records of some of them were kept and the results are presented in Tables 3.2.4 and 3.2.5 and described below. A few other insect pests were also seen infrequently, but they will not be discussed in detail.

Cicadellids, which were mostly the potato leafhopper, *Empoasca fabae* Harris, were occasionally found in fields at the beginning of the season, although no numerical records of them were kept until the end of July, when they started to appear frequently. Their numbers rose to peak levels frequently around mid-August (Fig. 3.2.10 a–e), except in the hay field near Teulon where the second hay cut delayed the peak occurrence by a week (Fig. 3.2.10 f). The numbers declined precipitously after the peak regardless of whether there was a hay-cut or insecticide application. In general, cicadellid numbers did not differ among field-types, between crop-types or between seed field-types (Table 3.2.4), however, hay fields provided catches 2–4 times of those in seed fields. Catches of cicadellids were greater in field-edges than in middles (Table 3.2.5)

Acridids (grasshoppers) were infrequently found, and most of them were *Melanoplus bivittatus* (Say). Numerical records of them were kept only from pitfall traps in which the numbers were low. Although hay fields tended to provide more catches than

seed fields ( $F_{1,10} = 4.21$ ; P < 0.05), acridid catches in pitfall traps did not differ among field-types (Table 3.2.4), between the two types of seed fields and between areas within a field (Table 3.2.5). Since the catches were low, acridids will not be discussed further.

Leafmines {assumed to be from the alfalfa blotch leafminer, *Agromyza frontella* (Rondani)} were also found in the present study. In 1999, mined leaflets were found occasionally, less than 3% on average, in fields near Dugald (Fig. 3.2.11 a, c, e). In fields near Teulon, the infestation was high: up to 50% of the middle leaflets were mined. There were apparently two peaks: the first in mid- to late June, and the second around mid-August, though in the hay field (Fig. 3.2.11 b) the second peak occurred a week earlier than in seed fields (Fig. 3.2.11 d, f).

The level of leafminer infestation did not differ significantly among field-types, between crop-types, between adjacent and non-adjacent seed fields (Table 3.2.4) or between field areas (Table 3.2.5). But leafminer infestation in hay fields was 1.3–3.5 times the infestation in seed fields. In 1999, although up to 53% of the middle leaflets were mined in mid-June, the infestation was generally infrequent. In 2000 and 2001, leafminer infestation was occasionally seen.

The occurrence of seed chalcids was determined based on infested seeds from samples of mature seeds collected in September, and did not allow description of seed-chalcid phenology in fields. On average about 2% of seeds was infested with seed chalcids (Table 3.2.4).

There were a few other insects found (Table 3.2.2), though records of their numbers were not kept because they usually appeared in low numbers, and are not known to cause significant damage to alfalfa in the Canadian Prairies. Of these insects, flea

beetles were noted throughout the study period, and sometimes in noticeable numbers. However, there was little damage of alfalfa attributable to flea beetles. Sweet clover weevil, *Sitona cylindricollis* Fåhraeus, occurred frequently until the end of July. Blister beetles in the genus *Lytta* occurred in fields from June to early August. Alfalfa loopers, *Autographa californica* (Speyer), were occasionally seen in all fields near Dugald, and in the hay field near Teulon. Elaterid beetles infrequently appeared in pitfall traps in all fields. Cutworms and June beetle adults were occasionally found in pitfall traps. The superb plant bug, *A. superbus*, and the alfalfa weevil, *H. postica*, were not found in any fields.

### Insect natural enemies

At least 20 taxa of predaceous and parasitic arthropod natural enemies were found, often in noticeable numbers, in fields of alfalfa (Table 3.2.15). Weekly mean catches of numerically recorded natural enemies are given in Table 3.2.16. Results associated with carabids will be discussed in a separate paper, Chapter 3.3.

# **Coccinellids**

Coccinellids occurred throughout the study period. They were mainly *Coccinella septempunctata* (L.) and *Hippodamia tredecimpunctata* Say. *Coccinella septempunctata* was the dominant species and occurred throughout the season, but *H. tredecimpunctata* was present usually during the later part of the season. Generally, the insecticide application markedly reduced the numbers of coccinellid adults and larvae, and the second hay-cut in late July or early August did not affect coccinellid numbers (Fig. 3.2.12).

In all years, coccinellid adults were present from the beginning of sampling (Fig. 3.2.12). The seasonal patterns of coccinellid occurrence appeared inconsistent. In general, there tended to be early peak populations of coccinellid adults, which were reduced and kept low for weeks by the first hay-cut or insecticide application. Then the numbers of coccinellid adults started increasing around mid-July and rose to peak levels usually around mid- to late August (Fig. 3.2.12). After the peak, numbers of coccinellid adults declined sharply regardless of whether there was a hay-cut or insecticide application. Deviation from the above trend was evident in the hay field and the non-adjacent seed field both near Riverton (2001), where coccinellid adults did not rise to peaks in August (Fig. 3.2.12 e).

Larvae were not counted separately in 1999. Larvae first appeared around mid-July in 2000 (Fig. 3.2.12 c), and in late July in 2001 (Fig. 3.2.12 f). Deviation from this trend was found in the hay field near Riverton (2001) where larvae first appeared in late August (Fig. 3.2.12 e). With the exception of high numbers in mid-August in the adjacent seed field near Riverton (2001) (Fig. 3.2.12 f), the numbers of larvae were usually low with peak occurrence during early to mid-August (Fig. 3.2.12 c, d).

Overall, coccinellid numbers did not differ among field-types, between croptypes, seed field-types (Table 3.2.16) or between field areas (Table 3.2.17). The results were similar in each of the years 1999, 2000 and 2001.

#### **Syrphids**

Syrphids collected in sweep net samples were usually adults, but they were not identified to species. In general, the hay-cut reduced syrphid numbers for longer than did

insecticide application. Syrphids were usually present at the beginning of sampling and continued to occur in low numbers in the majority of fields until mid-July. Then the numbers rose to peak levels in late July (Fig. 3.2.13 a, b, f); deviations from this pattern occurred in all fields sampled in 2000 and in the adjacent seed field near Teulon (1999) and in the hay field near Riverton (2001) where the peak population occurred around mid-August (Fig. 3.2.13 c–e). In all years, regardless of hay-cuts or insecticide applications, syrphid numbers declined precipitously after the peak (Fig. 3.2.13).

Overall, syrphid numbers did not significantly differ among field-types, but catches in hay fields were smaller than in seed fields ( $F_{1,10} = 7.08$ ; P < 0.01); there was no significant difference between the two types of seed fields (Table 3.2.16). There were also no differences between field-edges and field-middles (Table 3.2.17). When data from each year were analyzed separately, no difference among field-types, between croptypes, seed field-types or field-areas was found in any year, though catches were consistently smaller in hay fields than in seed fields each year.

#### Orius spp.

In general, the seasonal patterns of anthocorids, which were in the genus *Orius*, were not consistent among different fields across different years. Their first appearance in samples seemed to be very variable in different fields and years: from mid-June to the end of June in 1999 (Fig. 3.2.14 a, b), from early to mid-June in fields near Arborg in 2000 (Fig. 3.2.14 c) and from the end of June, when sampling was initiated in this locality. In 2001, the insects were found from the beginning of sampling in fields near Arborg (Fig. 3.2.14 f), although in the hay field near Riverton they were not found until

mid-July (Fig. 3.2.14 e). Although their first appearance varied among fields and years, in all years and fields their occurrence in peak numbers was usually during mid- to late August (Fig. 3.2.14) except in the non-adjacent seed field near Riverton (2001) where the peak occurrence was in late July.

There were differences in *Orius* numbers among field-types: fewer *Orius* were caught in hay fields than in seed fields ( $F_{1,10} = 6.25$ ; P < 0.05), but the adjacent and non-adjacent seed fields did not differ (Table 3.2.16). Field-edges and -middles also did not differ in *Orius* catches (Table 3.2.17). When data for each year was analyzed separately, similar but not significant trends were found in all years.

#### Nabis spp.

Damsel bug (nabids), mostly *Nabis alternatus* (Parshely), usually appeared from the beginning of sampling. *Nabis americoferus* Carayon were infrequently found. The numbers of *Nabis* spp. were reduced by the first hay-cut or insecticide applications. But soon after the second disturbances, the numbers began to increase. The seasonal patterns in different fields and years were variable: from mid-August to the beginning of September in 1999 (Fig. 3.2.15 a, b), from the end of August (Fig. 3.2.15 c, d) to mid-September in 2000, from the end of July (Fig. 3.2.15, e) to late August (Fig. 3.2.15 f) in 2001.

*Nabis* numbers differed among field-types: in hay fields there were more *Nabis* than in seed fields ( $F_{1,10} = 8.10$ ; P < 0.05), but catches in the two types of seed fields did not differ from each other (Table 3.2.16). Catches of *Nabis* spp. were smaller in field-edges than in field-middles (Table 3.2.17). Similar but not significant trends were found in all the above cases for *Nabis* in separate analyses for each year.

### **Pentatomids**

Numerical records of pentatomids (stinkbugs), which were in the genera *Euschistus* and *Cosmopepla*, were kept only in 1999. Pentatomids were present in fields from the beginning of sampling. Their numbers were low throughout the season, although their numbers rose to high levels in most cases during early to mid-September (Fig. 3.2.16 a–e). As indicated in the figure, the hay-cut seemed to affect pentatomids more than insecticide application, and the second insecticide application seemed to be the least harmful of the disturbances that were inflicted in this study (Fig. 3.2.16).

Generally, variation in pentatomid numbers was found among field-types: in hay fields, there were fewer pentatomids than in seed fields ( $F_{1,10} = 10.63$ ; P < 0.01). The non-adjacent seed fields provided the most pentatomids, which were followed by the adjacent seed fields, although these differences were not significant (Table 3.2.16). There were fewer pentatomids caught in field-edges than in field-middles (Table 3.2.17).

## **Chrysopids**

Chrysopids were common in sweep-net samples. They were not identified to species in 1999. In other years, they were mainly the adults of *Chrysoperla carnea* (Stephens), although adults of *Chrysopa oculata* Say were found occasionally. The effects of hay-cut or insecticide application on chrysopids were not consistent (Fig. 3.2.17).

In 1999 and 2001, chrysopid adults first appeared at the end of May or the beginning of June (Fig. 3.2.17 a, b, f), except that they started appearing in mid-July in the Riverton hay field (Fig. 3.2.17 e). In 2000, adult chrysopids were low in numbers, and

they first appeared from middle to end of July (Fig. 3.2.17 c, d). Adults usually peaked in numbers during early to mid-August (Fig. 3.2.17).

Larvae were not counted separately in 1999. In 2000 and 2001, larvae first appeared in majority of cases in mid-July (Fig. 3.2.17 c, d), except in seed fields near Riverton (2001), where they occurred from late June (Fig. 3.2.17 f). Larval numbers usually peaked during mid- to late July (Fig. 3.2.17 d–f), although the adjacent seed field near Arborg (2001) had peak larval occurrence at the end of August. In both years, larval numbers declined sharply after the peak regardless of disturbances (Fig. 3.2.17 c–f). It was noted that adult numbers could not usually be predicted from larvae.

The number of chrysopids significantly differed among field-types: in hay fields there were fewer chrysopids than in seed fields ( $F_{1,10} = 25.34$ ; P < 0.01) (Table 3.2.16), though there was no difference between seed field-types or field-areas (Table 3.2.17). Significant differences were found in 2001 ( $F_{2,2} = 122.79$ ; P < 0.01, and  $F_{1,2} = 217.13$ ; P < 0.01 for field-types and crop-types, respectively); the trend was similar but not significant in 1999 and 2000.

### **Spiders**

In 1999 and 2000, spiders were not identified to species. In 2001, a diverse assemblage of spiders comprising a total of 78 species representing 16 families of spiders was collected (Appendix II). Average catches in sweep-net samples were smaller than those in pitfall traps. Although the seasonal patterns varied among fields and years, and between sampling methods, spiders were usually present in fields when sweeping began (Fig. 3.2.18). The first insecticide application markedly reduced spider numbers, but the

effects of the second insecticide application were inconsistent (Figs. 3.2.18 and 3.2.19). Conversely, the hay-cut did not greatly affect spider populations, particularly those in pitfall traps (Fig. 3.2.18 a, c, e and 3.2.19 a, c, e). The patterns of catches in sweep-net and pitfall traps suggest that spiders probably were bimodal in their occurrence, the first modality during early to mid-June, and the second around mid- to late August.

In both sweep net and pitfall trap samples, spider numbers did not differ among field-types, between crop-types, seed field-types (Table 3.2.16) or field-areas (Table 3.2.17). A similar trend was found when data for each year were considered separately.

### **Opilionids**

Opilionids, which were not identified to species, were present in fields from the beginning of sampling (Fig. 3.2.20 a, b, e, f), except in 2000, when the first catch was in late June in hay fields (Fig. 3.2.20 c). The first insecticide applications often reduced opilionid numbers greatly (Fig. 3.2.20 b, f), but the effects of the second insecticide application were inconsistent (Fig. 3.2.20 b, d, f). The hay-cut usually did not affect opilionid numbers (Fig. 3.2.20 a, c, e). Generally, the seasonal patterns of opilionids appeared inconsistent among fields and years.

In1999 and 2000, their peak occurrence was around mid- to late August (Fig. 3.2.20 a, c), except in the non-adjacent seed fields near Teulon (1999) and Arborg (2000) where the peak occurrence deviated as shown in fig. 3.2.20 (b and d) and in the adjacent seed field both near Arborg (2000) where the peak was at the end of August. In 2001, the peak occurrence was during mid- to late July (Fig. 3.2.20 e, f), except in the non-adjacent seed field near Riverton where peak was in late June.

Opilionid numbers differed among field-types: the number was greater in hay fields than in seed fields ( $F_{1,10} = 10.63$ ; P < 0.01), but the two seed field-types did not differ (Table 3.2.16). Field-edges provided smaller catches than did field-middles (Table 3.2.17). The result of the overall analysis was dominated by the pattern in 1999 for which the individual analysis showed the same pattern. In 2000 and 2001, a similar but not significant trend was found.

## **Other natural enemies**

Records of robber flies (asilids) were kept, but their numbers were too low to analyze and present. Assassin bugs (reduviids) appeared occasionally in sweep net samples, though, no numerical records of them were kept. Parasitoids appeared infrequently in sweep net samples, but their numbers were not recorded. The species of these parasitoids are listed in Table 3.2.15. There were six species of aphidiid parasitoids, although in 2001 it was found that over 75% of these aphidiid parasitoids were *A. ervi*. Parasitoid assessments through dissection of *Lygus* and *A. lineolatus*, and rearing of *A. pisum* do not provide direct measures of the temporal and numerical occurrence of adult parasitoids.

**Migration of natural enemies after hay-cut**. Among predaceous arthropods, after the first hay-cut, no significant migration of coccinellids, chrysopids, *Orius* spp., *Nabis* spp., syrphids, spiders and opilionids was evident (Table 3.2.18). After the second hay-cut, the results were similar except that *Orius* spp. exhibited a significantly greater increase in adjoining edges of the adjacent seed fields (Table 3.2.19).

### Discussion

#### Pest occurrence and patterns

Three taxa, *Lygus* spp., *A. lineolatus*, and *A. pisum* occurred regularly and often numerously, indicating that they are the most common pest insects in alfalfa fields of Manitoba. These pests have been reported to cause damage to alfalfa crops in the other Prairie Provinces (Lilly and Hobbs 1962; Harper 1978; Goplen *et al.* 1987; Murrell 1987; Schaber and Entz 1988; Soroka 1991; Soroka and Murrell 1993).

### Lygus spp.

Although *Lygus* bugs were not identified to species, previous reports suggest that they were most likely to be *L. lineolaris*, *L. borealis* and *L. elisus*. Timlick *et al.* (1993) found that in alfalfa fields in the Red River region where Dugald is situated, *L. lineolaris* was the dominant species, and *L. borealis* was second to *L. lineolaris*. Whereas, in the Interlake region, where Arborg and Riverton are situated, *L. borealis* dominated over *L. lineolaris*. In both areas, few *L. elisus* were also found. However, Schwartz and Foottit (1992b) in a mid-July study found *L. borealis* dominated in alfalfa fields in the Red River region. From sweeping alfalfa fields in the Interlake and Red River regions of Manitoba during 2002 to 2004, Mostafa (unpublished, pers. comm.) found a predominance of *L. lineolaris* with occasional occurrences of *L. borealis* and *L. elisus*. In Saskatchewan, Braun *et al.* (2001) found that *L. lineolaris* was predominant from May to mid-June and from mid-July to late August, whereas *L. borealis* was most abundant from mid-June to early July. The temporal shift in dominance is possibly related to the availability of preferred host plants and their stages (Murrell 1987; Leferink 1991).

Lygus bugs overwinter as adults under leaf litter or debris outside alfalfa fields, and enter alfalfa fields as soon as alfalfa plants sprout in the spring (Goplen et al. 1987). In the present study, there was often a small peak of *Lygus* adults around mid-June (Fig. 3.2.4 c, e, f), and those adults were probably the overwintered adults, as suggested by Gerber and Wise (1995). During mid- to late July in the current study, there was a second peak of Lygus adults in some fields (Fig. 3.2.4 a, b, d-f), although in some fields such peaks were not detected, possibly because of the first hay-cut or an insecticide application. To reach adulthood, a *Lygus* nymph takes about four and five weeks at 20 and 16°C, respectively (Champlain and Butler 1967; Khattat and Stewart 1977). Therefore, it is likely that the adult peak during mid- to late July represented the first generation developed from nymphs that survived or hatched after the first hay-cut or insecticide application. Gerber and Wise (1995) reported that in Manitoba, the first generation adults peak during the first three weeks of July. In the present study, there was frequently a large adult peak during mid- to late August (Fig. 3.2.4 a, c, d), and in fields where no such peaks were found, the population trend indicated that had these fields not been disturbed, peaks would have been occurred in late August or in September. According to Gerber and Wise (1995) Lygus adults at that time of the season were probably of the second generation.

Although the second hay-cut or insecticide application affected populations, the numbers of *Lygus* bug adults frequently rebounded after disturbance (Fig. 3.2.4). It does not seem that the rebound was due entirely to survivors of the disturbance, as the late season adult numbers in these fields could not be predicted from the number of nymphs in the fields before the disturbance. The rapid rebound was probably caused by adults that

immigrated into alfalfa fields, which at that time remained green while most other host crops had senesced or been harvested. Murrell (1987) also found a late season influx of adults into alfalfa fields, which she attributed to immigration.

In some fields, there was a small peak of *Lygus* nymphs around mid-June, although the hay-cut or insecticide application did not allow such a rise in the majority of fields. In most fields, there was a second peak in number of *Lygus* nymphs around mid-August (Fig. 3.2.4). According to Gerber and Wise (1995), these first and second peaks were probably the first and second generations of *Lygus* nymphs, respectively. Murrell (1987), in an extensive field study in Saskatchewan, found similar seasonal patterns. As indicated in Fig. 3.2.4, nymphs were relatively more numerous later in the season than early in the season, a trend previously reported from Saskatchewan (Craig 1983; Murrell 1987) and Manitoba (Gerber and Wise 1995). Conversely, Craig (1983) and Braun *et al.* (2001) at latitudes >52°N in Saskatchewan found that *Lygus* nymphs were rarely as numerous late in the season as in the early period; this trend may be a consequence of cool summers causing the nymphal stage to be protracted (Craig 1983).

## Adelphocoris lineolatus

In addition to *Lygus* bugs, plant bugs in the genus *Adelphocoris* also damage alfalfa crops in Canada. Previous reports indicate that these pests in the genus *Adelphocoris* are mainly the alfalfa plant bug, *A. lineolatus* (Goeze), the superb plant bug, *A. superbus* (Uhler), and the rapid plant bug, *A. rapidus* (Say) (Hughes 1943; Lilly and Hobbs 1956; Craig 1963; Murrell 1987). However, in the present study, only *A. lineolatus* was found. Murrell (1987) in an extensive study in Saskatchewan also found *A. lineolatus* was virtually the only *Adelphocoris* species in alfalfa fields.

Adelphocoris lineolatus overwinters as eggs, frequently in alfalfa stems; and after hatching in spring, nymphs start to feed on alfalfa (Hughes 1943). Nymphs of A. lineolatus develop through five instars to reach adulthood, and this occurs in about 28 days at 17°C and in only about 18 days at 26°C (Hughes 1943). Seasonal occurrence of A. lineolatus (Fig. 3.2.5) in the present study indicates that there were likely two generations of nymphs. Although disturbed by the hay-cut or insecticide application, the first peak in numbers usually occurred in June, and probably represented the first generation. The second peak that frequently occurred in August was probably of the second-generation nymphs (Craig 1963). It was noted that during the second peak period, the rise from low numbers to the peak took three weeks suggesting that the hatch was completed mostly in three weeks, a trend also noted by Craig (1963). After the peak of the late season nymphs, numbers declined regardless of whether or not there was a disturbance (Fig. 3.2.5). Adelphocoris lineolatus has a narrow host plant range (Hughes 1943; Craig 1963), and prefers alfalfa plants (Murrell 1987); A. lineolatus adults are weak fliers and movement of its females and nymphs is limited (Hughes 1943). Therefore, it seems likely that these second generation nymphs give rise to adults in the same field.

In hay fields in 1999, the numbers of nymphs relative to adults in August was far greater than those in any other field or year (Fig. 3.2.5), but the number of the subsequent adults did not correspond. It is unclear whether the nymphs migrated away or died before reaching adulthood. The limited host range, lack of nymphal migration (Hughes 1943), and possible absence of other preferred host crops in surrounding fields this late in the season, suggest that death of nymphs before adulthood is most likely. But in other cases, unlike *Lygus* bugs, *A. lineolatus* adult numbers were usually predictable from previous

numbers of nymphs, and there was no late season resurgence (Fig. 3.2.5), suggesting that there was little migration of the adults late in the season. However, migration is required for increased reproduction of *A. lineolatus* (Hughes 1943), suggesting that adults might migrate early in the season, when they reproduce (Hughes 1943, Craig 1963). Hughes (1943) reported that *A. lineolatus* is a weak flier, and that the movement of reproductive females is usually limited. In such a case, the effect of migration could be of shorter range and the result that numbers of adults but not nymphs were greater in field-edges than in middles (Table 3.2.5) supports this. If not reproductive, adults of this bug generally migrate only when faced with shortage of food and absence of shelter (Hughes 1943). In addition, the food preference and the possible absence of alternative hosts suggest that the late-season population is not the result of migration late in the season.

The two generations of nymphs that were evident were not reflected in the number of peaks of *A. lineolatus* adults, except in the hay (Fig. 3.2.5 f) and non-adjacent seed fields near Dugald (1999). It is unlikely that the adults present during the second peak in late August developed from overwintered eggs. This is because, in the Canadian Prairies, although very limited hatching continues sporadically until about mid-July, hatching of overwintered eggs is mostly completed by mid-June (Craig 1963), and the hatched nymphs are likely to become adults by mid-July (Hughes 1943). Nymphs that occurred in high numbers around mid-June (Fig. 3.2.5) represents mostly older (3<sup>rd</sup> to 5<sup>th</sup>) instars, and a third instar nymph would reach adulthood in less than 19 and 10 days at 17 and 26° C, respectively (Hughes 1943). Given the temperatures, and cumulative degree-days above 10.6°C during the present study (Appendix I), it is unlikely that the adult peak in mid- or late August represented the nymphs found in early June. Rather, it is likely that

the first hay cut and insecticide application killed most first generation nymphs, and probably resulted in an undetectable population of corresponding adults. Craig (1963) reported that *A. lineolatus* is largely univoltine north of 53°N, and is bi- or multivoltine south of 51°N. Murrell (1987) found a partial second generation in southern Saskatchewan.

### <u>Acyrthosiphon pisum</u>

Acyrthosiphon pisum is a common pest of alfalfa in North America (App and Manglitz 1972). In the present study, numbers of *A. pisum* tended to be low early in the season, and high from mid-July to early-August, a trend consistent with reports from Alberta and Saskatchewan (Harper 1978; Soroka 1991). On field peas in Manitoba, Deneka (1992) found peak populations of *A. pisum* from mid-July to the beginning of August. In Saskatchewan, Murrell (1987) found *A. pisum* to occur in peak numbers from early to late August. After the peak, *A. pisum* populations declined precipitously, a trend noted previously (Murrell 1987; Murrell *et al.* 2002; Deneka 1992). The population decline may be the result of changes in *A. pisum*'s physiological responses to photoperiod (Sharma *et al.* 1973; Smith and MacKay 1990), aggregation, food quality (Kennedy and Forsbrooke 1972) or changes in natural enemy populations (Lilly and Hobbs 1962; Neuenschwander *et al.* 1975; Radcliffe *et al.* 1976; Frazer *et al.* 1981*a*, 1981*b*).

## **Other insect pests**

Although there were quite a few other insect pests found in this study, only those that occurred relatively frequently or in high numbers at some point of the season will be

discussed. In the present study field crickets, *Gryllus* spp., occurred in alfalfa fields, and was more frequent in hay fields than in seed fields. Little is known about seasonal occurrence and damage caused by crickets to established alfalfa, though *Allonemobius* spp. has been reported to damage alfalfa seedlings in Kentucky (Grant *et al.* 1982). However, according to Jacobs *et al.* (1992) field crickets are mostly litter-feeders and they do not prefer to feed on alfalfa.

In this study, the leafhopper, Empoasca fabae, regularly occurred and tended to be more frequent in mid- to late August. A similar trend was also reported from Ontario (Faris et al. 1981). In Manitoba – potatoes, which are one group of the primary hosts of E. fabae – are frequently attacked by E. fabae in maximum numbers during August (Manitoba Agriculture and Food 2004). In Manitoba, the recommended time to harvest potatoes is before September (Manitoba Agriculture and Food 2004), suggesting that potatoes were probably not suitable hosts for the pest in late August. The sudden rises of E. fabae populations in mid to late August and the result that they were more numerous in field-edges suggest that E. fabae probably invaded from other crops like potatoes that were either harvested or ready to be harvested. *Empoasca fabae* attacks alfalfa in Eastern Canada (Goplen et al. 1987), and causes substantial yield loss in Ontario (Ontario Ministry of Agriculture and Food 1980). In the north-central and northeastern United States, E. fabae attacks significantly reduce both quantity and quality of hay alfalfa (Kindler et al. 1973; Lamp et al. 1985). In Illinois, Lamp et al. (1985) found that high populations of *E. fabae* during late July caused a significant reduction of alfalfa hav production. In Minnesota, E. fabae attack may significantly affect the second and third hay crop (Cuperus et al. 1983). Generally, alfalfa plants are more vulnerable to E. fabae

attacks in the early stage of plant growth; and *E. fabae* cause little damage to the older and larger plants (Kouskolekas and Decker 1968). Cicadellids in the current study were prevalent in August, when the hay crop was either harvested or ready to be harvested; and the seed crop was maturing. Pest attack on alfalfa at this time of the year is unlikely to cause significant damage to alfalfa seed (Charnetski 1983*a*, 1983*b*).

Symptoms of leafminer infestation, assumed to be from the alfalfa blotch leafminer, *Agromyza frontella* (Rondani), were found in this study. Alfalfa blotch leafminer infests alfalfa throughout Eastern Canada (Guppy 1981). Leafminers are relatively recent pests, which have been spreading westward from eastern Canada (Harcourt and Binns 1980). As leafmines were infrequently found and in most cases, percentage of damaged leaflets were low, at its present level of infestation the insect seems to be of little importance to alfalfa in Manitoba.

The present study does not allow description of seed-chalcid phenology, as seed chalcid infestation was based on samples of mature seeds collected in September. Although evidence that alfalfa seeds were attacked by the seed chalcid was found, growers seem ignorant of the pest. In Saskatchewan, up to 6% seeds were infested by seed chalcids in both hay and seed fields (Soroka and Spurr 1998). Seed chalcid infestation may often be underestimated; deformed and darkened seeds with pathogen infections can be a frequent consequence of seed chalcid infestation, but are not always linked to the causal agent. The other insect pests found in this study do not seem to be causing any damage to alfalfa in Manitoba, and will not be discussed.

# Occurrence and patterns of insect natural enemies

Coccinellids were often found (Fig. 3.2.12) and Coccinella septempunctata was the most frequent species. Coccinella septempunctata is an introduced species and has been relocated to control aphids in several localities in the United States (Schaefer et al. 1987). Since its establishment, C. septempunctata has been spreading and has become the dominant coccinellid replacing many native coccinellids in agricultural habitats (Turnock et al. 2003). In Manitoba, the species has been found since 1989 (Matheson 1989), and in 1992, it was the predominant coccinellid species (Turnock et al. 2003). By sweeping alfalfa crops in southern Manitoba (49-51°N, 96-98°W), Turnock et al. (2003) found that C. septempunctata was more abundant than Hippodamia tredecimpunctata in 1999, but less abundant in 2000 and 2001. However, they did not provide information on seasonal patterns of these coccinellids. In the present study, coccinellid numbers were low most of the season, a similar trend to that in Saskatchewan (Murrell 1987). There were relatively more coccinellids late in the season (Fig. 3.2.12), a similar trend to that in Minnesota (Radcliffe et al. 1976) and California (Neuenschwander et al. 1975). In this study, H. tredecimpunctata was found abundantly late in the season, probably because the species is usually active at lower temperatures and migrates from field crops to overwintering sites later than C. septempunctata in the autumn (Turnock et al. 2003).

Syrphids collected in sweep-net samples were mostly adults, and they were frequently found from July with peak occurrence in August (Fig. 3.2.13). To mature their eggs, syrphid fly females require pollen as food, and so remain very mobile; they move from flower to flower and oviposit where food for offspring is present (Schneider 1969, and references therein). Such behaviour of adults is likely to lead to easy capture. The

seasonal patterns found in the present study are probably related to the presence and condition of alfalfa flowers, which were numerous and in full bloom in July. Numerous syrphid fly adults were present around mid-August; during that period in Manitoba, most other crop plants have senesced or are ready to be harvested, and flowers are few. There were still some flowers present on alfalfa plants at this time.

Syrphid fly larvae seldom appeared in sweep-net samples. In Canada, syrphid flies overwinter as pupae on foliage, among debris, or in the soil (Agriculture and Agri-Food Canada 2001). Syrphid adults can oviposit within four days of their emerging as adults. Pollard (1971), by using Brevicoryne brassicae (L.) infested Brussels sprout plants in pots, found that the syrphid, Episyrphus balteatus (de Geer), preferred to oviposit on infested plants in crop habitats rather than in woodland. Syrphid fly larvae usually hatch from eggs within two days of oviposition (Vockeroth 1992) and in favourable conditions, the larvae of some species can pupate in about eight days (Vockeroth 1992, and references therein). A short larval stage and long adult stage could lead to low numbers of larvae and high numbers of adults in a sweep-net, even if numbers in a cohort are the same. Syrphid fly larvae are apodous, and stick to the plants by coiling their bodies around leaf-petioles and plant shoots (Vockeroth 1992, and references therein). Therefore, occurrence of fewer larvae in sweep-net samples does not indicate that they were uncommon. Rather, it is likely that the sweep-net often failed to collect them.

The first appearance of *Orius* spp. varied across years and localities. Murrell (1987) also found such variation, which she attributed to sampling error. *Orius* spp. found in the present study were small in size, and bias may not be unusual when sampling such

small insects occurring in low numbers early in the season. However, the physical and management factors, which were different among fields and years, may also have contributed to variability. Numbers of *Orius* spp. peaked during mid to late August. In Minnesota, Godfrey and Leigh (1994) found low numbers of *Orius* spp. early in June, with the peak occurrence around mid-July.

Nabis spp. found in alfalfa fields comprised mainly Nabis alternatus (Parshley) and N. americoferus Carayon, and this is consistent with reports from elsewhere in Canada (Guppy 1958; Richards and Harper 1978). In southern Alberta, N. alternatus are more common (Richards and Harper 1978). Nabis spp. were found from the beginning of the sampling period, which agrees with Murrell (1987) in Saskatchewan. Peak occurrence of Nabis spp. was late in the season. Murrell (1987) did not provide information on the peak occurrence of Nabis spp., but in Minnesota, Godfrey and Leigh (1994) found peak numbers of Nabis spp. at the beginning of July.

*Chrysoperla carnea* and *Chrysopa oculata* Say were the two green lacewing species found in alfalfa fields in the present study. Generally numbers of larval and adult chrysopids were relatively low in samples, as was also observed by Murrell (1987). It was noted that adult numbers could not be predicted from larvae. This is possibly the result of the migration behaviour of young lacewing adults. In the first night after emergence, regardless of the presence of food, *C. carnea* and probably many other lacewing species perform an obligatory migration flight before they mate and oviposit, resulting in their leaving the natal habitat (Duelli 1988).

A diverse assemblage of spider species occurred in alfalfa fields (Appendix II). They represented both ground and plant canopy active spiders. For example, members in

the family Lycosidae are usually active on the soil surface, whereas, those in the Linyphidae are mostly plant-dwellers (Wise 1993). Little is known about the spider fauna from Canadian alfalfa fields. However, large and diverse spider communities containing species ranging from web-builders to active hunters, ground surface dwellers to plant canopy dwellers have been found in alfalfa fields in the United States (Fenton 1959; Muniappan and Chada 1970; Howell and Pienkowski 1971; Wheeler 1973). The seasonal patterns observed in the present study in which high numbers of spiders occurred from the beginning of the season agree with results from New York (Wheeler 1973).

In addition to the above taxa, there were some other predaceous taxa found in this study (Table 3.2.15). Among them carabid beetles were frequently present and results about carabid beetles have been described in a separate paper (Chapter 3.2.3). Little is known about the seasonal occurrence and importance of the other taxa in Table 3.2.15 in alfalfa fields. In the present study, they were found to be less frequent which suggests that these taxa could be of relatively minor importance in alfalfa fields of Manitoba. They will not be discussed further.

Parasitoids are integral part of agricultural habitat and contribute greatly toward natural suppression of pest populations in agricultural habitats (Debach and Rosen 1991; Pedigo 1999). In the current study, braconid parasitoids of mirids were found (Table 3.2.15) and evidence that these parasitoids killed mirids, particularly *Lygus* spp. (Table 3.2.3, Figs. 3.2.4 and 3.2.5) was detected. Two different methods were used for examining the parasitism of mirids. The method used in 1999 appeared to be inefficient. This is because alcohol-preserved tissues of both parasitoids and hosts often looked alike, and it became difficult to distinguish teased host-tissues from parasitoids that may have

been damaged despite careful dissection under the microscope. The method used in 2000 and 2001 appeared to be relatively effective and provides evidence that *Lygus* nymphs are attacked by parasitoids mainly through the middle part of the season (mid- June to August). The parasitoid larvae found in *Lygus* nymphs were of the genus *Peristenus*, though it was not possible to identify them to species. Six species of aphidiid parasitoids, which parasitize and kill aphids including the pea aphid, *A. pisum*, were found (Table 3.2.3, Fig. 3.2.6), and evidence they greatly contribute toward natural suppression of *A. pisum* populations was also detected.

# Factors affecting insect populations in alfalfa fields

Several abiotic and biotic factors influence population development of insect pests in agricultural habitats. Important abiotic factors include the temperature, humidity and light (Pedigo 1999; Norris *et al.* 2003). Important biotic factors include natural enemies comprising predaceous, parasitic and pathogenic agents, and competition. These factors are naturally present and frequently interact with human activities such as crop production practices.

Abiotic environmental factors through their influence on growth and development, natality, mortality and migration can significantly influence pest populations in agricultural habitats (Hughes 1943; Khattat and Stewart 1977; Norris *et al.* 2003). Population analyses in the present study indicate that temperature strongly influences population growth of all the three major insect pests regardless of their stages (Tables 3.2.6–3.2.8 and 3.2.11–3.2.14). Field characteristics in this study were second to temperature in influencing pest populations. The study fields were from different

localities and different years, and differed in properties (Table 3.2.1), which contributed greatly toward variations in pest populations. Although its effect was not assessed, moisture influences arthropod populations including carabids (Holland 2002).

There is evidence that photoperiod influences fecundity of mirids (Hughes 1943) and *A. pisum* (Sharma *et al.* 1973). No attempt was made to examine mirid fecundity in this study, and therefore mirids will not be discussed in this context.

Although a plethora of potential pest species occur in an agricultural habitat, cooccurring natural enemies prevent many of the pest species from reaching economically damaging levels. To increase benefits from these natural enemies, it is required that they be identified, their roles understood and efforts are made to conserve and augment them (Norris et al. 2003). As stated in the results and shown in Tables 3.2.6 and 3.2.13, there were some arthropod taxa (predators) which in interaction with CDD exerted negative relationships, while some other taxa exhibited positive relationships with the population growth of Lygus bugs, A. lineolatus (total, adults or nymphs) or A. pisum. The negative relationships suggest that the presence of those predators (the source of variability) either negatively affected population growth of related pests {Lygus bugs, A. lineolatus (total, and or adults or nymphs) or A. pisum} or that factors that favoured the pest population growth were unfavourable to the abundance of related predators and vice versa. Conversely, the positive relationship could mean either that the predators exhibited a positive density dependent response to corresponding pests or that the conditions that favoured pest population growths also favoured corresponding predator taxa. Such a density-dependent rises of predator populations could mean that they would affect the pest populations later.

## Lygus populations

In addition to temperature (Tables 3.2.6–3.2.8) and variation in field characteristics (Table 3.2.1) natural enemies, hay cutting or insecticide application also contributed toward Lygus population development. The seasonal patterns in the present study indicated that at their peak occurrence, coccinellids would frequently encounter adults and nymphs of Lygus spp. and it appeared that coccinellids influenced Lygus population development to some extent (Tables 3.2.6). Larval coccinellids feed on nymphs of Lygus bugs (Schaber 1992). In laboratory and field cage studies, coccinellids alone or as a member of the predator guild were found to reduce mirid numbers (Chapter 3.4). The cage studies also provided evidence that carabids, chyropids and nabids probably exerted some influence on Lygus populations. In 11 fields out of 18 in this study, the peak occurrence of nabids coincided with that of Lygus nymphs, suggesting that nabids frequently encountered Lygus prey. Predation of Lygus spp. by chrysopids (Schaber 1992) and nabids (Knowlton 1949; Clancy and Pierce 1966; Perkins and Watson 1972; Wheeler 1977; Tamaki et al. 1978; Schaber 1992) has been noted previously.

Loan and Craig (1976) reported that a univoltine parasitoid, *Peristenus pallipes* (Curtis) kills *Lygus* spp. in western Canada. Although different species of *Peristenus* were introduced from Europe and released into western Canada, no recoveries of the released species are known (Broadbent *et al.* 2002). In Saskatchewan, Braun *et al.* (2001) found *Lygus* nymphs to be parasitized by a univoltine parasitoid, which they assumed was *P. pallipes*. Since the pattern of parasitism is consistent with those reported from elsewhere in the Prairies (Braun *et al.* 2001), it is likely that a univoltine species is causing the nymphal mortality in Manitoba. In this case, the estimated maximum percent parasitism and mean percent parasitism expressed as a total over the season may be misleading with respect to the actual impact of parasitoids present only for a portion of the season (Braun *et al.* 2001). Loan and Craig (1976) found up to 83% of *Lygus* nymphs to host parasitoids in western Canada and Braun *et al.* (2001) found up to 70% of *Lygus* nymphs to host parasitoids in Saskatchewan. During mid-summer in the present study, as high as 68% of *Lygus* nymphs contained parasitoids, which certainly overestimates the overall impact of parasitoids on *Lygus* spp. in Manitoba. However, the result provides evidence that parasitoids play important role on *Lygus* populations in Manitoban alfalfa fields.

Hay cutting or insecticide application appeared to influence *Lygus* populations considerably. It appears that hay cutting reduced *Lygus* populations in alfalfa fields, although, high numbers of the pest often reoccurred from late July. Overall, numbers of *Lygus* bugs were relatively lower in hay fields than in seed fields, and the trend was pronounced for *Lygus* nymphs, particularly the older ones. This pattern may be attributable to the differential effect of hay cutting and insecticide application. In addition to killing or displacing inhabitants and destroying eggs laid in plants (Butler *et al.* 1971; Khattat and Stewart 1980; Harper *et al.* 1990; Schaber *et al.* 1990b), hay cutting removes plant stems with buds, flowers, and fruits, which serve as preferred feeding sites and favour reproduction of plant bugs (Hughes 1943; Tingey and Pillemer 1977). Therefore, removal of plants through hay cutting is likely to decrease the number of nymphs. The decrease was pronounced for the second generation *Lygus* nymphs: during mid-July and afterward when the second generation *Lygus* nymphs occurred (Gerber and Wise 1995)

there were fewer Lygus nymphs in hay fields compared with seed fields. Hay cutting also greatly affects microenvironment in the mown fields (Pinter et al. 1975; Harper unpublished, cited in Schaber et al. 1990b) and probably reduces their attractiveness to most arthropods until plants regrew. In contrast, insecticide applications, although killing the majority of insects, tend not to destroy the plant habitat in the way that hay cutting does, and this may account for seed fields having more Lygus bugs. The second insecticide application in seed fields was carried out in most cases when mirid nymphs, most of them older, would have reached their peak levels. In addition, seed fields usually received the first insecticide before hay fields were mown. This created an opportunity for the survivors and displaced Lygus individuals to move into seed fields after the haycut, as found in the present study (Table 3.2.9) and corroborated previous studies (Stern et al. 1964; Butler et al. 1971; Rakickas and Watson 1974; Summers 1976; Khattat and Stewart 1980; Schaber et al. 1990b). If the hay fields were not harvested or the seed fields were not sprayed with insecticides, it is likely that Lygus populations would have increased further.

### Adelphocoris lineolatus populations

As was the case with *Lygus* spp., in addition to temperature (Tables 3.2.11– 3.2.13) and variability in field characteristics (Table 3.2.1), some other factors including natural enemies and crop production practices influenced *A. lineolatus* populations on alfalfa. The seasonal patterns in the present study indicated that at their peak occurrence, coccinellids would frequently encounter nymphs of *A. lineolatus*, and it appeared that coccinellids affected to some extent *A. lineolatus* population development (Table 3.2.13).

In laboratory and field cage studies, coccinellids alone or as a member of the predator guild reduced mirid numbers (Chapter 3.4). The population trends suggest that chrysopids frequently encountered adults and nymphs of *A. lineolatus*; predation of *A. lineolatus* by chrysopids (Schaber 1992) and nabids (Knowlton 1949; Wheeler 1977; Schaber 1992) has been noted previously.

The present study is not conclusive about parasitism of *A. lineolatus*, as there was only one instance where one *A. lineolatus* nymph was found to be parasitized. It may be that the insect is occasionally parasitized at such a low level that more intensive sampling is required to detect parasitism. According to Day *et al.* (1992), the parasitism rate of *A. lineolatus* is comparatively low, less than one-third of that of *Lygus* bugs in the United States. In Saskatchewan, Craig and Loan (1987) found less than 5% nymphs of *A. lineolatus* to host parasitoids.

As was the case with *Lygus* spp., seasonal patterns in relation to hay cutting and insecticide application indicate that these operations influenced *A. lineolatus* populations on alfalfa. These operations immediately reduced the pest populations, although, high numbers of *A. lineolatus* reoccurred late in the season. Overall, *A. lineolatus* numbers tended to be relatively lower in hay fields than in seed fields, the possible reasons have been explained as for *Lygus* spp. previously.

## Acyrthosiphon pisum populations

As was the case with mirids, temperature (Table 3.2.14) and variation in field characteristics (Table 3.2.1) greatly influenced *A. pisum* populations. Populations of *A. pisum* are influenced by the temperature, availability and quality of food, photoperiod,

and activities of natural enemy populations (Craig 1973; Sharma *et al.* 1973; Neuenschwander *et al.* 1975; Frazer *et al.* 1981*a*, 1981*b*; Hutchinson and Hogg 1984; Lamb *et al.* 1987; MacKay 1987), and some of these factors probably contributed much to the population trends (Fig. 3.2.6) as found in the present study.

Hutchinson and Hogg (1985) suggested that emigration is one of the major factors affecting A. pisum populations in alfalfa fields in Wisconsin during late June to mid-July. Emigration of aphids from a field requires production of alates, which can occur in response to declining host quality or overcrowding (Kennedy and Forsbrooke 1972). The quality of plants as a host for aphids is dependent upon its concentration of secondary plant metabolites (Auclair 1976). Alfalfa plants contain saponins, concentration of which increases with increasing plant maturity, and saponins have been thought to influence A. pisum colonization (Horber et al. 1974). Conversely, Bournoville (Unpublished, cited in Febvay et al. 1988) found no relationship between concentration of saponins and A. pisum population development. In the current study, alfalfa plants were still green and succulent at the mid-July to early August phase of A. pisum population reduction. Therefore, the involvement of host quality degradation on population crashes of A. pisum in this study remains unclear. In Alberta, Hobbs et al. (1961) found that about 26% of A. *pisum* populations were winged in mid-June. Although no numerical records were kept, it was observed that numbers of winged A. pisum during mid-July to early August were relatively low compared to wingless forms, which suggests that crowding was not critical for the population crashes in this study.

In Quebec, the fecundity and thereby the population size of *A. pisum* gradually declines as the photoperiod decreases from 15 hours (Sharma *et al.* 1973). In a laboratory

trial, MacKay (1987) found that A. pisum adults from a clone founded by an individual collected from an alfalfa field in southern Manitoba, produced an average of about 98, 71 and 56 individuals at a photoperiod regime of 18:6, 15:9 and 14L:10D, respectively. However, she found the trend of fecundity not to be significantly related to photoperiod. In southern Manitoba (Latitude 49° 38'N, Longitude 97° 08'W), the critical photoperiod for ovipara production is near 14L:10D (Smith and MacKay 1990). The photoperiod is above 15 h during mid-July and early August in Manitoba, when populations of A. pisum declined precipitously. Therefore, it is unlikely that the population declines in the present study were due to physiological changes in response to photoperiod. This is further endorsed by results obtained through dissection of A. pisum adults, which revealed that although the numbers of early embryos tended to decline, numbers of late embryos were similar throughout late July to early August (Fig. 3.2.7). It is expected that the number of late embryos would immediately affect population changes. Therefore the trend of late embryo numbers suggests that the population crashes of A. pisum in this study were not solely due to decline in photoperiod-influenced fecundity. The trend for early embryos, however, indicates that the decline in fecundity would have an effect on populations of A. pisum later in the season.

In the present study, collapses of *A. pisum* populations frequently coincided with population increases of a predator complex including coccinellids, *Orius* spp. *Nabis* spp. and chrysopids (Fig. 3.2.8) suggesting that this natural enemy complex was responsible, at least to some extent, for the population collapses of *A. pisum*, as was found previously (Lilly and Hobbs 1962; Neuenschwander *et al.* 1975; Radcliffe *et al.* 1976; Frazer *et al.* 1981*a*, 1981*b*; Hutchinson and Hogg 1984). Coccinellids were one of the members of

this natural enemy complex, and coccinellids have been frequently found to suppress aphid populations in alfalfa fields. Frazer et al. (1981b) found that coccinellids alone can suppress aphid populations when the prey density was low, but at high prey densities, coccinellids in association with other predators suppress aphid populations. In Alberta, Lilly and Hobbs (1962) found a rapid decline of A. pisum populations in response to upsurges of coccinellids and other predators in a burnt alfalfa plot. In Oklahoma, Fenton (1959) found that coccinellids contributed to limiting aphid population development. Schaber (1992) stated that a coccinellid adult in the Prairies would consume over 4600 aphid individuals during the summer. Neuenschwander et al. (1975) showed that during the lifetime of a coccinellid, the adult and larva could destroy 5625 and 1295 aphid individuals, respectively. These authors did not provide any information about the species of those coccinellids. Neuenschwander et al. (1975) also reported that coccinellids reduce aphid populations in alfalfa fields in California. Coccinellids alone or as a component of the predator guild were also found to reduce A. pisum populations on caged alfalfa (Chapter 3.4).

Orius spp. feed on many arthropods including aphids and other soft-bodied insects (van den Bosch and Hagen 1966, cited in Godfrey and Leigh 1994; Wheeler 1974; Schaber 1992). Wheeler (1974) observed Orius spp. feeding on aphids on alfalfa. Results in this study provide circumstantial evidence that being a part of the predatory guild (Fig. 3.2.8), Orius spp. contributed toward the population crashes of *A. pisum*.

Nabis spp. feed on aphids in alfalfa fields (Wheeler 1977; Schaber 1992). Figure 3.2.8 indicates that *Nabis* spp. contributed to reduction of *A. pisum* populations as found in this study. Lilly and Hobs (1962) found that *Nabis* spp. as a member of the predator

guild reduced *A. pisum* populations in Alberta. In the laboratory and field cage studies, *Nabis* individuals alone or as a member of the predatory guild reduced *A. pisum* populations (Chapter 3.4).

Larvae of *C. carnea* and both larvae and adults of *C. oculata* feed on many softbodied arthropods including aphids (Principi and Canard 1984; New 1988; Schaber 1992). In the present study, the majority of lacewing catches were larvae. Chrysopid larvae are voracious predators (Principi and Canard 1984) and the effectiveness of their predation is well recognized (Neuenschwander *et al.* 1975; Senior and McEwen 2001). Fenton (1959) and Lilly and Hobbs (1962) found evidence that chrysopids contributed toward natural suppression of *A. pisum* populations. Chrysopid larvae alone or as a component of the predaceous guild reduced *A. pisum* populations on caged alfalfa in this study (Chapter 3.4).

In addition to the obligatory predators discussed above, facultative predation – where a phytophagous species may consume an insect – often occurs in field crops (Debach and Rosen 1991). Although *Lygus* bugs are one of the primary pests of alfalfa, they also feed on aphids in the fields (Lindquist and Sorensen 1970; Wheeler 1974). In the present study, *Lygus* adults were negatively related, although nonsignificantly, to *A. pisum* population growths, which suggest that *Lygus* adults affected to some extent the population growth of *A. pisum*. *Gryllus* spp. usually feed on leaf litter, but they frequently feed on soft-bodied arthropods on soil (Philip and Mengersen 1989). Little is known about consumption of *A. pisum* by *Gryllus* spp., although in this study *Gryllus* spp. were nonsignificantly negatively related to *A. pisum* population growth.

It appears that parasitoids also contributed greatly toward natural suppression of *A. pisum* populations. However, the present study does not provide information on parasitism when aphid populations were low, particularly in the later part of the season. High levels of parasitism often coincided with peak numbers of *A. pisum*, and seasonal trends of both host populations and parasitism levels suggest that parasitoids play important roles on *A. pisum* populations in alfalfa fields. There were at least six species of parasitoids (Table 3.2.15), but from the finding that 75% of the aphid parasitoids identified in 2001 were *A. ervi*, it appears that this species dominates the aphid parasitoid guild. In Manitoba, Matheson (1988) found five parasitoid species of *A. pisum*, and also noted the dominance of *A. ervi*.

Although previous reports suggest hay cutting effectively reduces *A. pisum* populations (Harper *et al.* 1990), greater numbers of *A. pisum* were present in hay fields than in seed fields in the present study. Harper *et al.* (1990) obtained control of *A. pisum* populations for 2–4 weeks when the hay crop was cut for the first time in the season before mid-July, and for at least 6 weeks when the second cut occurred after late July. In hay fields, there were fewer chrysopid, *Lygus* and *A. lineolatus* individuals, which feed on aphids (Lindquist and Sorensen 1970; Wheeler 1974, 1977) and were negatively related to *A. pisum* populations, and probably contributed to the result.

#### Agromyza spp.

*Agromyza* infestation was infrequent, and Harcourt *et al.* (1987, 1988) documented that pupation failure, intraspecific competition, predation and parasitization allow only 0.4–3% of eggs of the leafminer, *Agromyza frontella*, to survive to adulthood

in Ontario. This could also be the case in Manitoba. *Nabis* spp. and other predatory Hemiptera were often found in the study-fields, and they have been reported to cause 30% population reduction of *A. frontella* in Ontario (Harcourt *et al.* 1987).

### Roles of other potential natural enemies

The other arthropods that were frequently found did not appear to be significantly related to populations of the major insect pests in this study. Although these natural enemies were not found to influence populations of the major pest species, based on the population trends and previous reports, these arthropods deserve to be discussed. As described previously, results indicate that syrphid larvae were present, and they have been reported to reduce aphid populations. Neuenschwander *et al.* (1975) showed that a larva could destroy 1110 aphids in its lifetime. They also noted that rises in syrphid numbers coincided with population declines of aphids on alfalfa in California. The population collapse of *A. pisum* occurred in late July to early August, and the collapse often coincided also with population increases of syrphid fly adults.

Spiders are predaceous and can limit herbivore populations in field crops (Kajak *et al.* 1988). According to Wheeler (1973), they can consume mirids and *A. pisum*. Howell and Pienkowski (1971) reported consumption by different spider species of various pests including *Lygus* spp., *A. lineolatus* and *A. pisum*. In the present study, spiders were frequently present from the beginning of the season, when populations of other natural enemies were small. Thus, they could play an important role as early season suppressors of pest populations.

Opilionids feed on gastropods and various arthropods (Dixon and McKinlay 1989; Nyffelér and Symondson 2001). As they are generally active on the ground surface, opilionids along with other epigeic predatory arthropods, may have the potential to exert synergistic predation of insect pests. Opilionids are known to consume aphids (Dixon and McKinlay 1989; Nyffelér and Symondson 2001).

### Management for hay or seed production

The present study documents that alfalfa fields in Manitoba are subject to disturbances caused by crop production and management practices including cutting for hay crop or insecticide applications in seed fields. The purposes and requirements of these two operations are different, although both hay cutting and insecticide applications have implications for pest populations as described before.

## Hay cutting

It appears that alfalfa fields for hay are mown twice in a crop growing season in Manitoba. In southern Alberta, alfalfa is cut usually twice in a growing season, although sometimes there may be a third cut (Harper *et al.* 1990). In this study, the first hay cutting occurred around mid-June, and the second one frequently around mid-August. It is recommended that hay-cut be timed in relation to flowering condition of alfalfa plants: the quality of hay is greatest if harvested at the time 10% of plants in the fields are flowering (Goplen *et al.* 1987). The current practice of hay cutting, particularly in June, seems to satisfy this requirement. In addition, hay cutting often reduces the pest population levels, partly by causing surviving pests to move into nearby seed fields, as

found in the present study. Timing of the last hay cutting is important for stand persistence. The critical fall harvest period, when no cutting is recommended, is usually 4–6 weeks before the first killing frost (Goplen *et al.* 1987). The second hay cutting in this study occurred during late July to late August (Table 3.2.1). In Manitoba, harvesting alfalfa between mid-August and the first killing frost is not recommended, as it would result in depleted food reserves in roots and affect stand persistence (Goplen *et al.* 1987; Manitoba Agriculture and Food 2001). It appears in this study that the majority of growers followed this recommendation (Table 3.2.1).

It appears that hay cutting reduced pest populations and no pest control measures were needed, although in the 1960s, growers routinely applied insecticide to control aphids and other pests on hay crops of alfalfa in the Canadian Prairies (Harper *et al.* 1990). This was partly because during that time the hay crop was harvested quite late, approximately at 100% bloom, which allowed time for pest populations to build up to damaging levels before the crop was cut. Summers (1998) reviewed and concluded that resistant cultivars have been notably successful in suppressing aphid populations and eliminating the requirement for insecticide applications on hay crops of alfalfa in North America. Neuenschwander *et al.* (1975) stated that the widespread use of resistant cultivars helped successful control of spotted alfalfa aphids. It was also noted that the majority of growers used cultivars that are resistant to *A. pisum* and diseases, and this also probably contributed toward non-requirement of insecticide application on hay crop.

Although there were fewer mirids, hay fields had greater numbers of *Gryllus* spp. compared with seed fields. Generally, vegetationally diverse habitats provide more resources and are more attractive to herbivores (Root 1973), and to omnivorous insects,

the attractiveness is likely to be more pronounced. Tennis (1983) found a positive relationship between cricket density and plant diversity in the field. Alfalfa for hay was grown with companion crops including timothy, clover and other grasses. Despite being omnivorous (Tennis 1983), most field crickets feed on vegetative materials on the soil surface, and do not prefer alfalfa as food (Jacobs *et al.* 1992). Therefore, hay fields are likely to be more attractive than seed fields of alfalfa, and the occurrence of more *Gryllus* individuals in hay fields may not be surprising.

It appears that an alfalfa field for hay is sometimes grown adjacent to an alfalfa field for seed production, as was noted previously elsewhere in the Prairies (Schaber et al. 1990b). Hay cutting had implications for pest populations, particularly for mirids in seed fields; pre- and post-cut numbers of adult Lygus bugs at different areas of fields (Table 3.2.9) provide evidence that following the first hay-cut Lygus spp. adults migrated from the mown hay field into adjacent seed fields. After the second cut, however, there was a lack of significant adult invasion of adjacent seed fields. Lygus adults were previously found to migrate from mown hay fields into adjacent fields (Stern et al. 1964; Butler et al. 1971; Rakickas and Watson 1974; Summers 1976; Schaber et al. 1990b). Plant growth stage may influence Lygus invasion. Khattat and Stewart (1980) showed that Lygus adults do not invade fields unless the crop plants are in the bud stage, the primary feeding stage for the bug. Schaber et al. (1990b) also did not find significant invasion of Lygus adults when adjoining hay fields were mown after plants in the potential receiving field had passed the Lygus-preferred stage. This seems contradicted by the population trends of Lygus adults (Fig. 3.2.4 b, d) suggesting migration into seed fields occurs late in the season. In the present study, the sudden loss of habitat in mown

fields appears to displace *Lygus* from hay fields and it is surprising that after the second hay-cut, the displaced adults were not detected in the adjacent seed fields. At the time the second hay-cut occurred, most annual crop plants in fields were maturing or ready to be harvested, so *Lygus* adults are unlikely to have moved there. Perhaps at the time of the second cut, the large amount of adult dispersal from senescing crops masked the effect of the hay-cut on adjacent seed fields of alfalfa. Another possibility is that at the time the second mowing occurred, migration from mown fields was not local but relatively long range; this would elevate *Lygus* levels in all undisturbed fields, rather than just those adjacent to mown fields.

There was evidence of some *A. lineolatus* adults' migration into adjacent seed fields (Table 3.2.9). Schaber *et al.* (1990*b*) found that after the hay-cut *A. lineolatus* adults moved into adjacent seed fields, although such movement was absent when there were barriers of grassy strips, roads or ditchea. In Minnesota, Hughes (1943) found evidence of adult *A. lineolatus* migration following the hay-cut. However, he observed that unlike *Lygus* spp., *Adelphocoris lineolatus* adults are weak fliers, and movement of gravid females is limited. Therefore, detection of invasion of adult *A. lineolatus* is likely to be dependent upon the presence of barriers, population size, sex ratio and the state of females during the time the hay crop is swathed. There was evidence, which was striking in the field near Arborg, of *A. lineolatus* adult migration following the first hay-cut. Absence of significant migration in other fields probably resulted from the presence of wider margins with ditches and vegetations in between those hay and seed fields, as was noted previously (Schaber *et al.* 1990*b*). The result in this study suggests that probably *A.* 

*lineolatus* adults walked into the adjacent seed field (near Arborg) when the adjoining hay field was first mown in early July.

In the present study, significant movement of mirid nymphs following hay-cut was not detected. Mirid nymphs' ability to migrate is limited (Hughes 1943; Godfrey and Leigh 1994). Rakickas and Watson (1974) did not find movement of *L. hesperus* nymphs from cut field into adjacent field of alfalfa. Harper *et al.* (1990) stated that *Lygus* nymphs die following hay-cut, as they cannot leave the field and are subject to high soil surface temperatures that limit their dispersal. Temperatures in cut alfalfa fields may exceed 45°C (Pinter *et al.* 1975; Harper unpublished data, cited in Schaber *et al.* 1990b). Plant bugs suffer a high rate of water loss: daily water loss of a *Lygus* adult is at least 50% of its body weight, and the losses are greater in younger ones than in older ones (Cohen 1982). This would be exacerbated by the lack of plant fluids to consume and the high temperature in swathed fields. Therefore, it would be difficult for nymphs of *Lygus* spp. and *A. lineolatus* to survive and disperse after the hay-cut.

There was no evidence of a significant migration of *A. pisum* from mown hay fields into adjacent seed fields. Schaber *et al.* (1990*b*) studied dispersal of insects following the first (mid- June to July) and second hay-cut (Mid-August to early September) in three sites and found mixed results; in two of the sites, there were no signs of *A. pisum* dispersal into adjacent seed fields, but in another site, *A. pisum* numbers increased more dramatically after both the first and second hay-cuts. These authors did not separate alates and apterae, and thought that the increases could be the result of migration or differential reproduction of aphids. In the present study, alate *A. pisum* individuals were not found in fields when the first hay-cut occurred, and apterous

individuals have very limited dispersal ability (Losey and Denno 1998). Hobbs *et al.* (1961) found that about 26% of *A. pisum* populations were alate in mid-June. According to Hobbs *et al.* (1961, and references therein), when alfalfa approaches blossoming time, which is around mid-June in Manitoba, winged *A. pisum* individuals start appearing, and these alates tend to move from alfalfa fields into annual legume crop-fields. At the time the second hay-cut occurred, *A. pisum* populations were declining greatly. Aphids have low flight speed, and during migration, airflow determines their flight direction, altitude, and the distance traveled (Dixon 1971). In such a case, it is unlikely that the alate individuals, displaced by the hay-cut ended up in the adjacent seed fields.

Among the predaceous natural enemies studied in the present study, there was evidence for migration of *Orius* spp. only after the second hay-cut (Table 3.2.19). The finding is consistent with Rakickas and Watson (1974), who found *Orius tristicolor* (White) but not *Chrysopa*, coccinellids, *Collops*, *Geocoris*, *Nabis* or reduviids to migrate into half-grown alfalfa fields when full-grown alfalfa was cut in Arizona. Schaber *et al.* (1990*b*) in Alberta found only *N. alternatus* (Parshley) and *Orius tristicolor* to migrate into alfalfa fields after the adjacent hay field was swathed.

### **Insecticide application**

Insecticides were used once or twice in the season (Table 3.2.1). It seems that in June, seed growers applied insecticides as a clean-up treatment regardless of insect numbers and stages, and this was carried out 7–10 days before the projected date of leafcutting-bee release so that the residual effect of insecticide had dissipated at the time of pollinator introduction. A similar observation was made by Murrell (1987) in

Saskatchewan. Growers are usually busy and probably do not find it an attractive option to monitor the fields during summer months. Tradition may also influence this (Murrell 1987).

Nymphs of mirids, particularly of A. lineolatus, were likely to be hatching out during the time the June application was carried out, and unless most of the hatch had completed, application of control measures would unlikely be completely effective. Although pest populations rebounded within a few weeks after the June application of insecticides, given the lack of selective insecticides that are safe to beneficial organisms, the current timing appears to be appropriate in the sense that it usually reduced insect pest populations and helped maintain pest populations at levels low for a considerable period in the season. In addition, the pre-bloom nature of the treatment precluded harm to leafcutting bees introduced into fields as pollinators. Insecticide applications in June usually do reduce pest populations in prairie seed alfalfa fields for most of the season (Craig 1961; Murrell 1987). In most fields, three to five weeks following the June application of insecticides, there was resurgence of insect populations which reached high levels from late July to late August (Figs. 3.2.4–3.2.6). It is likely that the June application allowed survival of some nymphs of Lygus spp. and A. lineolatus that were in the egg stage at the time of application and some A. pisum. These survivors then formed the basis for the subsequent population build up. Immigrants may also have contributed to the build up. The present study does not provide information about the effect of insecticide application on parasitoids, but the population trend indicates that insecticides often affected predaceous arthropods. However, populations of predaceous arthropods often built up following the insecticide application, although the insecticide application

seems to have prolonged effects on *Nabis* spp., which were fewer in seed fields than in hay fields. Harper (1978) found evidence that although dimethoate greatly reduced populations of most predators including aeolothripids, anthocorids, coccinellids and nabids; the effect of the insecticide is much more prolonged on nabids. But nevertheless, there were more larvae to compensate for the reduced *Nabis* populations.

Growers appeared to be divided on whether to control pests late in the season. Most growers made the second application of insecticide as a routine application rather than following sampling and an economically-based decision (Table 3.2.20). Evidence that feeding by insect pests late in the season in Manitoba damages alfalfa seeds is scarce; alfalfa seedpods were at the ripening stage at the time the late season pest populations were controlled. It has been reported that late season insecticide applications produce no economic benefit (Charnetski 1983*a*, 1983*b*). However, in situations where pest populations are high enough to cause concentrated feeding during the late season, the infestation could be economically damaging, particularly in conditions of prolonged summer weather (Soroka and Murrell 1993). A very long frost-free period in the fall would mean that seeds developing late in the season could have matured into viable seeds. Intense feeding late in the season may also affect the viability of stands for the next season. In such cases, the second application of insecticide could be justified.

### Synthesis and Conclusions

The present study provides information on the seasonal occurrence and population levels of various arthropods, although it does not show these patterns in absence of disturbances. In this study, all the hay fields were swathed twice, and seed fields were

sprayed with insecticides once or twice each year. These operations affected arthropod populations, and the present study provides insight into the prevalence of arthropods in relation to these production practices of alfalfa in Manitoba.

The seasonal occurrence, population levels, and potential damage of insect pests indicate that *Lygus* spp., *A. lineolatus* and *A. pisum* are the most important insect pests in alfalfa fields of Manitoba. From personal talks with 31 growers in Manitoba, it is clear to me that these three insect taxa are of major concern in alfalfa production. Although infestations of leafminers and seed chalcids have also been noted, growers appeared to be ignorant of such infestations. This is not surprising as growers are usually concerned and aware of those species that cause conspicuous damage. Growers are likely to find it difficult to separate the mined leaflets from leaflets blemished by disease or for other reasons. Seeds damaged by seed chalcid infestation are usually lost with the chaff during threshing (Arnold 1960; Soroka and Spurr 1998).

A variety of predaceous arthropods that feed on *Lygus* spp., *A. lineolatus*, *A. pisum*, and other pests also occur in alfalfa fields. These include but are not limited to anthocorids, carabids, chrysopids, coccinellids, nabids, pentatomids, syrphids, spiders and opilionids. Both plant canopy- and ground-active predators were present in alfalfa fields, and this may result in additive predation as suggested by McAllister and Roitberg (1987) and Losey and Denno (1998).

Although temperature has been found to be the most influential factor of pest population growth, the present study provides circumstantial evidence that some predator taxa occurring in alfalfa fields can influence insect pest populations. However, no single species seems to be effectively restraining the pest population growth. The extent of

predation of a particular predator and the resulting effect on pest populations is usually difficult to determine in field conditions (Hagen and van den Bosch 1968; Debach and Rosen 1991). Predators are often generalists, and therefore, the extent of predation by a particular predator species in a diverse prey assemblage is often split, which can obscure the effects. In agricultural habitats, as found in the present study, various predator species occur simultaneously. In such cases, the overall effects could be great even when individual species contribute small effects, as multi-species predation effects are often additive (Frazer *et al.* 1981*a*, 1981*b*; McAllister and Roitberg 1987; Losey and Denno 1998; Sunderland 2002).

Parasitoids also contribute considerably toward suppression of pest populations, particularly of *Lygus* bugs and *A. pisum* in alfalfa fields. It was found that parasitism occurred mostly in the middle part of the season, when the pest infestation is critical for alfalfa: flowering, fruiting and seed setting occur during this period, and insecticide applications are problematic because of the presence of leafcutting bees in seed fields.

Both predators and parasitoids are simultaneously active in alfalfa fields, and it is difficult to determine which group contributes more to suppress pest populations. The exact mortality caused by predators is difficult to assess, because the victims are usually devoured (Hagen and van den Bosch 1968). Waage and Greathead (1988) proposed that the efficiency of different natural enemies is dependent on the type and distribution of pests. Pests with many generations, like *A. pisum*, might be best controlled with predators, whereas pests with single generation might be best controlled with parasitoids, which exhibit a non-random searching behaviour. This theory suggests that predators would be more effective against *A. pisum*, as found by Frazer *et al.* (1981*a*, 1981*b*).

Conversely, Snyder and Ives (2001) showed that although it reduces pest populations for a short time, predation by some taxa may lead to increased pest populations on a longterm basis. Examining the interaction among the predator, *Pterostichus melanarius*, the pest *A. pisum*, and aphid parasitoids in both field and laboratory cages, Snyder and Ives (2001) found that *P. melanarius* consumes parasitized aphids, which led to reduced parasitism rates and increased aphid populations on a long-term basis. In a choice test, they found that *P. melanarius* equally accepts healthy, parasitized yet mobile and mummified hosts. However, in situations where the parasitized hosts are mobile, such intraguild predation effects can be less intense. Both predators and parasitoids operate simultaneously in nature, and they both contribute toward reduction of pests in alfalfa fields (Neuenschwander *et al.* 1975; Radcliffe *et al.* 1976).

Crop production practices also seem to exert some influence on pest populations. Swathing hay crops affected most taxa and effectively reduced *Lygus* spp. and *A*. *lineolatus* populations to low levels so that no pest control measures were required in hay fields. Swathing at times also induced mirid adults to migrate into neighbouring fields.

It appears that insecticide application is required for controlling insect pests in alfalfa fields for seed production. The majority of growers have been found to apply insecticide twice in a crop season at times dictated by the presence of leafcutting bees for pollinating alfalfa flowers. However, there seems to be a lack of coordinated approach of scheduling for the insecticide application with hay cutting, despite the fact that hay cutting may induce adult mirids to migrate into neighbouring seed fields of alfalfa. It appears that a coordinated approach in which the first insecticide application is timed to coincide with the hay-cut would reduce the effect of immigration on the seed crop. In

addition, this would create the opportunity to eliminate plant bug nymphs more effectively, and increase the possibility of eliminating the second spray of insecticide. This, however, requires the availability of an insecticide that has a relatively short residual toxicity, and is effective against insect pests while being harmless to the pollinators. Availability of such insecticides would contribute toward more enlightened use of chemical insecticides.

	Localities			Field	S	Dates of mowing /	Sam	pling
Years	(Latitude; Longitude)	Field-types	Cultivars	Seeded (year)	Area (ha)	insecticide spray	Start	End
1999	Dugald	Hay	Algonquin	1996	20	14 Jun; 28 Jul	31 May	14 Sep
	(49° 52' N; 96° 49' W)	Adjacent seed	WL 317	1997	7	13 Jun; 18 Aug		_
		Non-adjacent seed	Key	1997	32	08 Jun; 22 Aug		
	Teulon	Hay	Canada 1	1995	22	30 Jun; 18 Aug	02 Jun	16 Sep
	(50° 25' N; 97° 15' W)	Adjacent seed	Centurion	1998	38	15 Jun; 27 Aug		-
		Non-adjacent seed	Runner	1996	44	15 Jun; 27 Aug		
2000	Arborg	Hay	Algonquin	1998	8	03 Jul; 08 Aug	23 May	29 Aug
	(50° 56' N; 97° 5' W)	Adjacent seed	Key	1996	18	29 Jun	-	-
		Non-adjacent seed	Ram	1997	24	29 Jun		
	Riverton	Hay	Algonquin	1993	56	13 Jul; 25 Aug	28 Jun	14 Sep
	(50° 59' N; 96° 59' W)	Adjacent seed	Algonquin	1997	44	24 Jun; 01 Aug		
	•	Non-adjacent seed	Algonquin	1999	40	24 Jun; 23 Aug		
2001	Arborg	Hay	Algonquin	1998	8	05 Jul; 12 Aug	30 May	28 Aug
	(50° 56' N; 97° 5' W)	Adjacent seed	Key	1996	18	25 Jun		
		Non-adjacent seed	B 330	2000	32	25 Jun		
	Riverton	Нау	Glory	2000	12	20 Jun; 21 Aug	29 May	28 Aug
	(50° 59' N; 96° 59' W)	Adjacent seed	Glory	1998	20	13 Jun; 24/25 Aug	-	•
		Non-adjacent seed	Glory	1997	64	13 Jun; 24/25 Aug		

Table 3.2.1. Study sites, locations, field characteristics, dates of mowing and insecticide application, and sampling periods, 1999–2001.

<u></u>	<u></u>				Loca	lities	
		Insects		Du	gald	Teulon	
Order	Family	Species	Common name	Hay	Seed	Hay	Seed
Hemiptera	Miridae	Lygus spp.	Lygus bug	÷	÷	+	+
		Adelphocoris lineolatus (Goeze)	Alfalfa plant bug	+	+	+	+
		Adelphocoris superbus (Uhler)	Superb plant bug	-	-	-	-
Homoptera	Aphididae	Acyrthosiphon pisum (Harris)	Pea aphids	+	+	+	+
	Therioaphis maculata		Spotted alfalfa aphids	+	+	+	+
	Cicadellidae	Various	Leafhoppers	+	+	+	+
Orthoptera	Acrididae	Various	Grasshoppers	+	+	+	+
-	Gryllidae	Gryllus spp.	Crickets	+	+	+	+
Diptera	Agromyzidae	Agromyza spp.	Alfalfa blotch leafminer	+	+	+	+
Hymenoptera	Eurytomidae	Bruchophagus roddi (Gussakovsky)	Alfalfa seed chalcid	?	+	?	+
Coleoptera	Chrysomelidae	Unidentified	Flea beetles	+	+	+	+
-	Curculionidae	Hypera postica (Gyllenhal)	Alfalfa weevil	-	-	-	-
		Sitona cylindricollis Fåhraeus	Sweet clover weevil	+	+	+	+
	Meloidae	<i>Lytta</i> spp.	Blister beetles	+	+	+	+
Lepidoptera	Noctuidae	Autographa californica (Speyer)	Alfalfa looper	+	+	+	-

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Table 3.2.2. Presence or absence of potential insect pests in alfalfa fields in Manitoba, 1999.

+ and - indicate the presence and absence, respectively of corresponding insects.

? indicates no examination was made.

Table 3.2.3. Estimated percent parasitism of *Lygus* nymphs and *A. pisum* collected by sweep netting alfalfa crops in 1999, 2000 and 2001 (only one nymph of *A. lineolatus* collected from a seed field near Dugald in 1999 was found to host parasitoid, and the result is not shown in this table).

			us spp.	Acyrthosiphon pisum					
	Years	Collected	Dissected	Parasitis	m (%)	Collected	Reared	Parasitism (%)	
Localities		(n)	(n)	Maximum	Mean	(n)	(n)	Maximum	Mean
Dugald	1999	179	101	20	4	259	234	53	28
Teulon	1999	345	225	20	3	410	259	62	32
Arborg	2000 2001	448 12	275 12	56 67	24 67*	900 1275	428 645	55 58	33 39
Riverton	2000 2001	430 305	261 189	64 68	38 29	800 740	390 395	48 58	24 33

\* Based on a single collection and dissection of 12 nymphs only.

				Field types		
Samples (units)	Insects	Insect stages	Hay	Adjacent seed	Non-adjacent seed	Statistics
Sweep net	Lygus spp.	All (total)	$13.6 \pm 1.2$	$20.5 \pm 4.0$	$14.4 \pm 2.2$	$F_{2,10} = 0.41$ ; ns
(number/30	29800 FF	Adults	$9.1 \pm 1.5$	$12.4 \pm 2.5$	$8.5 \pm 1.4$	$F_{2,10} = 0.21$ ; ns
sweeps)		Nymphs	$4.5 \pm 0.8^{b}$	$8.2 \pm 1.6^{a}$	$5.9 \pm 0.9^{ab}$	$F_{2,10} = 6.21; P < 0.014$
5	Adelphocoris	All (total)	$22.5 \pm 3.1$	$31.8 \pm 7.0$	$23.4 \pm 3.8$	$F_{2,10} = 0.51$ ; ns
	lineolatus	Adults	$10.9 \pm 1.4$	$12.2 \pm 2.5$	$8.4 \pm 1.2$	$F_{2,10} = 0.55$ ; ns
		Nymphs	$11.5 \pm 2.3$	$19.6 \pm 4.6$	$15.0 \pm 3.3$	$F_{2,10} = 1.41$ ; ns
	Acyrthosiphon pisum	Nymphs and adults	$143.1 \pm 29.8$	79.3 ± 14.1	94.4 ± 13.9	$F_{2,10} = 0.68$ ; ns
	Cicadellids <sup>1</sup>	Nymphs and adults	64.3 ± 17.1	$30.3 \pm 9.4$	$14.0 \pm 0.6$	$F_{2,2} = 4.55$ ; ns
Pitfall trap (number/	Acridids	Nymphs and adults	$0.5 \pm 0.2$	$0.1 \pm 0.0$	$0.1 \pm 0.0$	$F_{2,10} = 2.23$ ; ns
(number/ trap/week)	Gryllus spp.	Nymphs and adults	42.9 ± 16.4	$4.2 \pm 2.9$	3.2 ± 1.1	$F_{2,10} = 4.70; P < 0.05$
Leaflets mined (%)	Agromyza spp. <sup>1</sup>	-	9.0 ± 5.0	$6.7 \pm 3.4$	$2.6 \pm 1.2$	$F_{2,2} = 0.95$ ; ns
Infested seeds (%)	Bruchophagus roddi <sup>1</sup>	-	?	$2.0 \pm 0.2$	1.9 ± 0.3	

Table 3.2.4. Number per 30 sweeps or per trap/week (mean  $\pm$  SE) of various insect pests, and percentage (mean  $\pm$  SE) of middle-leaflets mined and alfalfa seed chalcid infested seeds in alfalfa fields in Manitoba, 1999–2001.

<sup>1</sup> Data for these insects were collected in 1999 only.

Means followed by the same letter do not differ (Tukey's test, experiment-wise alpha  $\leq 0.05$ ).

? indicates no examination was made.

			Areas with	in the field	
Samples (units)	Insects	Insect stages	Edge	Middle	Statistics
Sweep net	<i>Lygus</i> spp.	All (total)	$16.4 \pm 2.6$	$16.0 \pm 2.0$	$F_{1,15} = 0.13$ ; ns
(number/30 sweeps)		Adults	$10.3 \pm 1.8$	$9.7 \pm 1.3$	$F_{1,15} = 0.40$ ; ns
		Nymphs	$6.1 \pm 1.0$	$6.3 \pm 1.0$	$F_{1,15} = 0.04$ ; ns
	Adelphocoris lineolatus	All (total)	$28.6 \pm 4.4$	$23.2 \pm 3.5$	$F_{1, 15} = 4.76; P < 0.05$
	-	Adults	$11.8 \pm 1.6$	$9.2 \pm 1.3$	$F_{1,15} = 8.68; P < 0.01$
		Nymphs	$16.7 \pm 3.1$	$14.0 \pm 2.8$	$F_{1,15} = 1.76$ ; ns
	Acyrthosiphon pisum	Nymphs and adults	$105.8 \pm 17.1$	$105.5\pm18.6$	$F_{1,15} = 0.05$ ; ns
	Cicadellids	Nymphs and adults	$39.3 \pm 13.9$	$33.1 \pm 11.5$	$F_{1,3} = 14.01; P < 0.05$
Pitfall trap	Acridids	Nymphs and adults	$0.3 \pm 0.2$	$0.2 \pm 0.1$	$F_{1,15} = 2.73$ ; ns
(number/trap/week)	Gryllus spp.	Nymphs and adults	$17.6\pm8.1$	$15.9\pm9.7$	$F_{1,15} = 3.86$ ; ns
Leaflets mined (%)	Agromyza spp.	-	$6.4 \pm 3.2$	5.8 ± 2.8	$F_{1,3} = 1.53$ ; ns

Table 3.2.5. Number per 30 sweeps or per trap/week (mean  $\pm$  SE) of selected insect pests, and percentage (mean  $\pm$  SE) of middle-leaflets mined in field- edges and middles of alfalfa fields in Manitoba, 1999–2001.

Table 3.2.6. Descriptive statistics indicating the relationship between the population growth of total *Lygus* bugs in relation to cumulative degree-days (CDD) and the effect on this relationship of numbers of different predaceous arthropods.

Sources of variability	Coefficients / slopes (SE)	SS	df	F(df)	P <
Constant	0.247230				
CDD	0.002659 (0.000100)	40.35	1	706.68 (1,248)	0.01
Fields	-	5.64	17	5.82 (17,248)	0.01
CDD * Fields	-	10.30	17	10.61 (17,248)	
CDD * Carabids	0.000576 (0.000112)	2.00	1	9.27 (1,12)	0.01
CDD * Chrysopids	0.001068 (0.000320)	0.84	1	3.89 (1,12)	0.10
CDD * Coccinellids	- 0.000825 (0.000240)	0.90	1	4.17 (1,12)	0.10
CDD * Nabis spp.	0.001293 (0.000206)	2.99	1	13.65 (1,12)	0.01
CDD * Spiders	0.000895 (0.000249)	0.98	1	4.54 (1,12)	0.10
Residuals		2.59	12	3.78 (12,248)	0.01
Error		14.16	248		

Table 3.2.7. Descriptive statistics indicating the relationship between the population growth of *Lygus* bug adults in relation to cumulative degree-days (CDD) and the effect on this relationship of numbers of different predaceous arthropods.

Sources of variability	Coefficients / slopes (SE)	SS	df	F(df)	<i>P</i> <
Constant	0.226550				<u></u>
CDD	0.002597 (0.000107)	38.49	1	586.61 (1,248)	0.01
Fields	-	3.34	17	2.99 (17,248)	0.01
CDD * Fields	-	8.51	17	7.63 (17,248)	0.01
CDD * Carabids	0.000712 (0.000109)	3.12	1	26.44 (1,13)	0.01
CDD * Chrysopids	0.000614 (0.000312)	0.28	1	2.37 (1,13)	0.15
CDD * Nabis spp.	0.001132 (0.000187)	2.68	1	22.71 (1,13)	0.01
CDD * Spiders	0.000841 (0.000241)	0.90	1	7.63 (1,13)	0.05
Residuals		1.53	13	1.80 (13,248)	0.05
Error		16.27	248		

Table 3.2.8. Descriptive statistics indicating the relationship between the population growth of *Lygus* bug nymphs in relation to cumulative degree-days (CDD) and the effect on this relationship of numbers of different predaceous arthropods.

Sources of variability	Coefficients / slopes (SE)	SS	df	F (df)	<i>P</i> <
Constant	0.157555				
CDD	0.001738 (0.000109)	17.24	1	254.53 (1,248)	0.01
Fields	-	6.80	17	5.91 (17,248)	0.01
CDD * Fields	-	11.83	17	10.27 (17,248)	0.01
CDD * Chrysopids Residuals	0.001725 (0.000252)	1.77 11.06	1 16	2.56 (1,16) 10.21 (16,248)	0.15 0.01
Error		16.79	248		

Samples		First hay-	Adjacent s	eed field	Non-adjace	ent seed field	
(units)	Insects	cut	Edge	Middle	Edge	Middle	One-tailed test of contrast
Sweep net	Lygus adults	Precut	$1.0 \pm 0.4$	$1.3 \pm 0.5$	$0.8 \pm 0.2$	$0.8 \pm 0.4$	a) $t_5 = 1.97; P \approx 0.05$
(number/30		Post cut	$3.1 \pm 1.5$	$2.1 \pm 1.0$	$1.1 \pm 0.4$	$1.2 \pm 0.6$	b) $t_5 = 2.47$ ; $P < 0.05$
sweeps)	Lygus nymphs	Precut	$1.3 \pm 0.5$	$1.4 \pm 0.6$	$1.5 \pm 0.9$	$0.7 \pm 0.4$	a) $t_{,5} = 0.24$ ; ns
		Post cut	$1.8 \pm 1.0$	$1.7 \pm 0.8$	$1.7 \pm 0.9$	$0.8 \pm 0.4$	b) $t_5 = 0.28$ ; ns
	Adelphocoris	Precut	$0.8 \pm 0.5$	$2.5 \pm 1.9$	$0.9 \pm 0.7$	$0.8 \pm 0.6$	a) $t_5 = 1.02$ ; ns
	lineolatus adults	Post cut	$11.2 \pm 8.2$	$4.8 \pm 2.6$	$3.6 \pm 1.8$	$2.5 \pm 1.2$	b) $t_5 = 0.39$ ; ns
	Adelphocoris	Precut	$20.8 \pm 16.7$	$11.4 \pm 5.7$	$10.6 \pm 4.6$	$4.8 \pm 2.1$	a) $t_5 = 0.22$ ; ns
	lineolatus nymphs	Post cut	$4.4 \pm 3.1$	$5.0 \pm 3.5$	$2.2 \pm 1.4$	$1.2 \pm 0.6$	b) $t_5 = 0.17$ ; ns
	Acyrthosiphon	Precut	$20.0 \pm 11.4$	$12.1 \pm 5.5$	$15.2 \pm 8.2$	$12.0 \pm 9.2$	a) $t_{,5} = 1.15$ ; ns
	pisum	Post cut	$11.9 \pm 6.4$	$10.7 \pm 5.9$	$54.7\pm50.2$	$13.2 \pm 9.6$	b) $t_5 = 0.79$ ; ns
Pitfall trap	Acridids	Precut	0	0	0	0	Insufficient variability to analyze
(number/trap		Post cut	0	$0.1 \pm 0.1$	0	0	
/week)	Gryllus spp.	Precut	$0.1 \pm 0.1$	0	$1.4 \pm 1.4$	$0.3 \pm 0.2$	Insufficient variability to analyze
		Post cut	0	0	$4.3 \pm 4.3$	$1.4 \pm 1.4$	

Table 3.2.9. Pre- and post-cut (first cut) numbers per 30 sweep or per trap/week (mean ± SE) of various insect pests in field-edges and - middles of alfalfa fields in Manitoba, 1999–2001.

a) Test statistics for post-cut increases of numbers at edges of the adjacent seed fields compared with those at middles of the adjacent seed fields, and edge and middles of the non-adjacent seed fields.

b) Test statistics for post-cut increases of numbers in the adjacent seed fields compared with those in the non-adjacent seed fields.

Probability values are based on a one-tailed t-test.

•••••••		Second	Adjacent	seed field	Non-adjacen	t seed field		
Samples (units)	Insects	hay-cut	Edge	Middle	Edge	Middle	One-tailed test of contrast	
Sweep net	Lygus adults	Precut	$16.8 \pm 6.4$	$14.5 \pm 4.8$	$10.2 \pm 2.6$	$9.0 \pm 2.4$	a) $t_5 = 0$ ; ns	
(number/30	•••	Post cut	$24.2 \pm 7.6$	$17.8 \pm 2.9$	$15.2 \pm 4.7$	$19.3 \pm 4.5$	b) $t_5 = 0$ ; ns	
sweeps)	Lygus nymphs	Precut	$12.4 \pm 5.0$	$9.3 \pm 3.3$	$9.3 \pm 2.8$	$6.2 \pm 1.4$	a) $t_5 = 0.51$ ; ns	
		Post cut	$33.3 \pm 11.6$	$29.0 \pm 10.5$	$18.8 \pm 6.9$	$21.5 \pm 8.0$	b) $t_5 = 0.36$ ; ns	
	Adelphocoris	Precut	$23.1 \pm 7.2$	$17.3 \pm 6.3$	$16.2 \pm 5.4$	$9.3 \pm 2.2$	a) $t_5 = 0.20$ ; ns	
	lineolatus adults	Post cut	$32.1 \pm 12.6$	$25.6 \pm 12.2$	$19.4 \pm 3.1$	$14.0 \pm 3.4$	b) $t_5 = 0.20$ ; ns	
	Adelphocoris	Precut	$27.8 \pm 11.4$	$23.5 \pm 12.2$	$15.8 \pm 4.2$	$8.3 \pm 3.2$	a) $t_5 = 0.24$ ; ns	
	lineolatus nymphs	Post cut	$53.3 \pm 22.1$	$37.8 \pm 13.0$	$27.1 \pm 10.5$	$17.4 \pm 7.1$	b) $t_5 = 0.65$ ; ns	
	Acyrthosiphon	Precut	279.3 ± 191.3	$249.5 \pm 169.9$	$128.9 \pm 68.3$	$93.4 \pm 56.2$	a) $t_5 = 0.86$ ; ns	
	pisum	Post cut	$63.0\pm20.3$	$55.4 \pm 17.2$	$29.9 \pm 12.2$	$31.0 \pm 9.7$	a) $t_5 = 0.42$ ; ns	
Pitfall trap	Acridids	Precut	$0.3 \pm 0.2$	$0.1 \pm 0.1$	0	$0.1 \pm 0.1$	Insufficient variability to	
(number/trap		Post cut	$0.2 \pm 0.2$	$0.2 \pm 0.1$	$0.4 \pm 0.2$	$0.4 \pm 0.3$	analyze	
/week) *	Gryllus spp.	Precut	$3.5 \pm 2.1$	$0.6 \pm 0.3$	$1.6 \pm 0.8$	$4.6 \pm 3.0$	a) $t_4 = 0$ ; ns	
		Post cut	$29.8 \pm 26.9$	$1.7 \pm 1.2$	$22.6 \pm 12.6$	$13.0 \pm 9.5$	b) $t_4 = 1.76$ ; ns	

Table 3.2.10. Pre- and post-cut (second cut) numbers per 30 sweeps or per trap/week (mean ± SE) of various insect pests in field-edges and -middles of alfalfa fields in Manitoba, 1999–2001.

\* Data from one field is missing to compare before and after effect, consequently the error degree of freedom has changed.

a) Test statistics for post-cut increases of numbers at edges of the adjacent seed fields compared with those at middles of the adjacent seed fields, and edge and middles of the non-adjacent seed fields.

b) Test statistics for post-cut increases of numbers in the adjacent seed fields compared with those in the non-adjacent seed fields.

One-tailed t-test was used for determining any effects to be significant.

Table 3.2.11. Descriptive statistics indicating the relationship between the population growth of total *Adelphocoris lineolatus* bugs in relation to cumulative degree-days (CDD) and the effect on this relationship of numbers of different predaceous arthropods.

Sources of variability	Coefficients / slopes (SE)	SS	df	F(df)	P <
Constant	0.408213				
CDD	0.002763 (0.000154)	20.17	1	320.34 (1,196)	0.01
Fields	-	13.68	17	12.78 (17,196)	0.01
CDD * Fields	-	7.43	17	6.94 (17,196)	0.01
CDD * Chrysopids	0.001347 (0.000383)	1.20	1	10.99 (1,13)	0.01
CDD * Nabis spp.	0.001138 (0.000209)	2.87	1	26.27 (1,13)	0.01
CDD * Opilionids	0.000808 (0.000194)	1.67	1	15.29 (1,13)	0.01
CDD * Spiders	0.000464 (0.000275)	0.27	1	2.47 (1,13)	0.15
Residuals		1.42	13	3.32 (13,196)	0.01
Error		12.34	196		

Table 3.2.12. Descriptive statistics indicating the relationship between the population growth of *Adelphocoris lineolatus* adults in relation to cumulative degree-days (CDD) and the effect on this relationship of numbers of different predaceous arthropods.

Sources of variability	Coefficients / slopes (SE)	SS	df	F(df)	<i>P</i> <
Constant	0.283623				
CDD	0.002483 (0.000147)	16.29	1	284.74 (1,196)	0.01
Fields	-	8.87	17	9.12 (17,196)	0.01
CDD * Fields	-	6.44	17	6.63 (17,196)	0.01
CDD * Carabids	0.000364 (0.000159)	0.53	1	3.08 (1,14)	0.10
CDD * Nabis spp.	0.000751 (0.000177)	1.72	1	9.99 (1,14)	0.01
CDD * Opilionids	0.000411 (0.000188)	1.78	1	10.34 (1,14)	0.01
Residuals		2.41	14	3.01 (14,196)	0.01
Error		11.21	196		

Table 3.2.13. Descriptive statistics indicating the relationship between the population growth of *Adelphocoris lineolatus* nymphs in relation to cumulative degree-days (CDD) and the effect on this relationship of numbers of different predaceous arthropods.

Sources of variability	Coefficients / slopes (SE)	SS	df	F(df)	P <
Constant	0.046437				
CDD	0.002450 (0.000201)	15.86	1	147.90 (1,196)	0.01
Fields	-	18.05	17	9.90 (17,196)	0.01
CDD * Fields	-	9.51	17	5.22 (17,196)	0.01
CDD * Chrysopids	0.001778 (0.000461)	1.91	1	10.91 (1,12)	0.01
CDD * Coccinellids	- 0.000809 (0.000376)	0.59	1	3.37 (1,12)	ns
CDD * Nabis spp.	0.001618 (0.000292)	3.95	1	22.57 (1,12)	0.01
CDD * Orius spp.	0.000619 (0.000339)	0.43	1	2.46 (1,12)	0.15
CDD * Spiders	0.000665 (0.000326)	0.53	1	3.03 (1,12)	0.15
Residuals		2.10	12	1.63 (12,196)	0.15
Error		21.01	196		

ns = not significant at alpha  $\leq 0.05$ .

Table 3.2.14. Descriptive statistics indicating the relationship between the population growth of total *Acyrthosiphon pisum* in relation to cumulative degreedays (CDD) and the effect on this relationship of numbers of different predaceous arthropods.

Sources of variability	Coefficients / slopes (SE)	SS	df	F(df)	<i>P</i> <
Constant	0.113				
CDD	0.005704 (0.000302)	42.24	1	357.28 (1,133)	0.01
Fields	-	13.53	17	6.73 (17,133)	0.01
CDD * Fields	-	6.76	17	3.36 (17,133)	0.01
CDD * <i>Adelphocoris</i> <i>lineolatus</i> adults	0.001634 (0.000398)	1.64	1	6.03 (1,15)	0.05
CDD * <i>Lygus</i> spp. nymphs	0.001076 (0.000311)	1.04	1	3.82 (1,15)	0.15
Residuals		4.08	15	2.30 (15,133)	0.01
Error		15.72	133		

		Natural enemies	Near 1	Dugald	Near Teulon		
Order	Family	Species	Common name	Hay	Seed	Hay	Seed
Coleoptera	Carabidae	Various	Ground beetle	+	+	+	+
•	Coccinellidae	Coccinella septempunctata Linn.	Ladybird beetle	+	+	+	+
		Hippodamia tredecimpunctata Say	Ladybird beetle	+	+	+	+
Diptera	Asilidae	Unidentified	Robber fly	+	+	+	+
	Syrphidae	Unidentified	Hover fly	+	+	+	+
Hemiptera	Anthocoridae	Orius spp.	Minute pirate bug	+	+	+	+
•	Nabidae	Nabis spp.	Damsel bug	+	+	+	+
	Pentatomidae	Various	Stink bug	+	+	+	+
	Reduvidae	Unidentified	Assassin bug	+	+	+	+
Neuroptera	Chrysopidae	Chrysoperla carnea (Stephens)	Green lacewing	+	+	+	+
-		Chrysopa oculata Say	Green lacewing <sup>1</sup>	?	?	?	+
Araneae	Various	Unidentified	Spider	+	+	+	+
Opiliones	Various	Unidentified	Harvestmen	+	+	+	+
Hymenoptera	Aphidiidae	Aphidius ervi Haliday	-	+	+	+	+
		Aphidius pisivorous Smith	-	+	+	+	+
		Aphidius smithi Sharma & Subba Rao <sup>1</sup>	-	+	+	?	?
		Aphidius spp.	-	+	+	+	+
		Praon pequodorum Viereck	-	+	+	+	+
		Praon occidentale Baker	-	+	+	+	+
	Braconidae	Peristenus sp.*	-	+	+	+	+

Table 3.2.15. Presence of important insect natural enemies in alfalfa fields in Manitoba, 1999.

<sup>1</sup>Found in a later trial in 2001 (Chapter 3.5). ? indicates no examination was made. <sup>•</sup>Dissection of *Lygus* spp. nymphs revealed the presence of parasitoids, which is assumed to be caused by *Peristenus* sp. In addition, the aphid parasitoid, *Aphidius pulcher* Baker was found in Riverton in 2001.

Samples (units)	Arthropods	Hay	Adjacent seed	Non-adjacent seed	Statistics
Sweep net	Coccinellids	$1.4 \pm 0.2$	$2.1 \pm 0.4$	$1.9 \pm 0.4$	$F_{2,10} = 0.63$ ; ns
(number/30 sweeps)	Syrphids	$1.0 \pm 0.2$	$4.2 \pm 1.2$	$4.2 \pm 1.3$	$F_{2,10} = 3.54$ ; ns
(1141110 411 0 0 0 0 0 0 0 0 0 0 0 0 0 0	Orius spp.	$0.5 \pm 0.1$	$4.0 \pm 0.9$	$2.2 \pm 0.5$	$F_{2,10} = 4.21; P < 0.04$
	Nabis spp.	$3.3 \pm 1.0$	$1.5 \pm 0.6$	$1.0 \pm 0.3$	$F_{2,10} = 4.22; P < 0.05$
	Pentatomids <sup>1</sup>	$0.2 \pm 0.0$	$0.8 \pm 0.1$	$0.4 \pm 0.0$	$F_{2,2} = 24.05; P < 0.05$
	Chrysopids	$0.3 \pm 0.0^{b}$	$1.5 \pm 0.2^{a}$	$1.2 \pm 0.2^{\text{a}}$	$F_{2,10} = 13.41; P < 0.01$
	Spiders	$2.1 \pm 0.4$	$1.8 \pm 0.3$	$2.2 \pm 0.4$	$F_{2,10} = 0.64$ ; ns
Pitfall trap	Spiders	$4.1 \pm 0.6$	$3.6 \pm 0.5$	$4.1 \pm 0.7$	$F_{2,10} = 0.34$ ; ns
(number/trap/week)	Opilionids	$21.9 \pm 3.3^{a}$	$3.2 \pm 0.6^{b}$	$20.3 \pm 12.2$ <sup>ab</sup>	$F_{2,10} = 5.41; P < 0.02$

Table 3.2.16. Number per 30 sweeps or per trap/week (mean ± SE) of various natural enemy arthropods in alfalfa fields in Manitoba, 1999–2001.

<sup>1</sup> Data for this taxon were collected in 1999 only. Means within a row followed by the same letter do not differ (Tukey's test, experiment-wise alpha  $\leq 0.05$ ).

		Areas wit	hin the field	
Samples (units)	Arthropods	Edge	Middle	Statistics
Sweep net	Coccinellids	$1.6 \pm 0.3$	$1.9 \pm 0.3$	$F_{1,15} = 3.63$ ; ns
(number/30 sweeps)	Syrphids	$3.4 \pm 1.0$	$2.9 \pm 0.8$	$F_{1,15} = 2.54$ ; ns
	Orius spp.	$2.2 \pm 0.5$	$2.3 \pm 0.7$	$F_{1,15} = 0.12$ ; ns
	Nabis spp.	$1.6 \pm 0.4$	$2.3 \pm 0.7$	$F_{1,15} = 6.04; P < 0.05$
	Pentatomids <sup>1</sup>	$0.3 \pm 0.1$	$0.4 \pm 0.1$	$F_{1,3} = 30.00; P < 0.05$
	Chrysopids	$1.0 \pm 0.1$	$1.0 \pm 0.2$	$F_{1,15} = 0.18$ ; ns
	Spiders	$2.1 \pm 0.2$	$1.9 \pm 0.2$	$F_{1,15} = 3.01$ ; ns
Pitfall trap	Spiders	$3.7 \pm 0.4$	$4.1 \pm 0.6$	$F_{1,15} = 0.86$ ; ns
(number/trap/week)	Opilionids	$13.6 \pm 4.5$	$16.7 \pm 7.6$	$F_{1,15} = 6.19; P < 0.05$

Table 3.2.17. Number per 30 sweeps or per trap/week (mean ± SE) of various natural enemy arthropods in field- edges and middles of alfalfa fields in Manitoba, 1999–2001.

<sup>1</sup> Data for this taxon were collected in 1999 only.

Samples		First	Adjacent	seed field	Non-adjace	nt seed field	One-tailed test of contrast
(units)	Arthropods	hay-cut	Edge	Middle	Edge	Middle	
Sweep net	Coccinellids	Precut	$0.4 \pm 0.3$	$1.1 \pm 0.9$	$0.3 \pm 0.2$	$0.9 \pm 0.8$	a) $t_{1,5} = 0.56$ ; ns
(number/30	00000	Post cut	0	0	$0.1 \pm 0.1$	$0.1 \pm 0.1$	b) $t_{1,5} = 1.81$ ; ns
sweeps)	Orius spp.	Precut	$1.1 \pm 0.9$	$0.4 \pm 0.4$	$0.1 \pm 0.1$	$0.2 \pm 0.2$	a) $t_{1,5} = 0.41$ ; ns
5 <b>.</b>	or no opp	Post cut	$3.1 \pm 1.4$	$2.0 \pm 1.1$	$1.3 \pm 0.7$	$1.5 \pm 1.0$	b) $t_{1,5} = 0.37$ ; ns
	Nabis spp.	Precut	$0.2 \pm 0.2$	$0.1 \pm 0.1$	$0.2 \pm 0.2$	$0.3 \pm 0.2$	a) $t_{1,5} = 0.32$ ; ns
	THOSE OPP.	Post cut	$0.1 \pm 0.1$	$0.1 \pm 0.1$	0	$0.3 \pm 0.1$	b) $t_{1,5} = 0.14$ ; ns
	Chrysopids	Precut	$0.4 \pm 0.2$	$0.6 \pm 0.4$	$0.1 \pm 0.1$	0	Insufficient variability to
		Post cut	$0.1 \pm 0.1$	$0.2 \pm 0.1$	$0.1 \pm 0.1$	$0.2 \pm 0.1$	analyze
	Spiders	Precut	$1.7 \pm 0.6$	$1.4 \pm 0.6$	$1.5 \pm 0.5$	$1.5 \pm 0.8$	a) $t_{1,5} = 1.14$ ; ns
	Spiders	Post cut	$0.9 \pm 0.3$	$1.1 \pm 0.5$	$1.2 \pm 0.3$	$1.6 \pm 0.5$	b) $t_{1,5} = 0.62$ ; ns
Ditfall tran	Spiders	Precut	$2.8 \pm 1.4$	$5.0 \pm 2.7$	$5.6 \pm 2.8$	$3.0 \pm 1.4$	a) $t_{1,4} = 1.01$ ; ns
Pitfall trap (number/trap	Spiders	Post cut	$2.9 \pm 0.8$	$4.5 \pm 1.6$	$6.8 \pm 3.7$	$2.2 \pm 0.5$	b) $t_{1,4} = 0.39$ ; ns
/week) *	Opilionids	Precut	$1.6 \pm 0.9$	$0.3 \pm 0.2$	$4.8 \pm 2.7$	$4.3 \pm 2.0$	a) $t_{1,4} = 0$ ; ns
· ···,	Opmonius	Post cut	$2.8 \pm 1.9$	$6.1 \pm 0.5$	9.1 ± 4.5	5.2 ± 3.4	b) $t_{1,4} = 0.14$ ; ns

Table 3.2.18. Pre- and post-cut (first cut) numbers (mean ± SE) of various natural enemy arthropods in field-edges and -middles of alfalfa fields in Manitoba, 1999–2001.

\* Data from one field is missing to compare before and after effect, consequently the error degree of freedom has changed.

a) Test statistics for post-cut increases of numbers at edges of the adjacent seed fields compared with those at middles of the adjacent seed fields, and edge and middles of the non-adjacent seed fields.

b) Test statistics for post-cut increases of numbers in the adjacent seed fields compared with those in the non-adjacent seed fields.

One-tailed t-test was used for determining any effects to be significant.

Samples		Second	Adjacent	seed field	Non-adjacen		
(units)	Arthropods	hay-cut	Edge	Middle	Edge	Middle	One-tailed test of contrast
Sweep net	Coccinellids	Precut	$4.4 \pm 1.4$	$5.0 \pm 2.0$	$2.8 \pm 1.0$	$5.1 \pm 1.8$	a) $t_5 = 0.52$ ; ns
(number/30	Cooomennes	Post cut	$9.9 \pm 5.9$	$8.5 \pm 3.4$	$4.4 \pm 1.4$	$6.9 \pm 3.3$	b) $t_5 = 0.73$ ; ns
sweeps)	Orius spp.	Precut	$13.1 \pm 7.4$	$13.9 \pm 8.0$	$5.3 \pm 1.9$	$5.9 \pm 2.4$	a) $t_5 = 2.01; P \approx 0.05$
54 <b>C</b> CP5)	Ormo opp.	Post cut	$17.3 \pm 7.4$	$15.7 \pm 7.6$	$6.9 \pm 2.7$	$8.1 \pm 2.1$	b) $t_5 = 0$ ; ns
	Nabis spp.	Precut	$3.0 \pm 1.3$	$2.7 \pm 1.3$	$0.9 \pm 0.1$	$1.1 \pm 0.5$	a) $t_5 = 0.98$ ; ns
	Theore opp.	Post cut	$5.7 \pm 3.0$	$8.6 \pm 6.2$	$2.4 \pm 1.4$	$5.3 \pm 3.6$	b) $t_5 = 0.58$ ; ns
	Chrysopids	Precut	$1.6 \pm 0.6$	$1.1 \pm 0.4$	$1.4 \pm 0.4$	$1.5 \pm 0.8$	a) $t_5 = 0.91$ ; ns
	Chilybophao	Post cut	$1.9 \pm 0.9$	$1.5 \pm 0.8$	$2.6 \pm 0.8$	$2.0 \pm 1.0$	b) $t_5 = 0.57$ ; ns
	Spider	Precut	$2.3 \pm 0.7$	$2.1 \pm 0.7$	$1.4 \pm 0.3$	$0.9 \pm 0.3$	a) $t_5 = -1.88$ ; ns
	opidei	Post cut	$2.5 \pm 1.1$	$1.9 \pm 0.7$	$2.6 \pm 0.4$	$2.8 \pm 1.0$	b) $t_5 = -2.62$ ; ns
Ditfall tran	Spider	Precut	$2.1 \pm 1.0$	$1.8 \pm 0.2$	$2.1 \pm 0.7$	$2.3 \pm 1.2$	a) $t_4 = -0.76$ ; ns
Pitfall trap (number/	opiuci	Post cut	$1.7 \pm 0.8$	$1.3 \pm 0.3$	$2.4 \pm 0.3$	$3.6 \pm 1.6$	b) $t_4 = -3.86$ ; ns
trap/week) *	Opilionids	Precut	$7.4 \pm 3.0$	$1.5 \pm 0.8$	$22.4 \pm 21.9$	$44.5 \pm 44.5$	a) $t_4 = -1.41$ ; ns
	Opinionius	Post cut	$7.0 \pm 2.1$	$3.4 \pm 0.9$	$19.2 \pm 17.4$	60.4 ± 59.9	b) $t_4 = -0.26$ ; ns

Table 3.2.19. Pre- and post-cut (second cut) numbers (mean ± SE) of various natural enemy arthropods in field-edges and -middles of alfalfa fields in Manitoba, 1999–2001.

\* Data from one field is missing to compare before and after effect, consequently the error degree of freedom has changed.

a) Test statistics for post-cut increases of numbers at edges of the adjacent seed fields compared with those at middles of the adjacent seed fields, and edge and middles of the non-adjacent seed fields.

b) Test statistics for post-cut increases of numbers in the adjacent seed fields compared with those in the non-adjacent seed fields.

Probability values are based on a one-tailed t-test.

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a An the second second

					spray er/30 sweeps)		Second spray (mean number/30 sweeps)				
Localities	Years	- Fields	Mirids *	Suggested threshold	Acyrthosiphon pisum	Suggested threshold	Mirids *	Suggested threshold	Acyrthosiphon pisum	Suggested threshold	
Dugald	1999	Seed	17.0	150.0 (Soroka	7.0	3000-	84.0	Early season:	16.0	30006000	
0		Seed <sup>1</sup>	11.0	1991; Soroka and Murrell	18.0	6000 (Schaber	154.0	150.0 (Soroka 1991; Soroka	19.0	(Schaber 1992)	
Teulon	1999	Seed	13.0	1993)	19.0	1992)	146.0	and Murrell	12.0		
i curon		Seed <sup>1</sup>	9.0		60.0		197.0	1993)	8.0		
Arborg	2000	Seed	22.0		40.0		-	Late season: 360 – 480	-		
Ū.		Seed <sup>1</sup>	83.0		57.0		-	(Harris 1992, cited in Soroka	-		
	2001	Seed	136.0		4.0		-	and Murrell	-		
		Seed <sup>1</sup>	146.0		55.0		-	1993)	-		
Riverton	2000	Seed	-		-		59.0		2.0		
		Seed <sup>1</sup>	-		-		65.0		126.0		
	2001	Seed	119.0		85.0		72.0		29.0		
		Seed <sup>1</sup>	45.0		26.0				20.0		
							165.0				

Table 3.2.20. Insect density at which alfalfa seed fields were sprayed with insecticides in Manitoba, 1999–2001.

Samples were taken 1–8 days prior to insecticide sprays unless otherwise mentioned. \* Mirids include *Lygus* and *Adelphocoris lineolatus* pooled together. <sup>1</sup> Seed field adjacent to the hay field.

Fig. 3.2.1. Localities in Manitoba where the studies were undertaken.

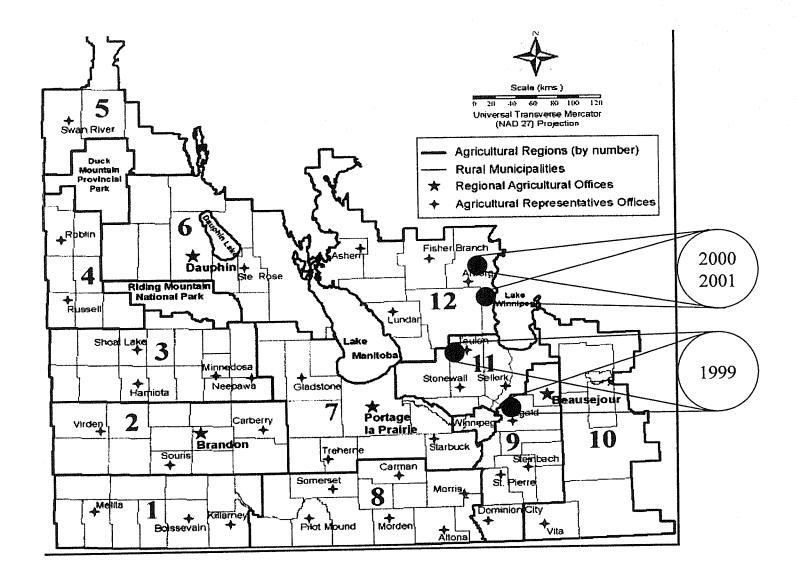
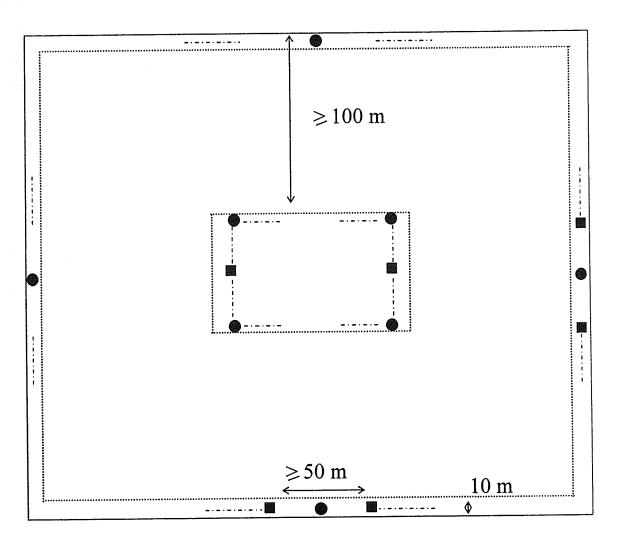


Fig. 3.2.2. Layout of the sampling plan in fields of alfalfa.



Sampling sites (1999) = Sampling sites (2000 and 2001)
 Sweeping tracks (Sampling spots)

Fig. 3.2.3. A pitfall trap and its cover.

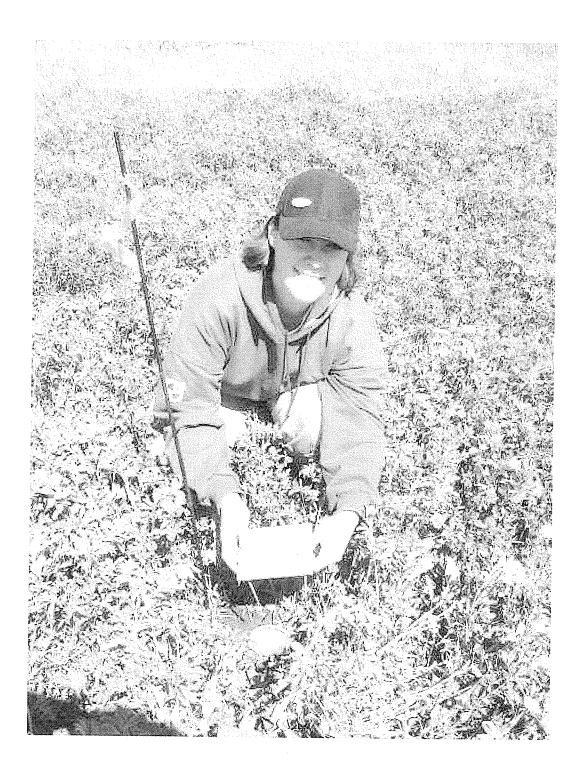


Fig. 3.2.4. Adults and nymphs, and estimated percent parasitism of *Lygus* spp. collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates hay crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

\_\_\_\_ Adults; \_\_\_\_ Nymphs; % Parasitism.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

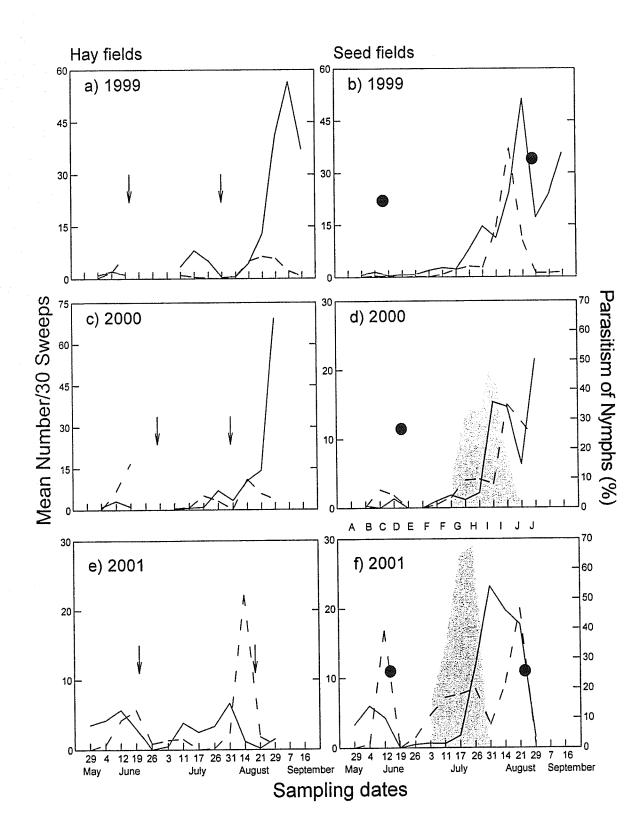


Fig. 3.2.5. Adults and nymphs of *Adelphocoris lineolatus* collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates hay crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

—— Adults; ––– Nymphs.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

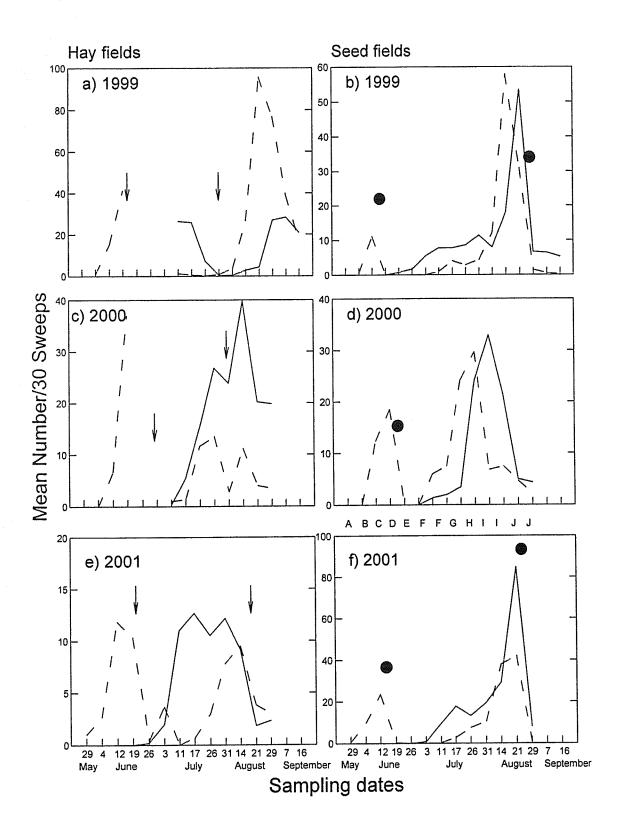


Fig. 3.2.6. Number and estimated percent parasitism of *Acyrthosiphon pisum* collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

—— Number; % Parasitism.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

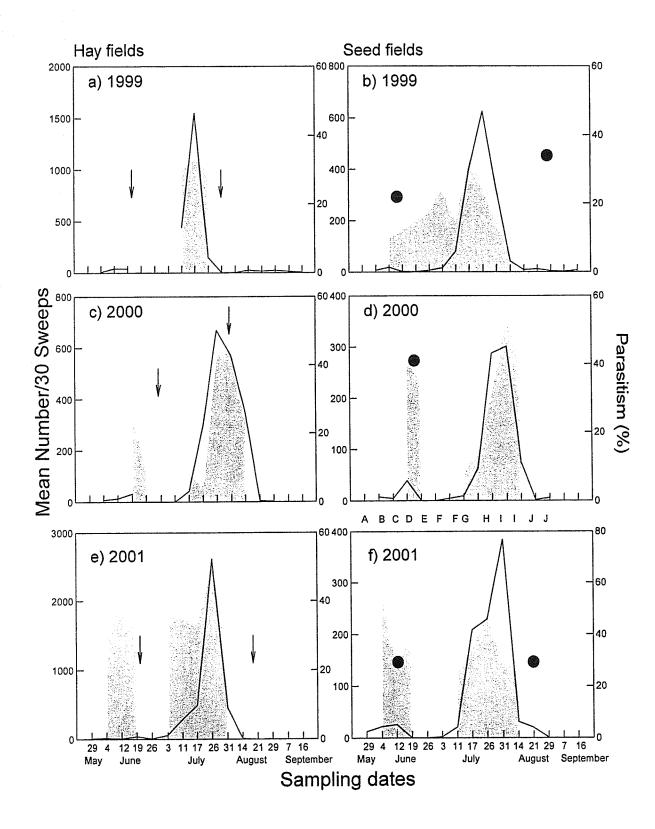


Fig. 3.2.7. Number of embryos found in field-collected *Acyrthosiphon pisum* adults, when they were dissected under a microscope in the laboratory.

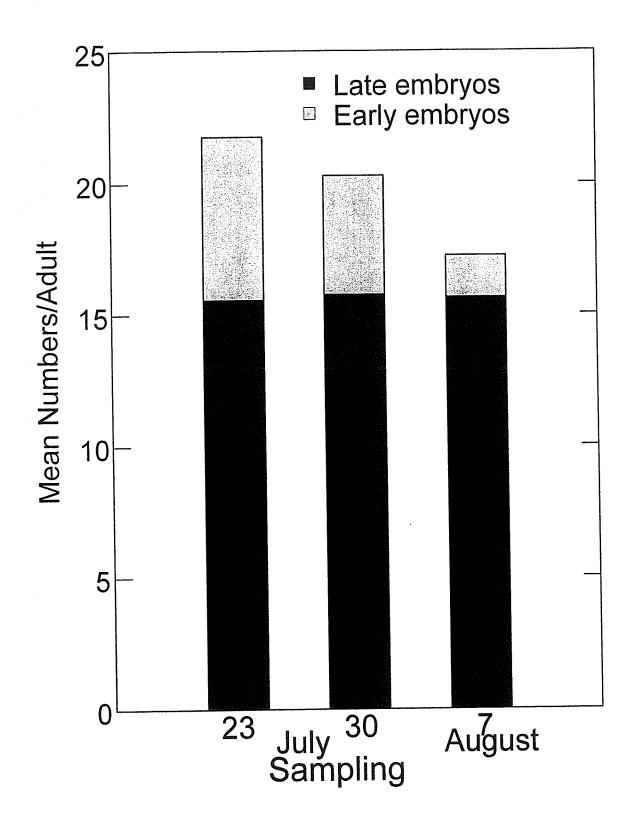


Fig. 3.2.8. Seasonal patterns of the populations of *Acyrthosiphon pisum* and common predaceous natural enemies in alfalfa fields near different localities in Manitoba during the crop growing seasons of 1999–2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

--- Acyrthosiphon pisum; ---- Predators

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

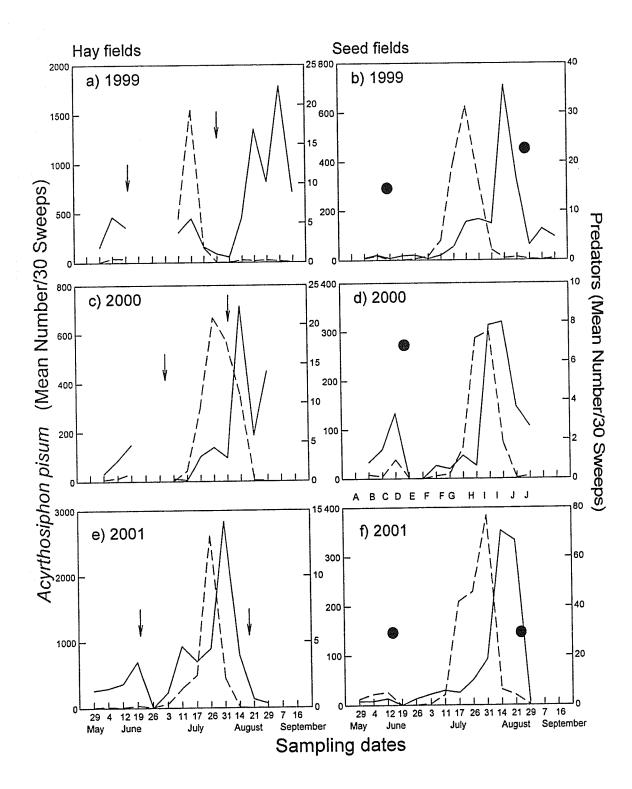


Fig. 3.2.9. Number of *Gryllus* spp. collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Note that the non-adjacent seed field near Arborg is repleaced by the non-adjacent seed field near Riverton (panel d), because catches were seldomly made in the non-adjacent seed field near Arborg.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

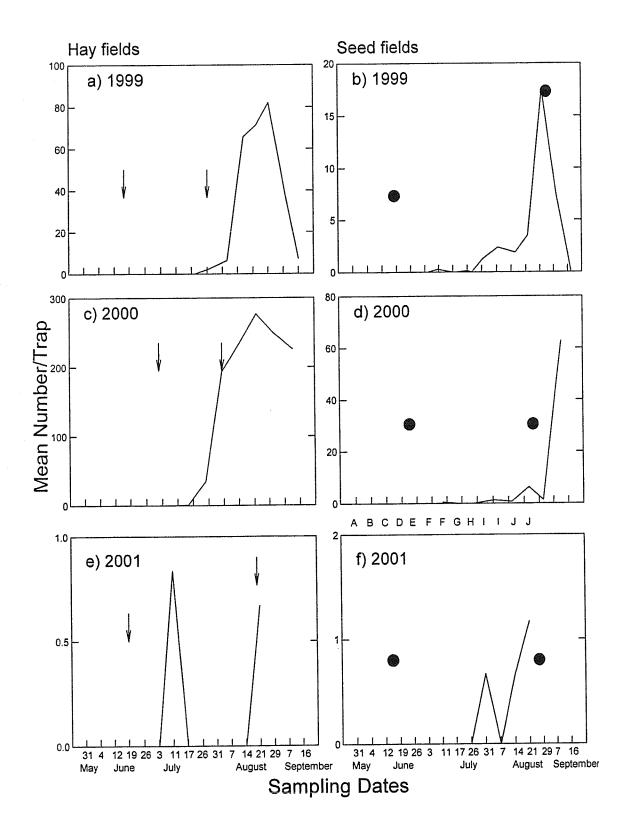


Fig. 3.2.10. Number of cicadellids collected in alfalfa fields near different localities in Manitoba during the crop season of 1999. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales, and no numerical records were kept until late July or early August.

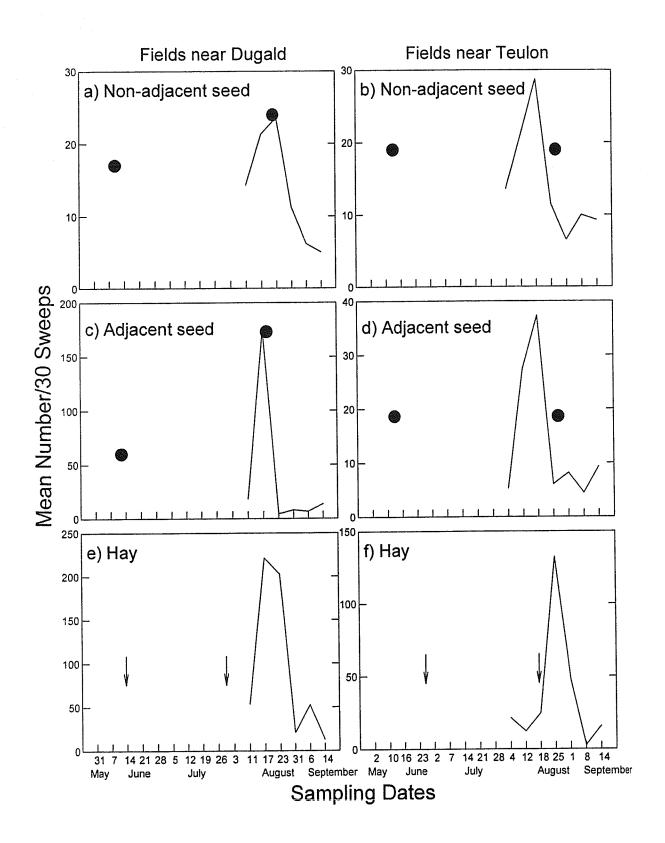


Fig. 3.2.11. Percentage of middle leaflets mined in alfalfa fields near different localities in Manitoba during the crop season of 1999. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines in b, c, d and e panels indicates that no samples were collected on corresponding dates.

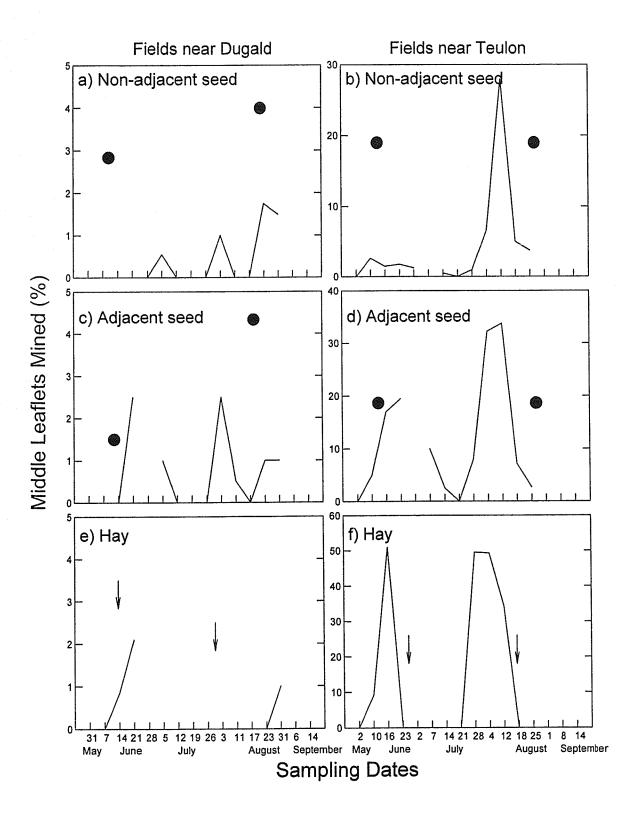


Fig. 3.2.12. Adults and larvae of coccinellids collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates. Numbers in 1999 represent adults and larvae together; For numbers in 2000 and 2001:

—— Adults; --- Larvae.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late)

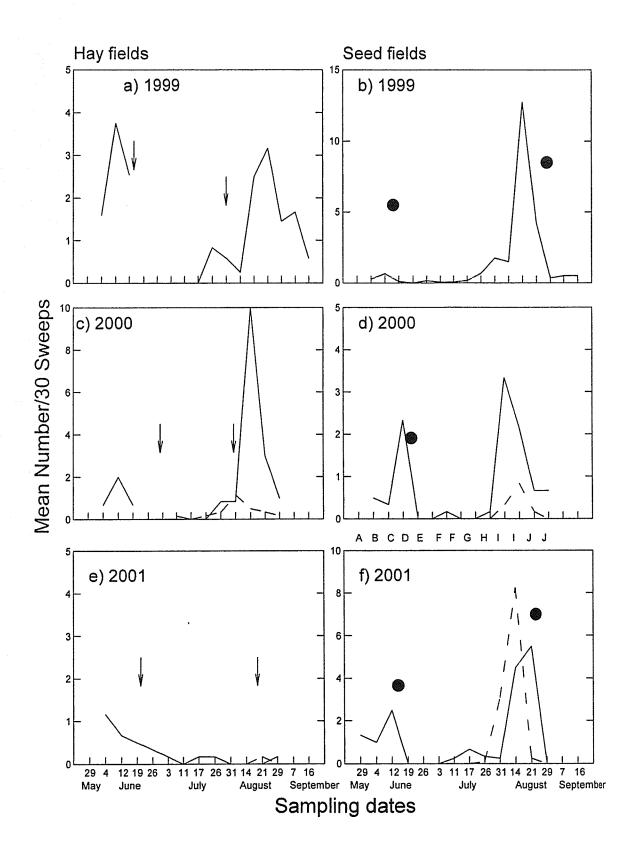


Fig. 3.2.13. Number of Syrphid flies collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

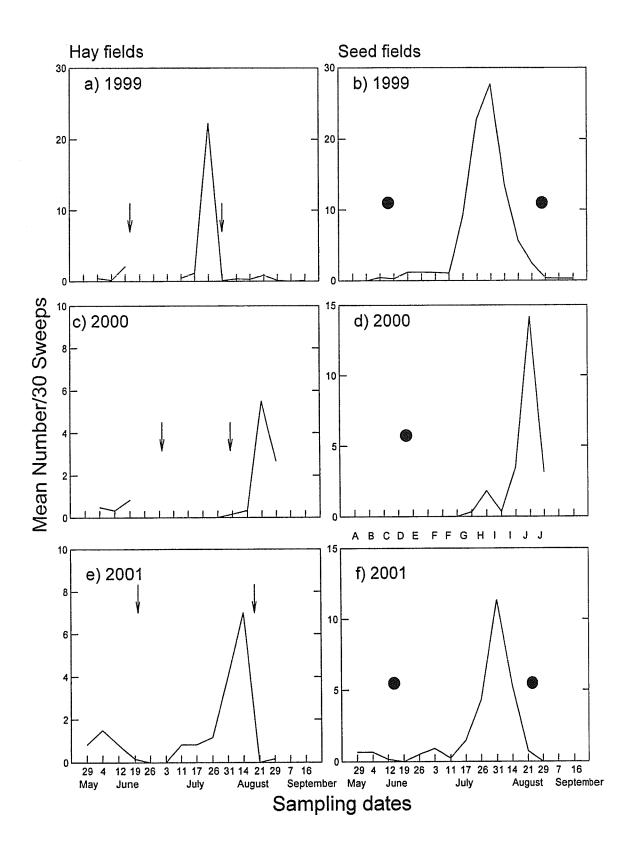


Fig. 3.2.14. Number of *Orius* spp. collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that scale on the vertical axis of panel f is different from those of the other panels. Discontinuation of lines indicates that no samples were collected on corresponding dates.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

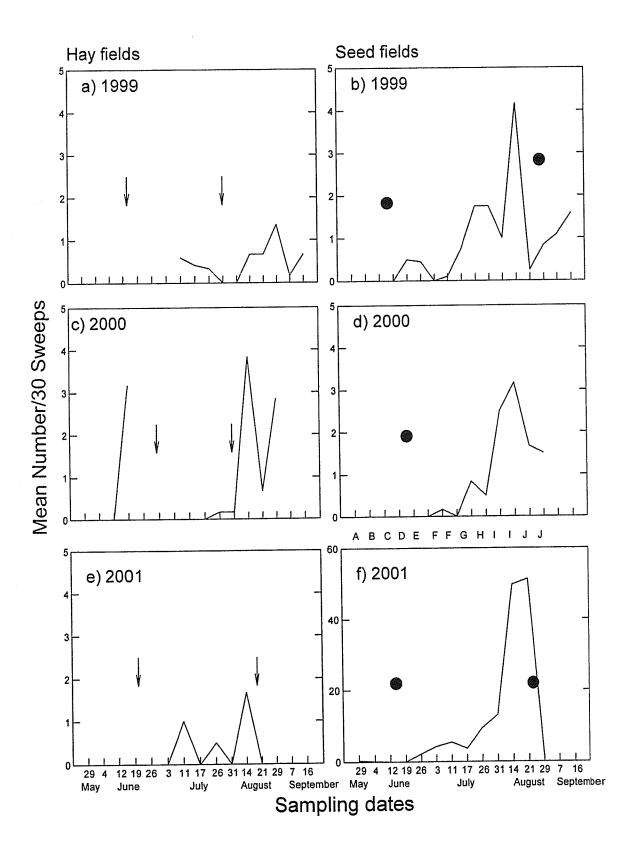


Fig. 3.2.15. Number of *Nabis* spp. collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

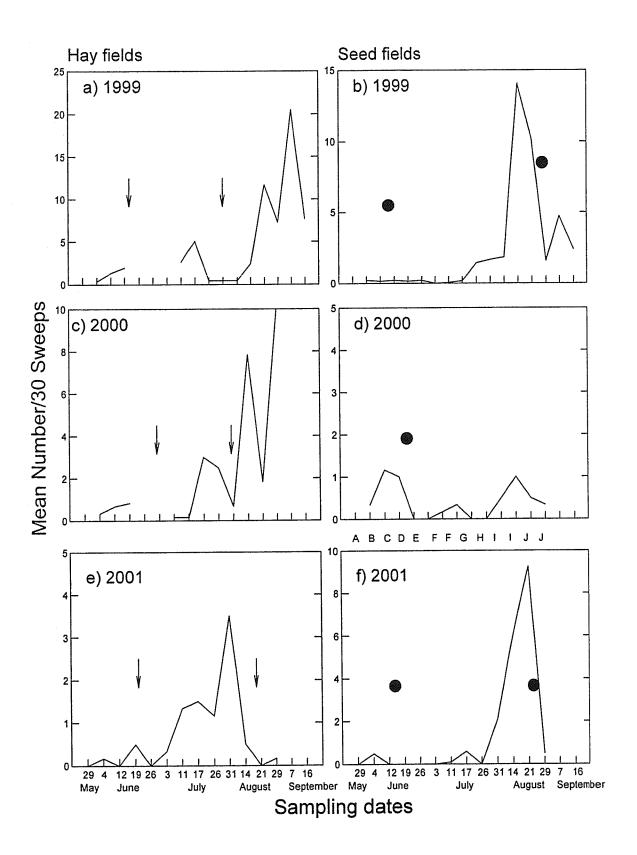


Fig. 3.2.16. Number of pentatomids collected in alfalfa fields near different localities in Manitoba during the crop season of 1999. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that scale on the vertical axis of panel c is different from those of the other panels. Discontinuation of lines indicates that no samples were taken during corresponding period.

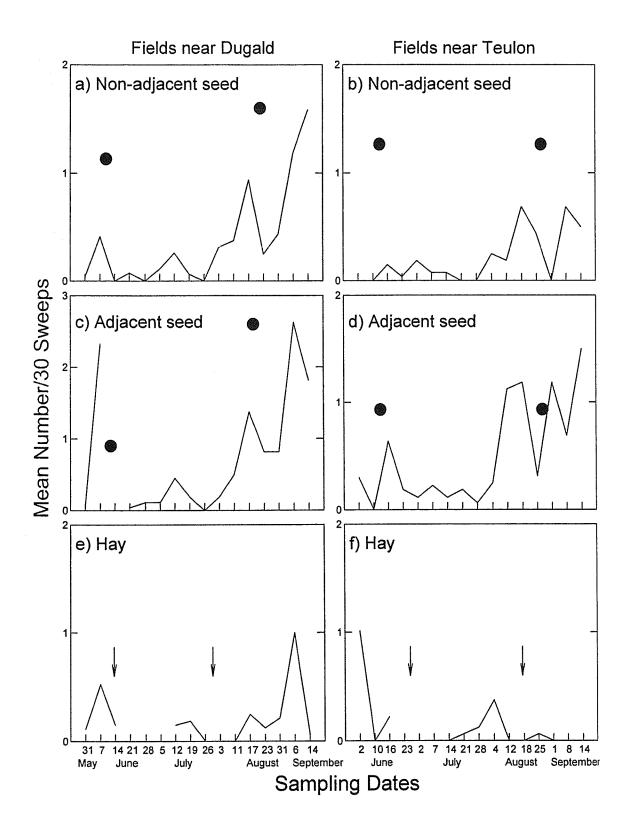


Fig. 3.2.17. Adults and larvae of chrysopids collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that scale on the vertical axis of panel f is different from those of the other panels. Discontinuation of lines indicates that no samples were collected on corresponding dates. Numbers in 1999 represent adults and larvae together. For numbers in 2000 and 2001:

----- Adults; ---- Larvae.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E

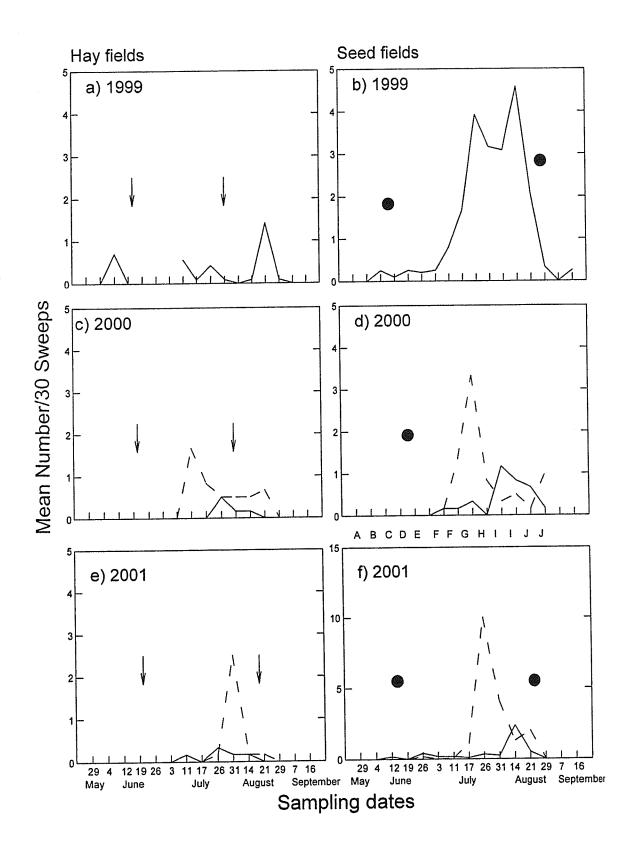


Fig. 3.2.18. Number of spiders collected by sweep-net sampling in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Discontinuation of lines indicates that no samples were collected on corresponding dates.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

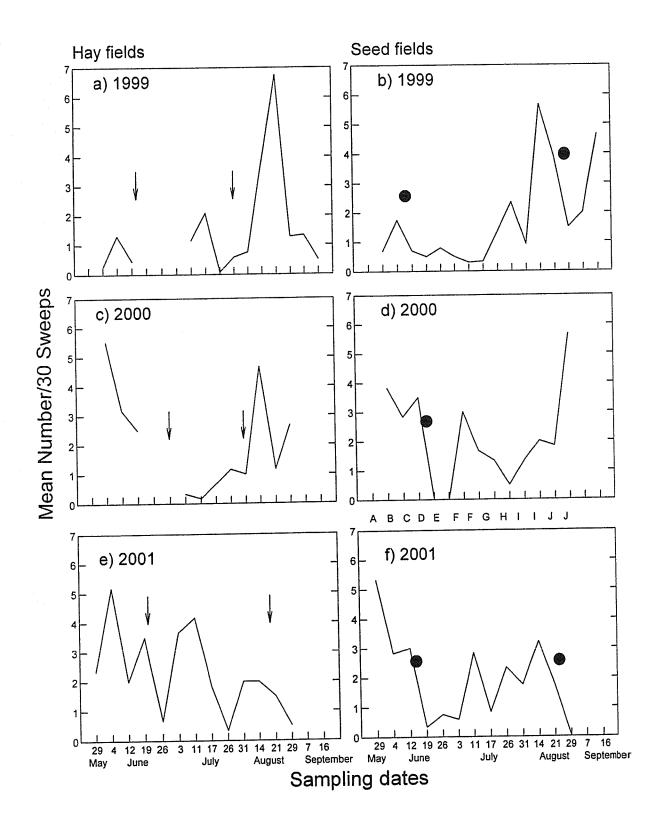


Fig. 3.2.19. Number of spiders collected by pitfall trap sampling in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.

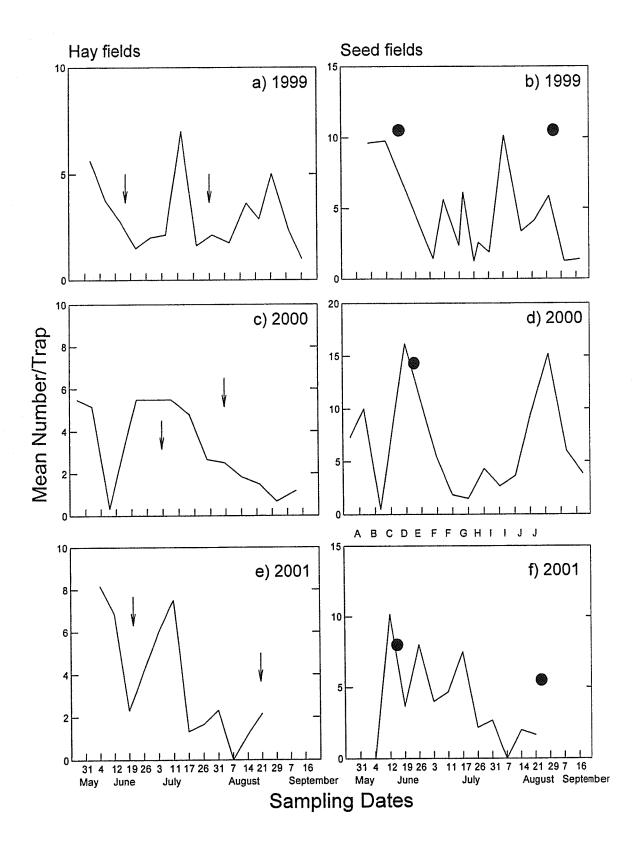
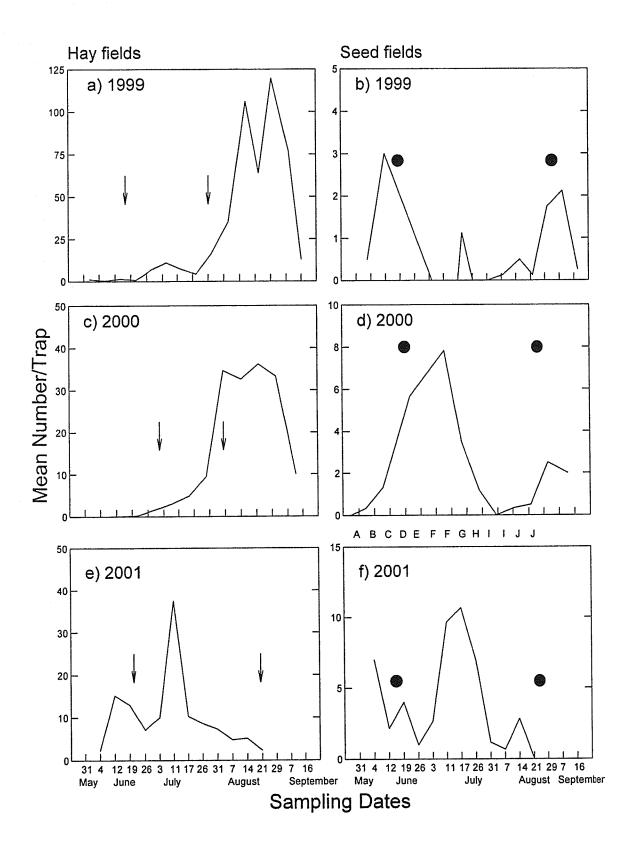


Fig. 3.2.20. Number of opilionids collected in alfalfa fields near different localities in Manitoba during the crop seasons of 1999 to 2001. Arrows indicate dates crop was harvested. Solid circles indicate dates fields received insecticides. Note that vertical axes are to different scales.

Growth stages of alfalfa plants in the field (Hall 1996):

A= Mid-vegetative, B = late vegetative, C = Early flower bud, D = Late flower bud, E = Early flower, F = Late flower, G = Early seedpod, H = Mid (between early and late) seedpod, I = Late seedpod, J = Ripe seedpod.



**CHAPTER 3.3** 

Abundance and diversity of ground beetles (Coleoptera: Carabidae) in relation to

production practices of alfalfa in Manitoba

#### Abstract

Carabid beetles are important components of crop fields, because they usually feed on either various plant-feeding insects or weed seeds. The abundance and diversity of carabid beetles in relation to production practices were examined by pitfall trap sampling in fields of alfalfa in Manitoba during the growing seasons of 1999-2001. Alfalfa fields were found to abound with a diverse assemblage of carabid beetles: a total of 101 species representing 25 genera was found during the study. However, only six species, Agonum cupreum, Amara littoralis, Amara torrida, Pterostichus corvus, P. lucublandus and P. melanarius were dominant and represented 92% of the total carabid beetles captured. The most dominant species was P. melanarius, which made up 65% of the total carabid beetles captured. Generally, hay fields tended to provide no more carabid species, but more carabid individuals than the crop grown for seed production. Hay cutting did not affect carabid beetle abundance and diversity. In contrast, insecticides applied in the early part of the season significantly reduced numbers of Agonum cupreum, Pterostichus corvus and P. lucublandus. Insecticide application late in the season did not affect carabid beetles. Within fields, field-edges usually had more individuals and more species of carabid beetles than did field-middles. As a result, field-edges tended to be more diverse.

#### Introduction

Alfalfa is forage legume crop, which can be grown for hay and seed production. Although alfalfa is a perennial plant, fields of alfalfa experience regular disturbances. In a growing season in Manitoba, alfalfa hay fields are usually mown twice, and alfalfa seed fields are sprayed with insecticides once or twice (Table 3.2.1). Both types of disturbances have implications for insect abundance and diversity. For example, alfalfa hay cutting reduces populations of insect species inhabiting the field (Harper *et al.* 1990; Chapter 3.2.2). The deleterious effect of insecticides on insect fauna is well known. Insecticide applications even deplete ground beetles (Coleoptera: Carabidae) that often live under leaf litter on the ground (Kuhlman 1974; Dritschillo and Erwin 1982; Reed *et al.* 1992).

Ground (carabid) beetles are important components of field crops. In temperate regions, they are the dominant group of epigaeic predators (Potts and Vickerman 1974), and many carabid beetle species feed on various plant-feeding insects (Los and Allen 1983). Significant predation by carabid beetles has been found on lepidopteran (Fuller 1988), homopteran (Chiverton 1988) and dipteran pest insects in agricultural lands (Floate *et al.* 1990). Some carabid beetles occurring in alfalfa fields have been found to feed on alfalfa weevil, *Hypera postica* Say, and its parasitoid, *Bathyplectes curculionis* (Thomson) (Barney *et al.* 1979). In addition, various carabid beetle species prey on weed seeds and have the potential to reduce weed populations in fields (Hengeveld 1980; Cardina *et al.* 1996).

The abundance and diversity of carabid beetles are influenced by the habitat structure and composition, the distribution of prey insects within the habitat, and

agricultural activities including but not limited to insect pest and weed management activities (Barney *et al.* 1979; Los and Allen 1983; Barney *et al.* 1984). In North America, the effects of agricultural practices on carabid beetles have been assessed (Cárcamo 1995). Although, carabid beetles were often found to be susceptible to disturbances including tillage and insecticide exposure (Cárcamo 1995), there is no consensus about the extent of the effects (Cárcamo *et al.* 1995).

In spite of being regularly disturbed, alfalfa fields have been found to harbor a diverse assemblage of arthropods including ground beetles (Pimentel and Wheeler 1973b; Harper 1988). Pimentel and Wheeler (1973b) recorded 11 species of carabid beetles among the 591 species of arthropods inhabiting an alfalfa field in New York. The abundance and diversity of carabid beetles have been studied in alfalfa fields in Virginia, Kentucky and Illinois (Los and Allen 1983; Barney et al. 1984; Barney and Pass 1986). Los and Allen (1983) caught 49 species of carabid beetles in fields of alfalfa in Virginia. In Canada, incidences of carabid beetles in relation to crop productions have been studied in cereals, beans, forages and pastures (Rivard 1964a; Cárcamo and Spence 1994; Cárcamo 1995; Cárcamo et al. 1995). But, little is known about the abundance and diversity of these beetles in relation to production practices in fields of alfalfa. Farming practices that conserve carabid beetles and other natural enemies may be practical alternatives to insecticides for suppressing pest populations (Cárcamo and Spence 1994). An understanding of the effect of production practices, particularly of hay cutting and insecticide sprays, on abundance and diversity of the carabid beetles will help develop conservation and augmentation strategies for this potentially beneficial insect taxon in fields of alfalfa (Barney and Pass 1986). Also, knowledge of the abundance and

distribution of these insects within the field and of the seasonal population fluctuations of the dominant species is important for strategic crop production and pest management activities that minimize adverse effects on these potentially beneficial insects. The present study was conducted to identify the species of carabid beetles and their patterns of temporal and spatial distribution in relation to alfalfa production practices in the Canadian Prairie context.

### Materials and Methods

The study was conducted in 1–5-year-old stands of commercially grown alfalfa, in Manitoba from 1999 to 2001. The field-sizes were 7–64 ha each. Each year, a hay field and two seed fields were studied in each of two different localities. Pitfall traps were used to monitor carabid beetles. The composition, construction, installation of pitfall traps and collection of samples have been described previously (Chapter 3.2).

# Hay cutting and insecticide sprays

Each season, the hay fields were mown twice during the study: in the period from mid-June to mid-July, and again between the end of July and late August. The seed fields received insecticide applications once or twice: during mid- to late June, and during mid-to late August except for one of the seed fields near Riverton in 2000, where the second spray was made on 1 August. Insecticide applications were of dimethoate 480 E at the rate of 425–1100 ml/ha except for the second spray in one seed field in Dugald in 1999, when Matador<sup>®</sup> 120 EC at the rate of 85 ml/ha was used. The hay cutting and insecticide applications have been summarized previously (Table 3.2.1).

# Data analysis

The abundance and diversity of carabid beetles in different types of fields, and also in the edge and middle areas within the fields were examined. The hay fields were cut twice, and seed fields were sprayed once or twice as stated previously (Table 3.2.1), and these sudden disturbances served as a time frame around which carabid beetle populations were examined and compared. Since different fields were disturbed at different times, rather than categorizing data by date of collection, data were categorized based on whether they were collected before the first disturbance, between the two disturbances or after the second disturbance. The period before the earliest disturbance within a season and locality was considered as the period before the first disturbance. The period between two disturbances included the period between the latest of the first disturbances and before the earliest of the second disturbances within a season and locality. Similarly, the period after the second disturbance included the period from the latest second disturbance to the end of sampling within a season and locality. Total catches of carabid beetle individuals and species in edge and middle areas of each field within each locality were calculated. This resulted in two values for each variable corresponding to each field within a locality and crop period. These values were used for calculating the corresponding Berger-Parker dominance index, and log series alpha diversity index (Fisher et al. 1943; Southwood 1978). The following log series alpha equation was used to calculate alpha diversity:

 $S = \alpha \ln(1 + N/\alpha)$ 

The log series alpha was calculated in two steps. In the first step, the logarithmic series parameter, x, was estimated by using least squares minimization in the NONLIN module of Systat<sup>®</sup> (Systat 2002) for the equation:

$$S / N = [(1 - x) / x] - \ln(1 - x)]$$

Where S in the total number of species and N is the total number of individuals in the samples. The values of x range between 0 and 1.

The log series alpha was derived from N and the estimate of x by using the equation:

$$\alpha = N(1-x)/x$$

Analysis of variance (ANOVA) was used to determine the significance of the effects of field-types, crop-types, field-areas on diversity parameters for carabid beetles. ANOVA was performed for a nested split plot design in which field-areas (edge and middle) was considered as subplots as explained previously (Chapter 3.2). Diversity measures were compared among field types (hay, adjacent seed field and non-adjacent seed field) and between crop types (hay and seed fields) over the entire season, before disturbances, in between disturbances and after the second disturbances. The experimental unit used in the overall analysis was the field, and variance was partitioned into components attributable to field-types, year-location (block) and their interactions. Regardless of it being significant, field-type was further partitioned into components to examine difference between crop types. Analyses resulted in no significant interactions between crop-type and area within the field. Hence, crop-type was ignored in presenting results for spatial distribution and vice versa. Paired t-tests were used to examine the immediate effect of disturbances caused by hay cutting and insecticide applications. Cumulative degree-days and maximum daily rainfall during April to September were used to examine

their relationship with carabid beetle catches. Little is known about threshold temperatures for carabid beetle development or activities. The presence and activities of carabid beetles vary with temperatures (Crist and Ahern 1999; Ayre 2001). Therefore, cumulative degree-days as calculated previously (Chapter 3.2) by arbitrarily using 10.6°C as the threshold temperature were used. Data were transformed to  $log_{10}(X)$  or  $log_{10}(X + 1)$ as appropriate to reduce heteroscedasticity in data. Interaction effects are not discussed unless significant. An  $\alpha$  level of 0.05 was used for considering any effect to be significant.

Although there were two hay fields and four seed fields sampled each year, only one hay field and one seed field was chosen to graphically illustrate the seasonal distribution of carabid beetle taxa in a year. There were occasions when data collection from some fields was interrupted due to hay cutting or insecticide application. Therefore, fields from which more complete and representative seasonal data were available were chosen to present the results graphically. These chosen fields were the hay field and its adjacent seed field each near Dugald (1999), Arborg (2000) and Arborg (2001).

#### Results

# Carabid beetle populations

A total of 30,546 carabid beetle adults representing 101 species from 25 genera was collected during the study (Appendix III). Among them, the hay fields provided a total of 13,147 carabid beetles at an average of 2191 individuals per field per season, and the seed fields provided 17,399 beetles at an average of 1450 individuals per field per season. Only six species, *Agonum cupreum* Dejean, *Amara littoralis* Mannerheim, *A*.

*torrida* Panzer, *Pterostichus corvus* Leconte, *P. lucublandus* Say, and *P. melanarius* Illiger were relatively numerous. These six species comprised 92% of the total carabid beetle catches during the study. The most abundant species was *P. melanarius*, which made up 65% of the total carabid beetles caught. *Agonum cupreum* made up 13%, whereas *P. corvus* and *P. lucublandus* represented 6% each, and *A. torrida* accounted for 1% of the total carabid beetles caught. Each of the remaining 95 species made up <1% of the catch, and together they made up 8% of the carabid beetles caught.

On a season-long basis, numbers of each of the most frequently occurring carabid beetle species did not differ among field types or crop types except for *A. cupreum*, which differed significantly among field types and crop types (Table 3.3.1). For *A. cupreum*, catches were significantly greater in hay fields than in both types of seed fields; there was no difference between the two types of seed fields (Table 3.3.1). Catches of *P. corvus*, and *A. littoralis* tended to be greater in hay fields, although this trend was not significant (Table 3.3.1).

During the period before the first disturbance, catches of none of the most frequently occurring carabid beetle species differed among field types or between crop types (Table 3.3.2). During the period between two disturbances, catches of *A. cupreum* were significantly greater in hay fields than in both types of seed fields; catches between the two types of seed fields did not differ (Table 3.3.2). During this period, none of the other frequently occurring carabid beetle species differed among field types or crop types, except for *P. lucublandus*, which occurred in significantly greater numbers in hay fields than in seed fields (Table 3.3.2). During the period after the second disturbances, catches of *A. cupreum* did not differ among field types, although catches in hay fields

were significantly greater than in seed fields (Table 3.3.2). The result was similar for *P*. *corvus*, but catches of the other species did not differ among field types or crop types during this late portion of the season (Table 3.3.2).

Total carabid beetle catches on a whole-season basis did not differ significantly among field-types and between crop-types, although the numbers in seed fields averaged 66% of those in hay fields (Table 3.3.3). *Agonum cupreum* and *P. lucublandus* probably contributed much to this result. When considering the period before the first disturbance, the total catches in hay fields averaged only 56% of those in seed fields, although this was not significant (Table 3.3.4). During the period between the first and second disturbances, total catches in hay and seed fields did not significantly differ, however catches in seed fields averaged 66% of those in hay fields (Table 3.3.4). During the postsecond disturbance period, carabid beetle catches did not differ among field types, however, they were significantly greater in hay fields than in seed fields (Table 3.3.4). During this period, catches in seed fields averaged 38% of those in hay fields.

Carabid beetle catches differed significantly across years: catches were the greatest in 2000 followed by those in 1999. In 2001, catches were the lowest, only 18 and 28% of those in 2000 and 1999, respectively ( $F_{2,10} = 8.03$ ; P < 0.01). The trials near Arborg in 2000 provided 2.5–14 times the number of carabid beetles in the other trials, and contributed much to this result.

#### Species diversity

The number of species collected was similar among field types, and between hay and seed fields (Table 3.3.3). In individual fields, there were 19–38 species in hay fields and 13–36 species in seed fields. However, a total of 65 carabid beetle species was

collected from hay fields, which was considerably lower than the 84 species collected from seed fields. Seventeen and 36 species were found exclusively in hay and seed fields, respectively (Appendix III). The trend was similar for the periods before disturbances, between the two disturbances, and also after the second disturbance (Table 3.3.4). The Berger-Parker dominance index, and log series alpha did not differ among field types or between hay and seed fields during any of the examined periods of the crop season (Tables 3.3.3 and 3.3.4), except for the period between two disturbances, when the Berger-Parker dominance index was marginally greater in seed fields than in hay fields (Table 3.3.4).

### Spatial distribution

There were no significant interactions between crop-type and areas within fields. Therefore, hay and seed fields were combined for analyses. Field-edges consistently provided more catches of *P. melanarius* than did field-middles during any examined period (Table 3.3.5). Field-edges also provided more catches of *P. lucublandus* and *A. littoralis* over the entire season and before the first disturbances (Table 3.3.5). Catches of the other species did not significantly differ between field-areas at any period as examined (Table 3.3.5). When the major species were pooled together, field-edges consitently provided significantly more catches of carabids. The result was similar for total carabids caught in different field-areas during any period as examined, except that the result was marginally significant after the second disturbances (Table 3.3.6).

As was the case with carabid individuals, significantly more carabid beetle species were caught in field-edges than in field-middles over the entire season (Table 3.3.6). A total of 89 species was caught from field-edges as opposed to 68 species from

field-middles. Among them, 32 species were exclusively found in edges as opposed to 13 species in middles. Field-edges also exhibited consistent results in terms of providing significantly more carabid beetle species than field-middles during the period before the first disturbance, between disturbances and after disturbance (Table 3.3.6). During the period before the first disturbances, a total of 47 species was found in field-edges as opposed to 35 species found in the middle of fields. During the period between two disturbances, field-edges provided 64 species as opposed to 43 species in the middle. During the period after the second disturbance field edges provided 31 as opposed to 21 carabid beetle species in field middles. The Berger-Parker dominance index did not differ significantly between field-areas during any examined period, but the log-series alpha diversity was significantly greater in field-edges on a season-long basis (Table 3.3.6).

## Seasonal distribution

Carabid beetles were present throughout the entire sampling period (Fig. 3.3.1). Generally, total carabid beetle catches were low until mid-July, after which their numbers gradually increased. Deviation from this trend occurred in the seed fields near Dugald and Teulon (1999), where more catches of carabids occurred at the end of May (Fig. 3.3.1 a). Catches in most cases tended to be the greatest around early to mid-August (Fig. 3.3.1 b, c), although in 1999, greatest catches usually occurred at the end of August, except in the adjacent seed fields where greatest catches occurred at the end of July (Fig. 3.3.1 a).

Seasonal occurrences of the most frequently captured species of carabid beetles are illustrated in Figs. 3.3.2–3.3.4. Variations occurred in the seasonal distribution patterns of different carabid beetle species. Of the most frequently caught species, *A*.

*cupreum* were more numerous early in the season in 1999. In hay fields, however, they were more numerous at the end of July near Dugald (Fig. 3.3.2 a) and at the middle of August near Teulon. In 2000, peak captures occurred around mid-August (near Arborg, Fig. 3.3.2 b) to mid-September (near Riverton). In 2001, the peak capture occurred during mid-July to early August (Fig. 3.3.2 c).

Catches of *A. littoralis* were generally low and inconsistent over the season in different years (Fig. 3.3.2 d–f), and they were mostly caught around mid-July.

*Amara torrida* were caught in low numbers, although they were caught regularly in 1999 (Fig. 3.3.3 a), when more were caught from fields near Dugald than those near Teulon. In the other years, catches were infrequent and no consistent patterns were observed (Fig. 3.3.3 b, c).

Catches of *P. corvus* were inconsistent in different years (Fig. 3.3.3 d–f). In 1999, they were caught more frequently in fields near Dugald than those near Teulon, and captures were greatest in seed fields when sampling was initiated at the end of May (Fig. 3.3.3 d) or at the beginning of June. In 2000, they were caught more frequently in hay fields than in seed fields, and the catches peaked in mid-August (Fig. 3.3.3 e), although in 2001, they were seldom caught (Fig. 3.3.3 f).

*Pterostichus lucublandus* were frequently caught during the study period. In most cases, catches were greatest around mid-August (Fig. 3.3.4 b, c), except in 1999, when the greatest captures were at the end of May or beginning of June, when sampling was initiated (Fig. 3.3.4 a).

*Pterostichus melanarius* was caught frequently during the study period (Fig. 3.3.4 d-f). The numbers caught were low until the middle of July after which the catch-sizes

increased gradually. Carabid catches were overwhelmingly dominated by *P. melanarius* and patterns of their seasonal occurrence were similar to those of total carabid catches as explained previously.

#### Effect of hay cutting on carabid beetles

The first hay cutting did not significantly influence catches of any of the most common species (Table 3.3.7) or total carabid beetles, species richness, Berger-Parker dominance index or the log series alpha (Table 3.3.8) as these measures in the week before cutting and in the week after cutting did not differ significantly. However, after the second hay cutting, catches of *A. torrida* significantly declined while those of *P. corvus* and *P. lucublandus* significantly increased (Table 3.3.7). The other major species and total carabid beetles remained unaffected after the second cut (Tables 3.3.7 and 3.3.8). The number of species captured, Berger-Parker dominance index and log series alpha were also unaffected by the second hay cutting (Table 3.3.8).

# Effect of insecticide application on carabid beetles

In contrast to the hay cutting, the first application of insecticides influenced most carabid beetle catches: catches of *A. cupreum*, *P. corvus*, and *P. lucublandus* declined, whereas catches of *A. torrida* increased. However, catches of *A. littoralis* and *P. melanarius* tended to remain unaffected by the first insecticide application (Table 3.3.9). This mixed influence resulted in a marginally significant reduction of total carabid beetle catches, although the number of species, Berger-Parker dominance index and log series alpha in the previous week did not significantly differ from those in the week following the first insecticide application (Table 3.3.10). After the second insecticide application,

however, most major carabid beetle species remained unaffected, except that *P. corvus* increased in the week following the insecticide application (Table 3.3.9). The second spray did not affect any of the measures of diversity (Table 3.3.10).

#### Discussion

As a perennial forage legume, alfalfa generally harbors a diverse assemblage of insect species, which may vary across locations and crop types (Pimentel and Wheeler 1973*b*; Los and Allen 1983; Barney and Pass 1986). In Alberta, Harper (1988) collected 37 species of carabid beetles from the interior of alfalfa fields. In Virginia, Los and Allen (1983) found 49 species, whereas in Kentucky, Barney and Pass (1986) found 40 species of carabid beetles in fields of alfalfa. Other studies in northwestern North American agricultural habitats have also reported diverse assemblages of carabid beetles. For example, in Alberta, Cárcamo (1995) found 29 carabid beetle species in fields of barley, and Cárcamo *et al.* (1995) obtained a total of 49 species in fields of barley, fababean, intercropped pea-barley and meadows, although, on an individual field scale, the range was 18–24 species. In Alberta, Frank (1971) found 63 species in a barley field. In Saskatchewan, Doane (1981) found 87 carabid beetle species in a wheat field. Kirk (1971*b*) collected 127 carabid beetle species in an extensive 4-year study of corn and corn in rotation with oats, wheat, soybeans, flax or alfalfa fields in South Dakota.

Despite the occurrence of a relatively large number of species in the present study, only six species made up 92% of the total carabid beetle catches; dominance by a few species is a common trend in agricultural systems (Luff 2002). Los and Allen (1983) found five of the 49 carabid beetle species present made up over 60% of the total carabid beetle catches in alfalfa fields in Virginia. In alfalfa fields in Kentucky, four species

accounted for 70% of the total catch, although 40 species were caught in total (Barney and Pass 1986). Similar trends were also found in fields of barley, fababean, pea-barley intercrop and meadows (Cárcamo 1995; Cárcamo *et al.* 1995). In the present study, *P. melanarius* overwhelmingly dominated the carabid beetle assemblage. *Pterostichus melanarius* is an introduced species, and is one of the commonest carabid beetles in North America (Luff 2002), and occurs in any type of vegetation (Rivard 1964*a*, Luff 2002). The species is the dominant carabid beetle in various crops including cereal, hay and pasture fields in Ontario (Rivard 1964*a*; 1966), in crops and meadows in Alberta (Cárcamo *et al.* 1995). The dominance of *P. melanarius* probably relates to its competitive superiority, as this rather large and active species can outcompete relatively smaller ones through competition and intraguild predation (Cárcamo *et al.* 1995; Luff 2002). Spence and Spence (1988) and Cárcamo *et al.* (1995) reported that *P. melanarius* negatively affect carabid beetle fauna in agroecosystems. *Pterostichus melanarius* is also extremely tolerant of insecticides (Hagley *et al.* 1980).

There were variations in carabid beetle numbers in different years. Carabid beetle abundance and activities are influenced by various interrelated biotic and abiotic factors (Thiele 1977). Most carabid beetles occurring in temperate agroecosystems are nocturnal (Luff 1978) including the dominant species, *P. melanarius* (Chapman *et al.* 1999), and temperature is negatively correlated with the occurrence of nocturnal carabid beetles (Kegel 1990). Carabid beetle survival and activities are highly influenced by temperature and moisture level (Kirk 1971*b*; Luff 1994). The crop season in 2000 was the coolest and driest of the three years of the study, whereas, in 1999 it was the warmest and wettest. In 2001, there was an intermediate level of temperature and rainfall (Appendix I) and in

July, a considerable portion of fields near Riverton remained flooded for more than a week. There was a negative correlation of temperature and maximum daily rainfall with carabid beetle catches indicating these factors influenced carabid beetle catches. In 2000 near Riverton, finding cooperating alfalfa growers was delayed, which delayed sampling initiation in that locality by a month, although, 54% of the total carabid beetles were caught that year. Trials in 1999 were conducted in different localities, while trials in 2000 and 2001 were in the same localities with some fields in common. These factors suggest that variation across years was probably more influenced by environment than localities. However, the relationship of temperature and maximum daily rainfall with carabid beetle catches explains only 22 and 42% of the total variability ( $r^2 = 0.22$  and 0.42, respectively), which suggests that some other factors also influenced carabid beetle catches. It is difficult without detailed investigation to pinpoint the other factors that influenced carabid beetle catches across years.

In a pitfall trapping study in crop fields in South Dakota, Kirk (1971*b*) found that high catches of carabid beetles started at the beginning of August and peaked at the beginning of September. In the present study, carabid beetle catches were high in August reaching a peak mostly during mid- to late August. This was presumably due to environmental conditions influencing the seasonal phenology of the most predominant species, *P. melanarius*, which overwinters as a larva (Lindroth 1966), and therefore, adults were seldom caught in the early part of the season.

Different species had different seasonal patterns, which has previously been reported for carabid beetle species in alfalfa fields in Virginia (Los and Allen 1983) and in parkland, boreal forest and meadows in central Alberta (Niemelä *et al.* 1992). *Amara* 

littoralis overwinter as adults (Lindroth 1968) and their greater catches from the end of June to the middle of July probably represents the adults that overwintered. Considering the overwintering stage and early peak, a peak in late season was expected, but not found. In apple orchards near Lake Ontario, Holliday and Hagley (1978) did not find a second peak for Amara spp., which they thought was because after emergence from pupation, new adults of Amara spp. remained below the ground until the following spring. This might also be true for A. littoralis. Migratory behavior may also cause this, and little concerning this is known about this beetle. Amara torrida overwinters as both larvae and adults (Lindroth 1968), which explains their low occurrence early in the season, and relatively high occurrence from the middle of the season. Pterostichus corvus and P. lucublandus exhibited similar bimodal patterns of seasonal distribution, although in 2001, the latter species were mostly absent from traps. Pterostichus lucublandus overwinter as adults (Kirk 1971a), and their bimodal occurrence as found in most fields was previously reported from South Dakota (Kirk 1971a). In Ontario, Rivard (1964b) found gravid females of P. lucublandus in May and June, which indicates that individuals of the species found in May and June were probably the reproductive adults that emerged from overwintering diapause, and catches were elevated from August possibly because of emergence of new adults. Little is known about the biology of P. corvus, however, the seasonal occurrence suggests that their life cycle may be similar to that of P. lucublandus. Pterostichus melanarius catches were greater during August and onward. The species overwinters as larvae (Lindroth 1966), which explains why adults were seldom captured early in the season. Little is known about the overwintering behavior of A. cupreum. However, their seasonal pattern indicates a similarity to *P. melanarius*.

Although, hay cutting changes the microenvironment of fields (Khattat and Stewart 1980), little is known about its effect on carabid beetles. However, various carabid beetle species are affected by insecticides (Hagley et al. 1980; Los and Allen 1983; Chiverton 1984). In the present study, the effect of hay cutting on carabid beetles was weaker than that of insecticide applications. Generally, catches of A. cupreum were greater in hay fields than in seed fields. Although not affected by hay cutting, the species was affected by the insecticide application in June, which probably caused the difference among crop types. Statistically similar captures in both hay and seed fields during the time before the first disturbance also confirms this. Insecticide applications in June also affected P. corvus and P. lucublandus, probably because of their high activity during that period. Although several species were affected by insecticide applications in June, the result was not reflected in the total carabid beetle captures, presumably due to the overwhelming appearance of P. melanarius, which were not affected by hay cutting or insecticide application. Although the probability of being exposed to insecticide applications is likely to increase with increases in size of insects, Hagley et al. (1980) found that larger carabids tend to be less susceptible to insecticides and P. melanarius were found to be unaffected by several insecticides (Hagley et al. 1980). The species showed moderate tolerance to dimethoate (Hagley et al. 1980), the insecticide commonly used in seed fields in the present study.

Los and Allen (1983) found higher dominance and lower diversity in insecticide treated alfalfa fields than in untreated fields, which was probably due to the reduction of the number of taxa and increasing dominance due to a few species tolerant to insecticide, as indicated by Menhinick (1962). But this was not the case in the present study, as the indices of dominance and diversity were usually similar in both hay and seed fields. The reason for no change in the dominance index was probably the insensitiveness of the dominant species, *P. melanarius* to both hay cutting and insecticide sprays. Although, the first insecticide spray exerted an immediate affect on a few species, recolonization and reproduction probably offset the effects on a long-term basis. Los and Allen (1983) in one of their trials did not find any effect of insecticide application on species abundance and diversity of carabids in alfalfa, which they thought was due to the recovery of carabid beetles from the influence of applied insecticide. Recovery of insects after insecticide application is common, and was noted for some insects within one to three months of insecticide application in alfalfa fields (Donalde 1972; Surgeoner and Ellis 1976). The second insecticide application did not affect the carabid community, possibly because the sensitive stage was not present, or because most neighbouring crops were harvested and the displaced carabid beetles from those fields could have moved into seed alfalfa fields.

Field-edges were more diverse than field-middles in terms of the numbers of individuals and species of carabid beetles caught, although the Beger-Parker dominance index did not differ between field-areas at any period examined in this study, and the log-series alpha was greater in field-edges on a season-long basis (Table 3.3.6). The results may not be surprising. Tischler's (1965) conclusion that only certain species of arthropods permanently inhabit fields of agricultural crops suggests that many of the insect species found in annual crop fields probably arrive and colonize from the outside. The same can be true in perennial crop fields like alfalfa, which receives regular disturbances in a growing season. There is abundant evidence that non-crop habitats including field margins are important sources of diversified arthropod communities in

agricultural fields (Kromp and Steinberger 1992). There were frequently strips or patches of woodland or tall-grass vegetation near most fields in the present study, although the vegetation type and structure in European field margins studied by Kromp and Steinberger (1992) were different from those in the present study. In the present study, the species found exclusively in edges were probably accidental visitors, which were in search of food or reproduction sites and appeared in field-margins that served as the gateway/corridor for these beetles (Maelfait and De Geer 1990), and resulted in significantly greater numbers of species in field edges. There were more individuals of total carabids caught from field-edges, which agrees with reported studies in different agricultural systems (Holland 1992 and references therein; Kromp and Steinberger 1992). Most of the major species found in this study had similar results and contributed to the trend as found in this study. However, the result that P. melanarius was caught more from field-edges – even during the early period of the season (before the first disturbances) - is striking, as the species overwinters inside the field (Wallin 1986) and after emerging from the overwintering site redistributes throughout the field as the season progresses (Winder et al. 2000). The population trends of P. melanarius in field-edges and middles during different periods of the season indicate that edges had about 3-times the numbers of *P. melanarius* in middles after the second disturbances, which were less than 1.5-times in the other periods examined in this study (Table 3.3.5). This suggests that after emergence from the overwintering sites inside the fields (Wallin 1986), the beetles dispersed away (Winder et al. 2000). It is not unusual that many of the beetles move from one field to the other while dispersing. In Manitoba, snow usually melts before the end of April (Department of Energy, Mines, and Resources 1974). In the

present study, sampling was initiated in the last week of May, by when *P. melanarius* beetles probably had emerged from diapause and dispersed throughout the habitat. In such a case, the edge effect as found in this study may not be surprising.

# Conclusion

The present study provides insight into the species composition and the abundance and diversity of carabid beetles in fields of alfalfa in Manitoba. The effect of usual alfalfa production practices like hay cutting and insecticide application on carabid beetle community is documented. The insecticide application in the early part of the season affected some species more than the others. Generally, the hay fields and seed fields did not differ in carabid beetle diversity. However, field-edges contained more individuals and numbers of species of carabid beetles than field-middles. A careful investigation of the resulting effect on insect pest populations needs to be conducted.

Table 3.3.1. Total numbers (mean  $\pm$  SE per field per year) of most common carabid beetle species caught in different types of alfalfa fields during the entire crop season in Manitoba, 1999–2001. (Means are based on total carabid beetle individuals caught in eight traps per field in 1999 and in six traps per field in the other years).

		Fields $(n = 6)$		
Species	Hay	Adjacent seed	Non-adjacent seed	Statistics
Agonum cupreum	488.5 ± 136.4	97.7 ± 50.3	$64.8 \pm 12.9$	$F_{2,10} = 8.73; P < 0.01 (F_{1,10} = 17.33; P < 0.01)$
Amara littoralis	$34.3 \pm 12.8$	$25.2 \pm 14.8$	$18.5 \pm 11.7$	$F_{2,10} = 1.03$ ; ns ( $F_{1,10} = 1.32$ ; ns)
Amara torrida	$7.3 \pm 3.4$	22.8 ± 19.9	$26.8 \pm 16.3$	$F_{2,10} = 1.13$ ; ns ( $F_{1,10} = 0.58$ ; ns)
Pterostichus corvus	142.3 ± 86.3	$106.8 \pm 88.7$	59.8 ± 39.8	$F_{2,10} = 0.27$ ; ns ( $F_{1,10} = 0.42$ ; ns)
Pterostichus lucublandus	$132.3 \pm 55.0$	74.3 ± 34.8	$64.0 \pm 34.4$	$F_{2,10} = 2.12$ ; ns ( $F_{1,10} = 3.89$ ; ns)
Pterostichus melanarius	$1267.0 \pm 550.1$	$725.8 \pm 182.8$	1339.7 ± 812.0	$F_{2,10} = 0.11$ ; ns ( $F_{1,10} = 0.22$ ; ns)

ns = not significantly different.

n refers to the number fields of each type sampled.

Statistics without parentheses refer to the comparison among the three field types. Statistics in parentheses refer to the comparison between hay and seed fields. Table 3.3.2. Total numbers (mean ± SE per field per sampling period in a season) of most common carabid beetle species caught in different types of alfalfa fields during different period of the crop season in Manitoba, 1999–2001. (Means are based on total carabid beetle individuals caught in eight traps per field in 1999 and in six traps per field in the other years).

Species	Before first disturbance			In t	In between disturbances			After second disturbances		
Species	Hay (n = 5)	Adjacent	Non-adjacent seed $(n = 5)$	Hay (n = 6)	Adjacent seed $(n = 6)$	Non-adjacent seed $(n = 6)$	Hay (n = 4)	Adjacent seed $(n = 5)$	Non-adjacent seed $(n = 5)$	
Agonum cupreum	74.6 ± 34.7	63.8 ± 39.3	36.4 ± 11.6	170.0 ± 56.1	23.7 ± 14.2	$12.5 \pm 7.1$	384.5 ± 226.4	12.0 ± 8.2	$6.3 \pm 4.1$	
Statistics	$F_{2,8} = 0$	0.24; ns ( $F_{1,8} =$	0.27; ns)	$F_{2,10} = 8.43; P$	$< 0.01 \ (F_{1,10} =$	16.87; <i>P</i> < 0.01)	$F_{2,8} = 2.31$	; ns ( $F_{1,8} = 4.47$	7; <i>P</i> < 0.05)	
Amara littoralis Statistics	$3.2 \pm 1.6$ $F_{2,8} = 0$	$3.8 \pm 1.5$ ).60; ns ( $F_{1,8} =$	3.6 ± 3.4 0.03; ns)	$10.3 \pm 3.9$ $F_{2,10} = 0$	$14.2 \pm 10.9$ 0.68; ns ( $F_{1,10}$ =	4.7 ± 1.8 = 1.00; ns)	$2.0 \pm 0.8$ $F_{2,8} = 0$	$4.5 \pm 3.9$ 6.62; ns ( $F_{1,8} = 0$	1.0 ± 1.0 ).33; ns)	
<i>Amara</i> torrida Statistics	$0 F_{2,8} = 0$	$0.8 \pm 0.8$ 0.67; ns ( $F_{1,8} =$	0.2 ± 0.2 0.79; ns)	$3.0 \pm 1.0$ $F_{2,10} =$	9.5 ± 7.7 0.90; ns (F <sub>1,10</sub> =	14.7 ± 9.8 = 0.60; ns)	$1.0 \pm 1.0$ $F_{2,8} = 0$	$5.6 \pm 5.6$ 0.08; ns ( $F_{1,8} = 0$	10.7 ± 10.7 ).14; ns)	
Pterostichus corvus Statistics		$100.6 \pm 88.8$ 0.37; ns ( $F_{1,8} =$	52.2 ± 44.5 0.69; ns)	$6.2 \pm 3.7$ $F_{2,10} =$	$6.5 \pm 6.1$ 0.81; ns ( $F_{1,10}$ =	1.7 ± 0.7 = 1.62; ns)	$273.0 \pm 202.0$ $F_{2,8} = 2.87$	$24.0 \pm 12.4$ ; ns ( $F_{1,8} = 4.96$	15.7 ± 10.8 5; <i>P</i> < 0.05)	
<i>Pterostichus lucublandus</i> Statistics	$21.6 \pm 7.9$ $F_{2,8} = 0$	$67.4 \pm 33.5$ 0.90; ns ( $F_{1,8} =$	46.6 ± 31.1 1.10; ns)	$13.5 \pm 5.9$ $F_{2,10} = 3.4$	$6.8 \pm 4.9$ 9; ns ( $F_{1,10} = 6$ .	2.0 ± 1.1 33; <i>P</i> < 0.05)	$267.5 \pm 142.6$ $F_{2,8} = 2$	$12.0 \pm 9.4$ 2.03; ns ( $F_{1,8} = 4$	30.3 ± 26.4 4.01; ns)	
Pterostichus melanarius Statistics	$21.0 \pm 8.9$ $F_{2.8} = 0$	61.8 ± 23.3 0.77; ns ( <i>F</i> <sub>1,8</sub> =	54.4 ± 39.6 0.76; ns)		$345.0 \pm 110.3$ 0.03; ns ( $F_{1,10}$ =	525.3 ± 337.1 = 0.01; ns)		$326.5 \pm 123.9$ .53; ns ( $F_{1,8} =$	966.0 ± 820.8 1.67; ns)	

ns = not significantly different.

n refers to the number fields of each type sampled during corresponding period.

Statistics without parentheses refer to the comparison among the three field types.

Statistics in parentheses refer to the comparison between hay and seed fields.

Note that df in different sampling periods differed. This was because in 2000, sampling fields near Riverton was initiated at the end of June. Whereas in 2001, sampling fields near Riverton was terminated in the third week of August. Therefore, no corresponding data from the location were available.

Table 3.3.3. Total numbers and species richness of carabid beetles (mean  $\pm$  SE per field per year) caught in different types of alfalfa fields in the entire crop season in Manitoba, 1999–2001. (Means are based on total carabid beetle individuals caught in eight traps per field in 1999 and in six traps per field in the other years).

		Fields $(n = 6)$		
Diversity measures	Hay	Adjacent seed	Non-adjacent seed	Statistics
Individuals	2191.2 ± 700.3	1159.3 ± 359.7	$1740.5 \pm 872.8$	$F_{2,10} = 1.34$ ; ns ( $F_{1,10} = 2.30$ ; ns)
Species	$25.7 \pm 2.7$	$21.8\pm3.3$	$28.7 \pm 2.3$	$F_{2,10} = 3.11$ ; ns ( $F_{1,10} = 0.11$ ; ns)
Berger Parker dominance index	$0.6 \pm 0.1$	$0.7 \pm 0.1$	$0.6 \pm 0.1$	$F_{2,10} = 0.43$ ; ns ( $F_{1,10} = 0.51$ ; ns)
Log series alpha	$4.6 \pm 0.6$	$4.0 \pm 0.4$	$5.8 \pm 0.8$	$F_{2,10} = 2.44$ ; ns ( $F_{1,10} = 0.26$ ; ns)

ns = not significantly different.

n refers to the number fields of each type sampled.

Statistics without parentheses refer to the comparison among the three field types.

Statistics in parentheses refer to the comparison between hay and seed fields.

Table 3.3.4. Total numbers and species richness of carabid beetles (mean ± SE per field per sampling period) caught in different types of alfalfa fields during different periods of the crop season in Manitoba, 1999–2001. (Means are based on total carabid beetle individuals caught in eight traps per field in 1999 and in six traps per field in the other years, respectively).

	Before first disturbance			In between disturbances			After second disturbance		
Diversity		Adjacent	Non-adjacent	-	Adjacent	Non-adjacent		Adjacent	Non-adjacent
measures	Hay (n = 5)	seed $(n = 5)$	seed $(n = 5)$	Hay (n = 6)	seed $(n = 6)$	seed $(n = 6)$	Hay $(n = 4)$	seed $(n = 5)$	seed $(n = 5)$
Individuals	$164.6 \pm 62.5$	333.0 ± 167.9	$252.2 \pm 80.2$	808.8 ± 324.8	$449.2 \pm 127.1$	$616.7 \pm 360.8$	$2130.0 \pm 1126.8$	$331.6 \pm 132.5$	$1278.4 \pm 1076.7$
Statistics			$F_{2,10} = 0$	.34; ns ( $F_{1,10} =$	0.50; ns)	$F_{2,8} = 3.51$	; ns $(F_{1,8} = 6.91)$	; <i>P</i> < 0.05)	
Species	12.6 ± 3.5	$13.6 \pm 2.8$	$13.6 \pm 1.7$	$15.8 \pm 2.0$	$13.5 \pm 3.5$	18.3 ± 2.2	$9.8 \pm 1.7$	$7.4 \pm 2.4$	8.8 ± 2.9
Statistics	$F_{2,8} = 0$	).45; ns ( $F_{1,8} =$	0.71; ns)	$F_{2,10} = 3.72$ ; ns ( $F_{1,10} = 0.33$ ; ns)			$F_{2,8} = 0.92$ ; ns ( $F_{1,8} = 1.20$ ; ns)		
BP index	$0.4 \pm 0$	$0.5 \pm 0.1$	$0.5 \pm 0.1$	$0.6 \pm 0.1$	$0.8 \pm 0.1$	$0.7 \pm 0.1$	$0.7 \pm 0.1$	$0.7 \pm 0.1$	$0.6 \pm 0.1$
Statistics	$F_{2,8} = 0$	).10; ns ( $F_{1,8} =$	0.20; ns)	$F_{2,10} = 2.39$ ; ns ( $F_{1,10} = 4.13$ ; ns)		$F_{2,8} = 0.26$ ; ns ( $F_{1,20} = 0.04$ ; ns)		0.04; ns)	
Log series									
alpha	$3.5 \pm 0.8$	$3.3 \pm 0.5$	$3.3 \pm 0.2$	$3.5 \pm 0.6$	$2.8 \pm 0.7$	$4.8 \pm 1.1$	$1.5 \pm 0.1$	$1.4 \pm 0.4$	$2.4 \pm 0.4$
Statistics	$F_{2,8} = 0$	.06; ns ( $F_{1,22}$ =	0.11; ns)	$F_{2,10} = 2$	.37; ns ( $F_{1,27}$ =	0.19; ns)	$F_{2,8} = 2$	.24; ns $(F_{1,8} = 0)$	).81; ns)

ns = Not significantly different

n refers to the number fields of each type sampled.

BP index = Berger Parker dominance index

Statistics without parentheses refer to the comparison among the three field types.

Statistics in parentheses refer to the comparison between hay and seed fields.

Note that df in different sampling periods differed. This was because in 2000, sampling fields near Riverton was initiated at the end of June. Whereas in 2001, sampling fields near Riverton was terminated in the third week of August. Therefore, no corresponding data from the location were available.

Species								
	Entire sea	son (n = 18)	Before first dis	turbance $(n = 15)$	In between distu	(n = 18)	After second dis	sturbance $(n = 14)^*$
	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle
Agonum							0	
cupreum	$119.6 \pm 39.4$	$97.4 \pm 29.0$	$26.3 \pm 7.2$	$31.9 \pm 10.3$	$35.0 \pm 13.0$	$33.7 \pm 13.7$	$76.4 \pm 51.1$	$39.6 \pm 24.4$
	$F_{1,15} =$	2.50; ns	$F_{1,12}$	= 0; ns	$F_{1,15} = 0$	).84; ns		0.39; ns
Amara			-,		1,12	,	~ 1,7	
littoralis	$18.2 \pm 5.4$	$7.8 \pm 3.1$	$2.6 \pm 1.0$	$0.9 \pm 0.4$	$5.7 \pm 1.9$	$4.1 \pm 2.3$	$1.6 \pm 0.7$	$0.7 \pm 0.6$
	$F_{1,15} = 3.2$	8; <i>P</i> < 0.09	$F_{1,12} = 5.59; P < 0.05$		$F_{1,15} = 2.76$ ; ns		$F_{1.9} = 2.00$ ; ns	
Amara					- ,		1,7	,,
torrida	$11.6 \pm 4.8$	$7.4 \pm 4.2$	$0.1 \pm 0.1$	$0.3 \pm 0.2$	$5.5 \pm 2.6$	$3.6 \pm 1.6$	$4.4 \pm 3.7$	$2.0 \pm 1.4$
	$F_{1,15} =$	2.02; ns	$F_{1,12} =$	2.00; ns	$F_{1,15} = 1$	.20; ns	$F_{1,0} =$	0.85; ns
Pterostichus					-,		1,2	
corvus	$61.4 \pm 26.0$	$41.6 \pm 16.1$	$35.0 \pm 21.9$	$22.2 \pm 11.4$	$1.9 \pm 1.2$	$2.8 \pm 1.4$	$55.0 \pm 35.4$	$36.6 \pm 26.1$
	$F_{1,15} =$	0.14; ns	$F_{1,12} =$	0.02; ns	$F_{1,15} = 2$	2.68; ns		0.32; ns
Pterostichus					- ,		1,2	,
lucublandus	$52.3 \pm 15.5$	$37.9 \pm 10.5$	$22.7 \pm 6.8$	$22.5 \pm 8.7$	$4.6 \pm 1.6$	$2.8 \pm 1.3$	$62.1 \pm 34.3$	$30.7 \pm 16.0$
	$F_{1,15} = 6.4$	0; P < 0.05	$F_{1,12} =$	1.95; ns	$F_{1,15} = 1$	.71; ns	$F_{1,9} = 4.2$	4; $P \approx 0.07$
Pterostichus					.,		•,,	
melanarius	$707.9\pm232.0$	$402.9 \pm 114.6$	$26.1 \pm 7.7$	$19.6 \pm 8.2$	$277.6 \pm 87.4$	$200.0 \pm 73.9$	$615.6 \pm 300.9$	$217.6 \pm 107.1$
W	$F_{1,15} = 12.7$	<b>'8</b> ; <i>P</i> < 0.01	$F_{1,12} = 8.2$	0; <i>P</i> < 0.05	$F_{1,15} = 11.40$	6; <i>P</i> < 0.05		3; <i>P</i> < 0.05

Table 3.3.5. Total numbers of the most common carabid beetle species (mean ± SE per trap per period) caught in edges and middles of alfalfa fields during different periods of the season in Manitoba, 1999–2001.

ns = not significantly different.

n refers to the number fields of each type sampled.

\*Data from the hay field at Teulon (1999) are missing, so data from seed fields at Teulon (1999) were excluded from the analaysis. Note that df changed in different sampling periods. This was because in 2000, sampling of fields near Riverton was initiated at the end of June. In 2001, sampling fields near Riverton was terminated in the third week of August. Therefore, no corresponding data from the location were available.

Table 3.3.6. Numbers (mean $\pm$ SE) per trap per period and species richness of carabid beetles (mean $\pm$ SE) caught in edge and
middle areas within fields of alfalfa in different periods of the season in Manitoba, 1999–2001.

Diversity measures	Entire seas	Entire season (n = 18)		Before first disturbance $(n = 15)$		In between disturbances $(n = 18)$		disturbance	
	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	
Individuals	$1046.8 \pm 273.3$	$650.2 \pm 139.1$	$136.0 \pm 34.4$	$113.9 \pm 30.1$	$356.8 \pm 99.2$	$268.1 \pm 87.0$	$842.4 \pm 371.8$	341.1 ± 138.1	
Statistics	$F_{1,15} = 12.1$	5; $P < 0.01$	$F_{1,12} = 8.59; P < 0.01$		$F_{1,15} = 10.0$	$F_{1,15} = 10.02; P < 0.01$		$F_{1,9} = 3.97; P \approx 0.08$	
Species Statistics	$21.3 \pm 1.3 \\ F_{1,15} = 16.6$	$16.2 \pm 1.3$ 6; <i>P</i> < 0.01	$10.9 \pm 1.0$ $F_{1,12} = 8.5$	$8.7 \pm 1.2$ 8; <i>P</i> ≈ 0.01	$12.8 \pm 1.2$ F <sub>1,15</sub> = 11.8	9.9 ± 1.1 89; <i>P</i> < 0.01	$7.1 \pm 1.2$ $F_{1,9} = 7.22$	5.1 ± 0.9 ; <i>P</i> < 0.05	
BP index Statistics	$0.6 \pm 0.0$ $F_{1,15} = 0$	$0.6 \pm 0.1$ 0.29; ns	$0.5 \pm 0$ $F_{1,12} =$	0.5 ± 0.0 1.42; ns	$0.7 \pm 0$ $F_{1,15} = 0$	0.7 ± 0.1 0.56; ns	$0.7 \pm 0.1$ $F_{1,9} = 0$	0.7 ± 0.1 .54; ns	
Log series alpha Statistics	$4.3 \pm 0.3$ $F_{1,15} = 4.39$	$3.7 \pm 0.4$ $P; P \approx 0.05$	$3.2 \pm 0.3$ $F_{1,12} = 1$	2.9 ± 0.3 0.68; ns	$3.3 \pm 0.4$ $F_{1,15} = 0.4$	3.0 ± 0.5 0.75; ns	$1.5 \pm 0.2$ $F_{1,9} = 0$	1.2 ± 0.2 .22; ns	

ns = not significantly different.

n refers to the number fields of each type sampled. \*Data from the hay field at Teulon (1999) are missing, so data from seed fields at Teulon (1999) were excluded from the analaysis. Note that df in different sampling periods differed. This was because in 2000, sampling of fields near Riverton was initiated at the end of June. In 2001, sampling fields near Riverton was terminated in the third week of August. Therefore, no corresponding data from the location were available.

Species	First hay	cutting	Second hay cutting		
Species	Before	After	Before	After	
	$2.1 \pm 0.4$	$1.4 \pm 0.4$	$11.5 \pm 2.4$	$18.7 \pm 4.8$	
Agonum cupreum	$t_{34} = 1.1$	2. ns	$t_{30} = 0.1$	15; ns	
Statistics	$2.2 \pm 0.7$	$1.1 \pm 0.4$	$0.1 \pm 0$	$0.2 \pm 0.1$	
Amara littoralis	$t_{34} = 1.3$		$t_{30} = 0.74$ ; ns		
Statistics	0	$0.1 \pm 0$	$0.4 \pm 0.2$	0	
Amara torrida	$t_{34} = 0.4$		$t_{30} = 2.33; P < 0.05$		
Statistics	$0.4 \pm 0.3$	$0.1 \pm 0$	$0.6 \pm 0.2$	$15.0 \pm 6.4$	
Pterostichus corvus	$t_{34} = 0.9$		$t_{30} = 3.24; P < 0.01$		
Statistics Pterostichus lucublandus	$0.6 \pm 0.2$	$0.6 \pm 0.2$	$0.6 \pm 0.2$	$6.5 \pm 2.3$	
	$t_{34} = 0.2$		$t_{30} = 2.23;$	<i>P</i> < 0.05	
Statistics	$0.9 \pm 0.3$	$1.3 \pm 0.3$	$37.4 \pm 9.7$	$29.1 \pm 6.3$	
Pterostichus melanarius Statistics		$0.9 \pm 0.3$ $1.5 \pm 0.5$ $t_{34} = 0.88; \text{ ns}$		53; ns	

Table 3.3.7. Influence of hay cutting on numbers (mean ± SE) of most common carabid beetle species caught per trap in alfalfa fields in Manitoba, 1999–2001. (Significance of differences is tested with a paired t-test).

ns = not significantly different.

Table 3.3.8. Influence of hay cutting on numbers and species richness (mean  $\pm$  SE) of carabid beetles caught per trap in alfalfa fields in Manitoba, 1999–2001. (Significance of differences is tested with a paired t-test).

	First hay cutting		Second h	ay cutting
Diversity measures	Before	After	Before	After
Individuals	$47.7 \pm 14.1$	$43.5 \pm 13.3$	$320.6 \pm 101.7$	$443.0 \pm 193.0$
Statistics	$t_5 = 0.$	32; ns	$t_4 = 0$	.05; ns
Species	$8.2 \pm 1.3$	$7.5 \pm 1.1$	$6.8 \pm 0.8$	$6.4 \pm 1.0$
Statistics	$t_5 = 0.$	50; ns	$t_4 = 0.33$ ; ns	
Berger Parker				
dominance index	$0.5 \pm 0.1$	$0.4 \pm 0.1$	$0.7 \pm 0.1$	$0.7 \pm 0.1$
Statistics	$t_5 = 1$ .	55; ns	$t_4 = 0$	.44; ns
Log series alpha	$3.1 \pm 0.5$	$4.0 \pm 1.4$	$1.4 \pm 0.2$	$1.2 \pm 0.2$
Statistics	$t_5 = 0.70;  \mathrm{ns}$		$t_4 = 0.71$ ; ns	

ns = not significantly different.

Note that df in the first and second hay cutting differed. This was because in 2001, sampling fields near Riverton was terminated in the third week of August. Therefore, no data associated with the second hay cutting were available from the location.

Table 3.3.9. Mean number (mean  $\pm$  SE) of most common carabid beetle species caught per trap as influenced by insecticide applications in fields of alfalfa in Manitoba, 1999–2001.

Species	First insecticid	e application	Second insecticide application		
~	Before	After	Before	After	
Agonum cupreum	$3.6 \pm 0.7$	$1.2 \pm 0.5$	$1.3 \pm 0.6$	$0.6 \pm 0.3$	
	$t_{63} = 5.58;$	P < 0.1	$t_{43} = 1$	.57; ns	
Amara littoralis	$0.2 \pm 0.1$	$0.5 \pm 0.2$	$0.3 \pm 0.1$	$0.2 \pm 0.1$	
	$t_{63} = 1.2$	33; ns	$t_{43} = 0.30$ ; ns		
Amara torrida	0	$0.4 \pm 0.2$	$0.2 \pm 0.1$	$0.3 \pm 0.1$	
	$t_{63} = 2.67;$	<i>P</i> < 0.01	$t_{43} = 0.87$ ; ns		
Pterostichus corvus	$2.7 \pm 0.7$	$0.5 \pm 0.2$	$0.3 \pm 0.2$	$1.3 \pm 0.4$	
	$t_{63} = 3.88;$	<i>P</i> < 0.01	$t_{43} = 3.37$	7; <i>P</i> < 0.01	
Pterostichus lucublandus	$2.8 \pm 0.7$	$0.4 \pm 0.1$	$0.3 \pm 0.2$	$0.7 \pm 0.4$	
	$t_{63} = 5.31; P < 0.01$		$t_{43} = 1$	l.02; ns	
Pterostichus melanarius	$5.2 \pm 1.1$	$7.6 \pm 3.0$	$12.5 \pm 2.5$	$15.4 \pm 3.9$	
1 lei osticitus metunui tus	$t_{63} = 1.78$ ; ns		$t_{43} = 0.50; \text{ ns}$		

ns = not significantly different.

 ${\cal C}_{\mathcal{C}}^{*}$ 

Note that df in the first and second insecticide application differed. This was because in 2001, sampling fields near Riverton was terminated in the third week of August. Therefore, no data associated with the second insecticide application were available from the location

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Table 3.3.10. Number and species richness (mean  $\pm$  SE) of carabid beetles caught per trap as influenced by insecticide applications in fields of alfalfa in Manitoba, 1999–2001.

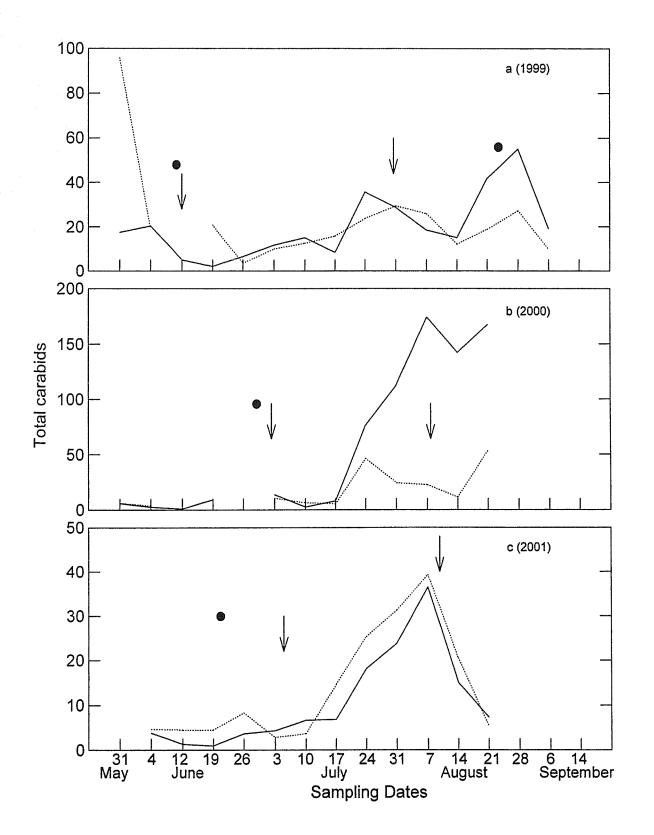
	First insecticide application		Second insectic	ide application	
Diversity measures	Before	After	Before	After	
Individuals	$109.7 \pm 34.2$	82.7 ± 50.9	$121.2 \pm 37.7$	$143.7 \pm 59.3$	
	$t_9 = 1.98; H$	P < 0.08	$t_5 = 0.2$	27; ns	
Species	÷ .	$6.8 \pm 1.1$	$7.3 \pm 1.5$	$7.2 \pm 1.8$	
- <b>F</b>	$t_9 = 0.9^{\circ}$	7; ns	$t_5 = 0.15$ ; ns		
Berger Parker					
dominance index	$0.5 \pm 0.1$	$0.5 \pm 0.1$	$0.7 \pm 0.1$	$0.6 \pm 0.1$	
	$t_9 = 0.1$	0; ns	$t_5 = 0.6$	67; ns	
Log series alpha	$3.0 \pm 0.5$	$3.7 \pm 0.5$	$2.0 \pm 0.4$	$4.0 \pm 2.1$	
	$t_8 = 1.12$ ; ns		$t_5 = 0.83$ ; ns		

ns = not significantly different.

Note that df in the first and second insecticide application differed. This was because for the first insecticide application, calculation of log series alpha was not possible in one sample. Fields near Arborg did not receive the second insecticide application. Sampling of fields near Riverton was terminated in the third week of August in 2001. Therefore, no corresponding data were available for the analysis.

Fig. 3.3.1. Total carabid numbers (mean/trap) captured in pitfall traps in alfalfa fields in different localities of Manitoba, 1999–2001.Arrows indicate dates the hay crop was mown.

Solid circles indicate dates insecticides were applied in respective seed fields. Solid and dotted lines correspond to hay and seed fields, respectively. Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.



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Fig. 3.3.2. Numbers (mean/trap) of *Agonum cupreum* (a--c) and *Amara littoralis* (d-f) captured in pitfall traps in alfalfa fields in different localities of Manitoba, 1999–2001.

Arrows indicate dates the hay crop was mown.

Solid circles indicate dates insecticides were applied in respective seed fields.

Solid and dotted lines correspond to hay and seed fields, respectively.

Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

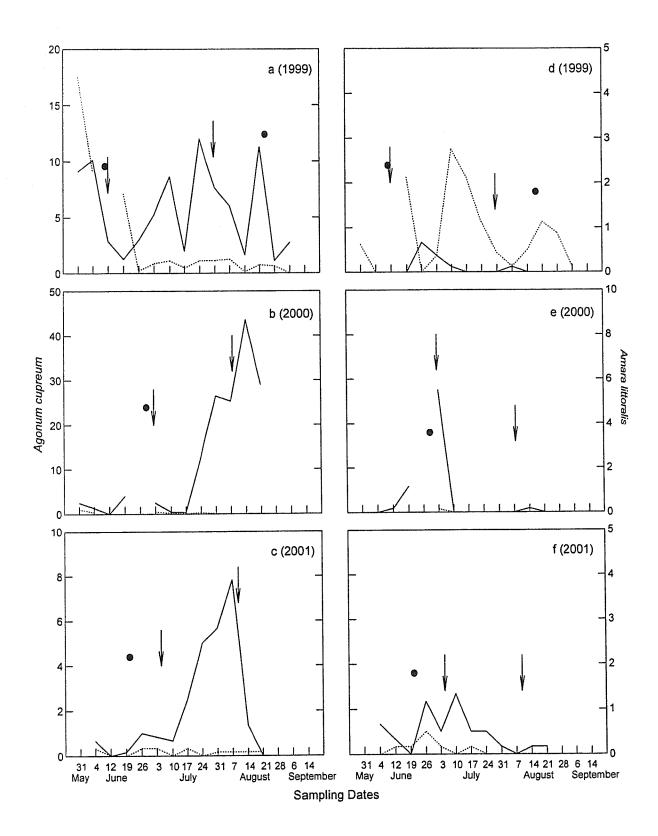


Fig. 3.3.3. Numbers (mean/trap) of *Amara torrida* (a–c) and *Pterostichus corvus* (e–f) captured in pitfall traps in alfalfa fields in different localities of Manitoba, 1999–2001.

Arrows indicate dates the hay crop was mown.

Solid circles indicate dates insecticides were applied in respective seed fields.

Solid and dotted lines correspond to hay and seed fields, respectively.

Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.

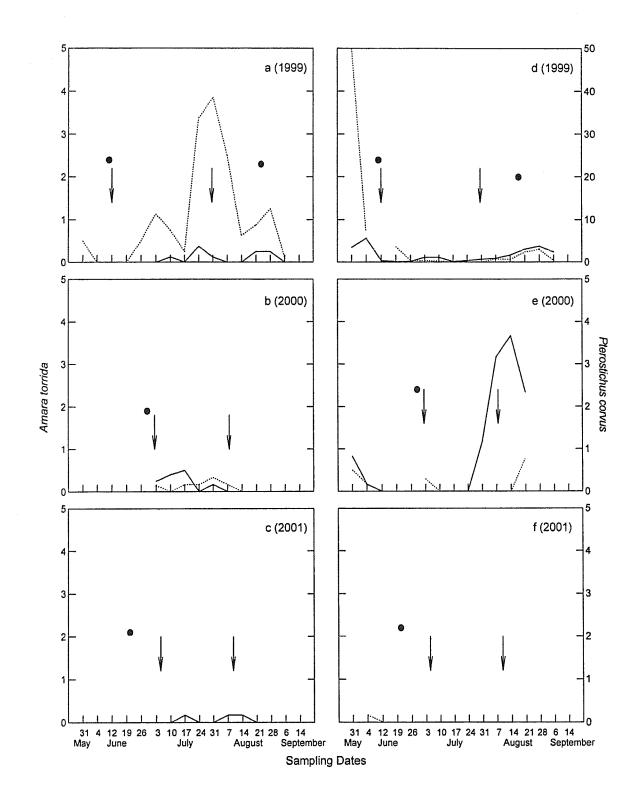


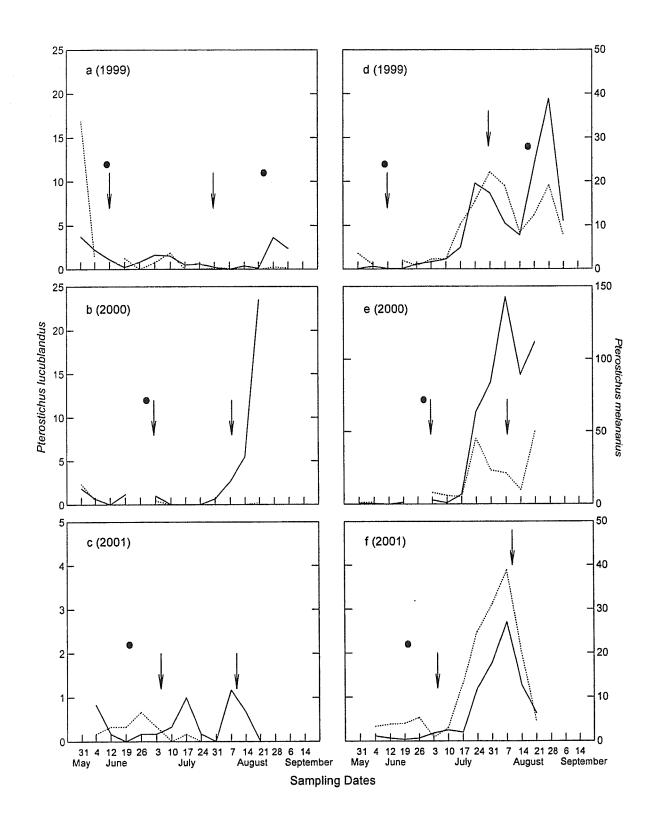
Fig. 3.3.4. Numbers (mean/trap) of *Pterostichus lucublandus* (a–c) and *P. melanarius* (e–f) captured in pitfall traps in alfalfa fields in different localities of Manitoba, 1999–2001. Solid and dotted lines correspond to hay and seed fields.

Arrows indicate dates the hay crop was mown.

Solid circles indicate dates insecticides were applied in respective seed fields.

Solid and dotted lines correspond to hay and seed fields, respectively.

Note that vertical axes are to different scales. Discontinuation of lines indicates that no samples were collected on corresponding dates.



# **CHAPTER 3.4**

Influence of some common predaceous insects on populations of insect pests of

alfalfa

#### Abstract

The effect of larval *Chrysoperla carnea*, adult and larval *Coccinella* septempunctata, adult and nymphal *Nabis alternatus* and adult *Pterostichus melanarius* on three pest insects, *Lygus* spp., *Adelphocoris lineolatus* and *Acyrthosiphon pisum*, was studied on caged alfalfa plants in field and laboratory cages. In field cages, mixed populations of these predatory insects reduced the numbers of the nymphs of *Lygus* spp. and *A. lineolatus* and numbers of *A. pisum*. In the laboratory, *N. alternatus* was the greatest consumer of *Lygus* spp. nymphs, and *N. alternatus* and *P. melanarius* consumed more *A. lineolatus* nymphs than did the other tested predators. Adult and larval *C. septempunctata* consumed more *A. pisum* than did the other tested predators. *Chrysoperla carnea* larvae consumed 20% less *A. pisum* than did *C. septempunctata*. It was concluded that predators can reduce major pests in alfalfa, and *N. alternatus* is probably the most effective predator in situations where the studied insects cohabit in the field.

## Introduction

Naturally occurring biological control is ubiquitous, but may be improved by conservation or augmentation of natural enemies. Predators are important natural agents and are usually the best agents for conservation biological control (Hagler 2000). Predation often reduces insect pest numbers and may cause local extinction of insect pests (Huffaker *et al.* 1976). Most insect predators are generalists; in agricultural crops, they feed on a variety of insect pests and so may suppress prey populations (Huffaker *et al.* 1976; Debach and Rosen 1991; Hagler 2000).

Alfalfa fields are complex habitats that favor assemblages of a variety of insects (Pimentel and Wheeler 1973b; Harper 1988). Like most field crops, however, alfalfa is damaged by only a few of these insects (Pimentel and Wheeler 1973b; Schaber and Entz 1991; Summers 1998). In the Canadian Prairies, the most damaging insects in alfalfa fields include lygus bugs, *Lygus* spp., the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), the pea aphid, Acyrthosiphon pisum (Harris) (Homoptera: Aphididae), and the alfalfa weevil, Hypera postica (Gyllenhal) (Coleoptera: Curculionidae) (Lilly and Hobbs 1962; Goplen et al. 1987; Schaber and Entz 1988; Harper et al. 1990; Schaber et al. 1990a; Schaber 1992). However, H. postica is not reported to damage alfalfa crops in Manitoba, though the insect may occasionally be found. Alfalfa fields also contain a variety of predatory insects (Whitcomb and Bell 1964; Pimentel and Wheeler 1973b; Wheeler 1977; Harper 1988). In the Canadian Prairies, the most common predators in alfalfa fields are the lacewing, Chrysoperla carnea (Stephens) (Neuroptera: Chrysopidae), ladybird beetles including the non-native Coccinella septempunctata (Linnaeus) (Coleoptera: Coccinellidae) (Turnock et al. 2003) and the

western damsel bug, *Nabis alternatus* (Parshley) (Hemiptera: Nabidae) (Harper 1978; Frazer *et al.* 1981*b*; Harper *et al.* 1990; Schaber *et al.* 1990*b*; Schaber 1992). The ground beetle, *Pterostichus melanarius* (Illiger) (Coleoptera: Carabidae) which is an introduced species, has become ubiquitous in this region (Niemelä and Spence 1991). These four predators are abundant in Manitoban alfalfa fields (Chapter 3.2), and therefore have the potential to influence insect pest populations in these fields.

The benefits of predatory insects are well recognized, though often they are difficult to quantify (Lapchin et al. 1987; Hagler 2000). Predatory insects were found to reduce populations of aphids including A. pisum in alfalfa fields in Alberta and California (Hagen and van den Bosch 1968; Frazer and Gilbert 1976; Baumgaertner et al. 1981; Frazer et al. 1981a, 1981b). Chrysopids (Neuenschwander et al. 1975; Wheeler 1977; Schaber 1992), coccinellids (Rogers et al. 1972; Neuenschwander et al. 1975; Wheeler 1977; Frazer et al. 1981a, 1981b; Schaber 1992; Elliott et al. 2000), nabids (Knowlton 1949; Clancy and Pierce 1966; Perkins and Watson 1972; Wheeler 1977; Tamaki et al. 1978; Schaber 1992) and carabids (Scheller 1984) are reported to consume insect pests including aphids and Lygus bugs. However, there is little information on the effects of these predatory insects on populations of the most common pests, Lygus spp., A. lineolatus, and A. pisum in alfalfa fields in the Canadian Prairie Provinces. For a holistic pest management scheme to be successful and sustainable, it is essential that it capitalizes on the roles of commonly occurring predators as mortality factors for populations of specific insect pests or the pest complex as a whole. The present study examined whether the commonly occurring predator insects have any effect on populations of the common

pest insects, Lygus spp., A. lineolatus and A. pisum, on alfalfa plants in field and laboratory cages.

# **Materials and Methods**

The effects of larval *C. carnea*, adult and larval *C. septempunctata*, adult *N. alternatus* and adult *P. melanarius* on populations of *Lygus* spp., *A. lineolatus* and *A. pisum* were investigated in field and laboratory cages. The field-cage study was conducted in a hay field near Arborg, Manitoba (Latitude 50° 56' N; Longitude 97° 5' W). A 15 x 20 m strip of alfalfa was marked, where the study was conducted on plants that were left uncut and caged until completion of the study.

In 2000 and 2001, field-cage trials were used to assess whether the guild of predators typical in alfalfa fields in Manitoba influences populations of the three major pest species. Population change of each of the pest species was assessed in the presence and absence of a mixed-species group of predators. All trials took place in 1 (width) x 1 (length) x 1.2 (height) m field cages constructed of nitex monofilament screen (24 meshes/cm) supported at each vertical corner by conduit pipe (2 cm diameter). At the base, the mesh of the cage was reinforced by metal plates which were buried in the soil to a depth of 6 cm. Coarse sand was poured around the plates to seal any gaps through which insects could move in and out of the cages. Access to the cage was provided by a zipper in one side of the cage. In a cage, there were 18–21 alfalfa plants with plant heights ranging from 60 to 80 cm. On the day of cage deployment, and again a week later, caged plants were sprayed with synergized pyrethrins (allethrin 0.176%, tetramethrin 0.09%, piperonyl butoxide 1.25%) using an aerosol spray can. The week

following the second insecticide spray, caged plants were sprayed with the fungicide benomyl 50% WP (5 ml per 2 l water). About 24–48 hours after the fungicide spray, the treatments were applied.

In 2000, the trial was conducted using Lygus spp. and A. pisum. There were five treatments: no insects, Lygus bugs alone, Lygus bugs with predators, A. pisum alone, and A. pisum with predators. Treatments were applied in four replicate blocks. Thirty nymphs of third and fourth instar Lygus bugs were released in each cage for treatments containing Lygus spp. Two hundred aphid individuals, mostly older nymphs and adults, were released in each cage for treatments containing A. pisum. At least six hours after releasing the pest insects into cages, the guilds of predators collected from alfalfa fields were introduced. A guild of predators released into each predator treatment cage consisted of eight C. carnea second or third instar larvae, 12 C. septempunctata adults, eight N. alternatus adults, and six P. melanarius adults. Pterostichus melanarius adults were collected in dry pitfall traps. All other insects were collected by sweeping alfalfa fields. Only were undamaged insects introduced into cages. All introductions were made on 7 July. Leafcutting bees were maintained in each cage so that pollination could occur. Bees were reared from cocoons in the laboratory (Richards 1989) and 15–20 adults, mostly females, were released in each cage every second week from cage erection until mid-August. Before the first introduction, a nesting site was provided in each cage for the leafcutting bees. A laminated grooved (25 mm) polystyrene block, which is commercially available for constructing leafcutter beehives, was used to make the nesting site. A nesting site comprised 17 (length) x 15 (width) x 7.5 (height) cm of the polystyrene block, to the back of which a plywood sheet was glued. The block was then tied at alfalfa

plant height with a wooden stick that was standing inside the cage. The nesting site faced southeast, and was protected from rain by a roof made of thin iron sheet.

One month after introducing insects into the cages (7 August), data on insects were collected from cages in two of the replicate blocks. To do so, without removing cages, plants in cages were cut 6–8 cm above the ground and immediately put into large plastic garbage bags {25 (height) x 90 (width) cm. About half an hour later, the numbers of insects on the cage-floor, on plant remnants, and inside cage surfaces were recorded. A pitfall trap was sunk in each cage and operated for one week. In the laboratory, insects in the bags were counted. Until yield assessment, insects in cages in the remaining two blocks were left undisturbed and the caged plants were allowed to grow to seed maturity and the seeds were harvested on 12 September to provide yield data. Percent healthy seed was determined and the amount of seed was measured as the weight (g) of dry seeds.

In 2001, the trial was repeated using *Lygus* spp. and *A. lineolatus* bugs. There were four treatments: *Lygus* bugs alone, *Lygus* bugs with predators, *A. lineolatus* alone and *A. lineolatus* with predators. As before, there were four replications for each treatment, but an additional eight cages were erected in the four blocks to gather additional data on the effect of *Lygus* bug treatments on yield. *Lygus* bug and predator introductions were as described for 2000. In treatments containing *A. lineolatus*, 30 third and fourth instar *A. lineolatus* nymphs were introduced into each cage. The guilds of predators were introduced into assigned cages as explained previously. These introductions took place on 17 July, 2001. Alfalfa leafcutting bees were introduced into the cages (14 August, 2001), data on insects were collected as described for 2000 from 16 of the 24 cages

leaving the additional eight cages undisturbed. Until yield assessment, insects in the remaining eight cages were left undisturbed, and plants were allowed to grow to seed maturity. As described for 2000, data on yield were collected in mid-September from these cages.

For the laboratory-cage study, one prey type was offered to one predator type on a small, caged alfalfa shoot in order to investigate the predatory capabilities of individual species of predators. Five predator types, third-instar larvae of C. carnea, adults of C. septempunctata, third-instar larvae of C. septempunctata, adults of N. alternatus, and adults of P. melanarius were assessed against three pest types: nymphs of Lygus spp., nymphs of A. lineolatus, and adults of A. pisum. An additional set of control treatments had pests but no predators. The resulting 18 combinations of treatments were replicated four times in 2001. In 2002, the control treatment for Lygus spp. and A. lineolatus bugs was excluded due to unavailability of specimens, and the remaining treatment combinations were replicated two to six times depending on insect availability. All of the insects except A. pisum were collected by sweeping in alfalfa fields. The insects were sorted by taxon in 250 ml opaque polyethylene containers {10 (diameter) x 4.5 (height) cm honey containers}, each containing one or two 10 cm alfalfa shoots and sealed with a perforated lid. Insects in containers were transferred to the laboratory in a picnic cooler. Insects in the laboratory were kept at 5°C for 24 h before use in trials the next day. Acyrthosiphon pisum were from a laboratory colony, and reproductive adults 2–3 days after their final moult were used.

A cage containing an alfalfa shoot potted in a plastic cup was used as an experimental unit. A 15 cm long field-collected fresh alfalfa shoot was inserted into a 25

ml plastic vial full of water. The mouth of the vial was closed with paper towel strips rolled around the alfalfa stem. The vial with the shoot was placed centrally in a 11 (height) x 9 (diameter) cm plastic beer cup to which Metromix<sup>®</sup> plant growth medium was added until about 8 cm of alfalfa shoot was above the surface of the medium in the cup. Water was then sprayed in the cup to moisten and compress the Metromix<sup>®</sup>. For trials with A. pisum, 10 aphid individuals were placed on the alfalfa shoot with forceps and the shoot was immediately covered by a perforated 25 x 15 cm polyethylene freezer bag, which was attached to the top rim of the beer cup with transparent adhesive tape. Two hours after placing aphids on alfalfa shoot, a small slit was cut in the freezer bag and one individual of the assigned predator was introduced in the cage. The slit was sealed with transparent adhesive tape. For trials with Lygus spp. and A. lineolatus, through a slit in the freezer bag three third to fourth instar nymphs of a taxon were released in each of the cages assigned to the treatment. Two hours after the prey introductions, one individual of the assigned predator was introduced in the cage. No predator was released in the units assigned to the control treatment. Once set up, cages were placed in an environment chamber at 24  $\pm$  2°C and 70  $\pm$  5 % relative humidity with a light regime of 16:8 L:D. Cages were checked every 24 h for four days and numbers of insects, including aphid nymphs, were recorded. Dead individuals were examined microscopically to determine whether the death was caused by predator attack.

All data were subject to analysis of variance using Systat 10 (SPSS 2000). When necessary, a logarithmic or arcsine transformation was used to stabilize the variance. For data from the trial in field cages, General Linear Model analysis of variance was performed to determine the effects of predator introduction on insect pests and seed yield

inside cages regardless of pest species introduction. The t-test was used to determine any effects that predators had on pest insects in cages into which respective pests were introduced. Similarly, to determine whether the introduced predators were successfully established and retained in the cages, a one-tailed t-test was used regardless of insect pest introduction in cages with or without predator introduction. For data from the trials conducted in the laboratory, repeated measure analysis of variance was performed. In the laboratory trial in 2002, there were instances in which some predators escaped from cages with A. lineolatus. Data from these cages were excluded from the analysis. A one-tailed ttest was used to examine whether the introduction of predators into cages resulted in greater mortality of corresponding prey species. Then, data from control (no predator) cages were excluded, and those from cages including predators were analyzed. Tukey's test was used to separate treatment means. An alpha level of 0.05 was used for experiment-wise level of significance. In the preliminary analyses, no significant effect of years was found on predation in the laboratory cage studies. Therefore, pooled results of the two-year laboratory studies were presented.

#### Results

In field cages in 2000, the introduction of the predators with nymphs of *Lygus* bug did not significantly reduce adults and nymphs of *Lygus* bug (Table 3.4.1). However, the numbers of *Lygus* adults and nymphs in the presence of predators were considerably lower than in their absence: about 42% and 65%, respectively of their numbers in absence of predators. In 2001, the predators in field cages significantly reduced the number of *Lygus* bug nymphs. The predators reduced, although not significantly, *Lygus* bug adults to 55% of the number in their absence (Table 3.4.2).

Predators significantly reduced the number of *A. pisum* in cages: the number of *A. pisum* was significantly fewer in the presence of the predator guild than in its absence (Table 3.4.1). The cages into which no *A. pisum* were released had a level of infestation similar to that of the cages into which predators were introduced with *A. pisum* (Table 3.4.1).

Like for the case of caged *Lygus* bugs in 2001, the predators did not cause a significant reduction of the number of *A. lineolatus* adults, but they reduced the number of *A. lineolatus* nymphs (Table 3.4.2).

At the end of the trials in both years, there were significantly higher numbers of predators present in cages in which predators were released than in cages in which no predators were released (Tables 3.4.1 and 3.4.2). In cages in which predators were released, up to 94, 50, 30 and 43% of the numbers of released *C. carnea*, *C. septempunctata*, *N. alternatus* and *P. melanarius*, respectively, were captured at the end of the trials. However, no *N. alternatus* were captured at the end of the trials in 2000. In this year, cages into which predators were released had more than four times the number of predators in cages into which no predators were released (Table 3.4.1).

Introduction of the predators with *Lygus* bugs produced mixed results for seed yield, as the amount of seed production was significantly higher with predators in 2000 trials only, and a greater, though not significantly so, percentage of these seeds was healthy (Table 3.4.3). In other cases, the introduction of predators did not result in either a significant difference in seed production, or a significant increase in the production of healthy seeds (Table 3.4.3).

In laboratory trials, there was no mortality of *Lygus* nymphs in the absence of predators (Table 3.4.4). Introduction of predators did not result in significant mortality of *Lygus* nymphs one day after the introduction ( $F_{1,40} = 2.73$ ; ns). However, compared with the control treatment, predators inflicted significant mortality of Lygus nymphs two ( $F_{1,40}$ = 4.36; P < 0.05), three ( $F_{1,40} = 6.82$ ; P < 0.05) and four ( $F_{1,40} = 24.59$ ; P < 0.01) days after the introduction. When data from control (no predators) cages were excluded, different predators caused marginally different mortality of *Lygus* nymphs (Table 3.4.4): *N. alternatus* inflicted the greatest mortality followed by *P. melanarius*, coccinellid adults, chrysopid larvae, and coccinellid larvae (Table 3.4.4). There was a significant progression of Lygus nymph consumption by the predators over the four days ( $F_{3,120} =$ 67.78; P < 0.01). The average consumption was 14% on the first day, and almost doubled and quadrupled on the second and fourth days of exposure, respectively. There was no significant interaction between the predator species and days the prey species were exposed to predators ( $F_{12,120} = 1.35$ ; ns). In the first three days, the patterns of consumption by most predators were similar. However, on the fourth day, the total consumption by adult C. septempunctata and N. alternatus increased more compared with the other predators (Fig. 3.4.1).

In the case of *A. lineolatus* nymphs, although there was occasional mortality of the pest in the absence of predators, the mortality was significantly greater in the presence of predators (Table 3.4.4). Introduction of predators did not result in significant mortality of *A. lineolatus* nymphs one day after the introduction ( $F_{1,31} = 1.31$ ; ns). However, compared with the control treatment, predators inflicted significant mortality of *A. lineolatus* nymphs two ( $F_{1,31} = 6.24$ ; P < 0.05), three ( $F_{1,31} = 7.52$ ; P < 0.05) and four  $(F_{1,31} = 12.66; P < 0.01)$  days after the introduction. When data only from predatorincluded treatments were considered, different predators inflicted statistically similar levels of mortality of *A. lineolatus* nymphs (Table 3.4.4). However, adults of *P. melanarius* and *N. alternatus* inflicted double the mortality that chrysopid larvae did (Table 3.4.4). There was a significant progression of host consumption by the predators over the four days ( $F_{3,93} = 47.44; P < 0.01$ ): average consumption almost doubled every day (Fig. 3.4.2). There was no significant interaction between the predator species and days the preys were exposed to predators ( $F_{12,93} = 1.71;$  ns). In the first day, consumption by the predators was <20% of the available number of preys, and the consumption increased over the four days (Fig. 3.4.2).

There was marginal mortality of *A. pisum* adults in the absence of predators (Table 3.4.4). Mortality of *A. pisum* adults was significantly higher than the control in all treatments with predatory insects ( $F_{1,54}$  =110.82; 210.92; 321.74; and 359.13; *P* < 0.01 in all cases, one, two, three, and four days after introduction, respectively). When data only from predator included treatments were considered, different predators inflicted significantly different levels of prey mortality (Table 3.4.4). Both adult and larval *C. septempunctata* consumed all of the *A. pisum* prey adults available. The consumption of *A. pisum* adults by larval *C. carnea* was less than 100%, but did not significantly differ from that of the other tested predators. *Acyrthosiphon pisum* adult consumption by *N. alternatus* and *P. melanarius* was less than that by adult and larval *C. septempunctata*, although, *P. melanarius* adults consumed the fewest *A. pisum* adults (Table 3.4.4). There was significant progression of prey consumption by the predator insects over days ( $F_{3,162}$  = 55.85; *P* < 0.01), and a significant interaction of predator species with days ( $F_{12,162}$  =

1.85; P < 0.05). Coccinella septempunctata adults and larvae consumed over 85% of A. pisum adults in the first day. The remaining predators consumed most A. pisum adults by the second day, when consumption by N. alternatus and P. melanarius increased sharply (Fig. 3.4.3). In cages with larval and adult C. septempunctata, no newborn aphids were found. In the presence of the remaining predator types, the mean numbers of newborn aphids was 36–57% of that in the control cages (Fig. 3.4.4).

### Discussion

At the end of the field trials, there were more predators present in cages in which predators were released than in those where no predators were released. Similarly, more *Lygus*, *A. lineolatus* or *A. pisum* were present in cages into which they were released. The results indicate that the integrity of cages was successfully maintained and the released insects were adequately contained in cages. Therefore, it is unlikely that results were significantly biased due to migration of predators and pests in or out.

The failure of predators in field cages to significantly reduce the number of *Lygus* and *A. lineolatus* adults is probably attributable to the size of potential prey insects. The size of prey items influences the extent of predation (Price 1997, and references therein). Although larger prey can be easily detected, successful predation depends on the ease with which the prey can be captured and subdued (Cogni *et al.* 2002). Perkins and Watson (1972) found that the consumption of *Lygus hesperus* nymphs by *N. alternatus* diminished with increasing age and size of the nymphs. Older nymphs of *Lygus* and *A. lineolatus* were used in the present study, and they would have reached adulthood within a few days of their release (Sorensen 1939; Khattat and Stewart 1977). The optimum

temperature regime for the fourth instar nymphs of *L. lineolaris* is 24–28°C, and at that temperature a fourth instar nymph reaches adulthood in 7–8 days (Khattat and Stewart 1977). During the time the present experiment was conducted, the average temperature was near 18–20°C (Appendix I), and at a temperature regime of 16–20°C a fourth instar nymph of *Lygus lineolaris* can reach adulthood in less than 15 days (Khattat and Stewart 1977). Once nymphs reached adulthood, they were likely to be less vulnerable to predation (Clancy and Pierce 1966), probably due to enhanced mobility and defensive behaviour of adult prey. *Pterostichus melanarius* adults are also size-dependent predators (Hagley *et al.* 1982).

The presence of predators in field cages reduced the numbers of nymphs of *Lygus* and *A. lineolatus* by  $\geq$ 36%, and this indicates the effectiveness of predators on younger individuals. The nymphs that were present at the time of data collection were late instars and were probably not the result of introductions but instead developed from eggs that were present at the time the insecticide was applied in the cages. In the present study, data were collected one month after *Lygus* and *Adelphocoris* were released as late instar nymphs. Offsprings of the newly emerged *Lygus* adults take about four and five weeks to reach fourth instar at 20 and 24°C, respectively (Khattat and Stewart 1977), and a new adult of *A. lineolatus* requires more than five weeks to produce fourth instar nymphs at about 22°C (Hughes 1943). Therefore, there was insufficient time for late-instar nymphs to develop as offsprings of the released nymphs. Results in the present study suggest that the predators are more efficient consumers of young nymphs than of older nymphs and adults, as was stated by Clancy and Pierce (1966). Note that although the numbers of *Lygus* nymphs released in field cages were the same in both 2000 and 2001, the number

of *Lygus* adults captured during data collection was much lower in 2000 and than in 2001. The reason for this difference is unclear. During the time when the study was conducted, it was cooler in 2000 than in 2001 (Appendix I), which probably slowed down nymphal development (Khattat and Stewart 1977) allowing more time, for predators to prey on nymphs in 2000. Although, temperature reduction may affect the predation, the effect was probably overcompensated by longer availability of nymphs as preys. In addition, field-collected predators were used in these studies, and the physiological conditions of predators used in 2000 were likely to differ from those in 2001. At the end of the trial, comparatively more predators were present in both control and predator introduced cages in 2000 (Table 3.4.1) than those in 2001 (Table 3.4.2). All these might have contributed to the result.

Results of the laboratory trials suggest that among the species tested, *N. alternatus* was the most effective *Lygus* bug predator, and this is consistent with previous reports with *Nabis* spp. (Knowlton 1949; Clancy and Pierce 1966; Perkins and Watson 1972; Tamaki *et al.* 1978).

Pterostichus melanarius and N. alternatus appeared as the most voracious predators of A. lineolatus nymphs. In an observational field study, Wheeler (1977) found Nabis spp. to consume A. lineolatus nymphs. Pterostichus melanarius is a polyphagous predator and can reduce numbers of pests in field crops (Coaker and Williams 1963; Johnson and Cameron 1969; Plotkin 1981; Wallin *et al.* 1992; Wallin and Ekbom 1994). The behaviour of insects is influenced by their habitats (Tukahirwa and Coaker 1982; Fournier and Loreau 2001). In the present laboratory trials, predators were in a simple habitat with no prey choice, so the results do not necessarily represent the effects of these

predators in complex natural habitats in which a variety of predator and prey species cooccur.

The tested predators also significantly reduced the populations of A. pisum in field cages. Cages in which no A. pisum were released also had some A. pisum (Table 3.4.1). However, as indicated by the large value of the standard error, most A. pisum individuals in cages in which they were not released were found in one cage. These A. pisum individuals probably represented colonies developed from a few fortuitous survivors of insecticide sprays. In general, the presence of predators reduced A. pisum numbers, and this is consistent with previous observations (Taylor 1949; Cooke 1963; Smith and Hagen 1966; Neuenschwander et al. 1975; Frazer and Gilbert 1976; Baumgaertner et al. 1981; Frazer et al. 1981b; Chambers et al. 1983; Messina et al. 1997). In a different part of this study (Chapter 3.2), numbers of predaceous insects were found to be correlated with population growth of A. pisum in alfalfa fields. Results in the laboratory trials further complement those from the field cages. In the laboratory, both adult and larval C. septempunctata, and larval C. carnea were voracious feeders on aphids. Some researchers contend that green lacewing larvae are usually voracious predators of aphids (Canard and Duelli 1984; Messina et al. 1997; Canard 2001). Neuenschwander et al. (1975), on the other hand, documented through laboratory cage trials that larval chrysopids are less voracious than larval and adult coccinellids.

Except on one occasion, the presence of predators did not result in a significant effect on the total amount of seed production, or the percentage of healthy seeds. This may be attributable to the late time and short duration of the exposure of plants to the insects. Published reports suggest that late season population of mirids have little influence on seed alfalfa yield (Charnetski 1983*a*, 1983*b*). Caging one *A. lineolatus* with two alfalfa stems from late July until seed harvest, Soroka and Murrell (1993) also did not find significant yield reduction, although they found seed production to be reduced at higher pest populations. Furthermore, the difference in the number of the insects, particularly of *Lygus* and *A. lineolatus* caused by the predators may not have been great enough to cause a significant effect on yield and yield associated attributes. Exposing plants to pest insects from relatively early in the season and for a longer duration might have produced more effect on yield. Nevertheless, the introduction of predators with *Lygus* bugs promoted 30-79 kg/ha more seeds, which indicates economically significant benefits of predator presence.

The present field studies showed that predators can reduce numbers of *Lygus* spp., *A. lineolatus* and *A. pisum*, which are the major damaging insects in alfalfa fields, and the laboratory trials indicated that some of these predators were more effective than the others in reducing the numbers of specific insect pests, which is not surprising (Banks 1999). In natural habitats, there may be competition, predation and interference among co-existing predators (Phoofolo and Obrycki 1998). However, an assemblage of multi-species predators that work in different strata within the plant canopy in the field can reduce pest populations further than a single species, as the effects of multiple predators on pest populations are usually additive (Chang 1996). For example, predator activities on plants may provoke an escape response in which aphids fall to the ground (McAllister and Roitberg1987; Losey and Denno 1998), where they become available to epigaeic predators. Carabid beetles are mostly active on the ground, and many pest insects including *Lygus* spp. overwinter under leaf litter. Pests overwintering under leaf litter

may be preyed upon by the ground-active predatory carabid beetles. However, the extent of such predation and the resulting effects on overall pest populations remain unclear. Although carabid beetles are active in relatively colder conditions (Holland 2002), they also overwinter, suggesting that predation on overwintering pest insects might not be that great. Sunderland (2002) reviewed the roles of carabid beetles on pest populations in crop fields, and concluded that carabids alone seldom inflict significant mortality on pest populations; however, there has been frequent evidence that carabid beetles in assemblage with other predators often reduce pest populations greatly. Results of the present studies provide evidence that the predator guild can play an important role in reducing populations of insect pests in alfalfa fields.

Pest	Predators	Lygus spp.		Acyrthosiphon pisum	Predators	
		Adults	Nymphs			
No	No	$2.0 \pm 1.0$	$11.5 \pm 5.5$	$470.5 \pm 161.5^{ab}$	$5.0 \pm 4.0$	
Lygus spp.	No	$9.5 \pm 2.5$	$8.5 \pm 0.5$	-	$2.5 \pm 0.5$	
	Yes	$4.0 \pm 1.0$	$5.5 \pm 2.5$	-	$19.5 \pm 10.5$	
Acyrthosiphon	No	-	-	$2821.0 \pm 261.5$ <sup>a</sup>	$3.5 \pm 2.5$	
pisum	Yes	-	-	$201.5 \pm 82.5$ <sup>b</sup>	$11.5 \pm 4.5$	
Statistics		$F_{2,3} = 5.64$ ; ns	$F_{2,3} = 0.83$ ; ns	$F_{2,3} = 17.84; P < 0.05$	$F_{1.8} = 12.92; P < 0.01$ (overall)	
		$(F_{1,2} = 5.37; \text{ns})$	$(F_{1,2} = 1.24; \text{ ns})$	$(F_{1,2} = 37.59; P < 0.05)$	$F_{2,3} = 2.91$ ; ns (Lygus)	
		. ,			$(F_{1,4} = 7.17; P \approx 0.05)$ (Lygus)	
					$F_{2,3} = 1.61$ ; ns (A. pisum)	
					$(F_{1,4} = 3.93; \text{ ns})$ (A. pisum)	

Table 3.4.1. Number (mean ± SE) of pests and predator guild in field cages one month after their introduction, 2000.

Means within columns marked with the same letter were not significantly different (Tukey's test at experiment-wise alpha < 0.05).

Statistics in parentheses corresponding to pest species resulted from the analysis of data excluding cages that received no pest or predators.

Statistics in parentheses in the column for predators resulted from the analysis of data including those from cages that received no pest or predators.

Pest	Predators	Lygus spp. or Adelphocoris lineolatus				Predators		
		Adults	Statistics	Nymphs	Statistics	Total	Statistics	
Lygus spp.	No Yes	$43.8 \pm 17.4$ $24.3 \pm 6.9$	$F_{1,6} = 1.08;$ ns	$7.3 \pm 3.3$ $1.3 \pm 0.5$	$F_{1,6} = 13.16;$ $P \approx 0.01$	$0.8 \pm 0.5$ 14.8 ± 2.2	$F_{1,6} = 55.60;$ P < 0.01	When considered
Adelphocoris lineolatus	No Yes	$22.8 \pm 6.5$ $18.0 \pm 5.5$	$F_{1,6} = 0.31;$ ns	$\begin{array}{c} 4.0 \pm 1.4 \\ 0 \end{array}$	$F_{1,6} = 8.94;$ P < 0.05	$1.5 \pm 0.3$ $13.0 \pm 1.1$	$F_{1,6} = 147.32;$ P < 0.01	together: $F_{1,14} = 126.18;$ P < 0.01

Table 3.4.2. Number (mean ± SE) of pests and predator guild in field cages four weeks after their introduction, 2001.

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Treatments		2000				2001			
Insects	Predators	Seeds (g/m <sup>2</sup> )	Statistics	Healthy seeds (%)	Statistics	Seeds (g/m <sup>2</sup> )	Statistics	Healthy seeds (%)	Statistics
No	No	$6.5 \pm 1.7$		$73.0 \pm 6.0$					
<i>Lygus</i> spp.	No Yes	$4.3 \pm 0.9$ $12.2 \pm 1.3$	$F_{2,3} = 9.25; P \approx 0.05$ ( $F_{1,2} = 24.79; P < 0.05$ )			$16.0 \pm 1.8$ $18.3 \pm 2.8$	$F_{1,6} = 0.48;$ ns	$65.5 \pm 3.7$ $68.0 \pm 4.3$	$F_{1,6} = 0.20;$ ns
Acyrthosiphon pisum	No Yes	$9.7 \pm 1.5$ $6.0 \pm 3.5$			$F_{2,3} = 0.86$ ; ns ( $F_{1,2} = 7.54$ ; ns)				

Table 3.4.3. Yield (mean ± SE) of alfalfa seeds as influenced by the introduction of predators into field cages, 2000 and 2001.

Statistics in parentheses corresponding to pest species resulted from the analysis of data excluding cages that received no pest or predators.

	Insect pests								
Predators	Lygus spp.	Statistics	Adelphocoris	Statistics	Acyrthosiphon	Statistics			
			lineolatus		pisum adults				
No (control)	0 (4)	$F_{5,40} = 4.16;$ P < 0.01	8 ± 8 (4)	$F_{5,31} = 3.40;$ P < 0.05	$1 \pm 1$ (10)	$F_{5,54} = 84.02;$ P < 0.01			
<i>Chrysoperla carnea</i> larva	$58 \pm 12^{ab}$ (8)	$(F_{4,40} = 2.60; P \approx 0.05)$	$40 \pm 13$ (5)	$(F_{4,31} = 1.59;$ ns)	$89 \pm 5^{ab}$ (10)	$(F_{4,54} = 84.02; P < 0.01)$			
Coccinella septempunctata adult	$58 \pm 8^{ab}$ (8)		$50 \pm 11$ (8)		$100 \pm 0^{a}$ (10)				
Coccinella septempunctata larva	$41 \pm 7^{b}$ (9)		$58 \pm 12$ (8)		$100 \pm 0^{a}$ (10)				
Nabis alternatus adult	$88 \pm 9^{a}$ (8)		$79 \pm 10$ (8)		$83 \pm 5^{b}$ (10)				
Pterostichus melanarius adult	$67 \pm 10^{ab}$ (9)		83 ± 10 (4)		$79 \pm 5^{b}$ (10)				

Table 3.4.4. Percent mortality (mean  $\pm$  SE) of different insect pests four days after exposure to predators in the laboratory.

Means within columns and among predator species marked with the same letter were not significantly different (Tukey's test at experiment-wise alpha < 0.05).

Values in parentheses represent the total number of replication from which corresponding mean and SE were calculated. Statistics in parentheses represent data from predator-included treatments only (data from the control treatment were excluded). Fig. 3.4.1 Consumption of *Lygus* bugs by different species of predaceous insects in laboratory cages.

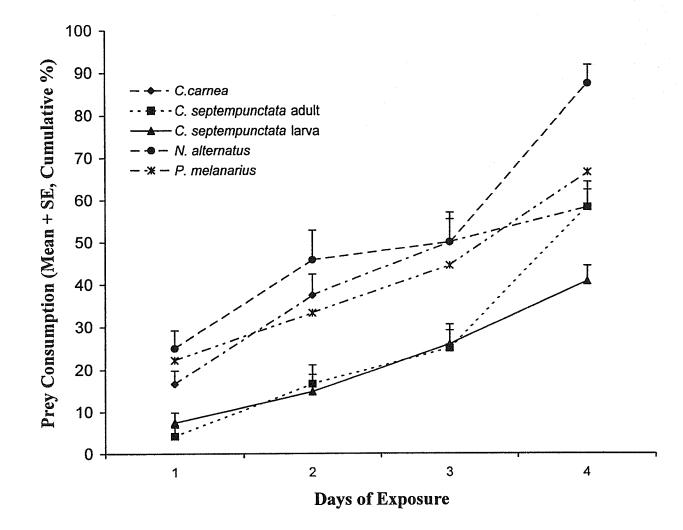


Fig. 3.4.2 Consumption of *Adelphocoris lineolatus* bugs by different species of predaceous insects in laboratory cages.

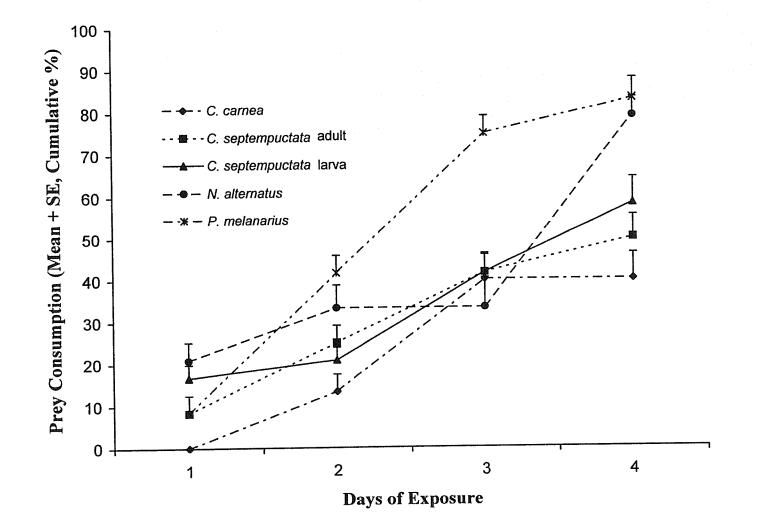


Fig. 3.4.3 Consumption of *Acyrthosiphon pisum* adults by different species of predaceous insects in laboratory cages.

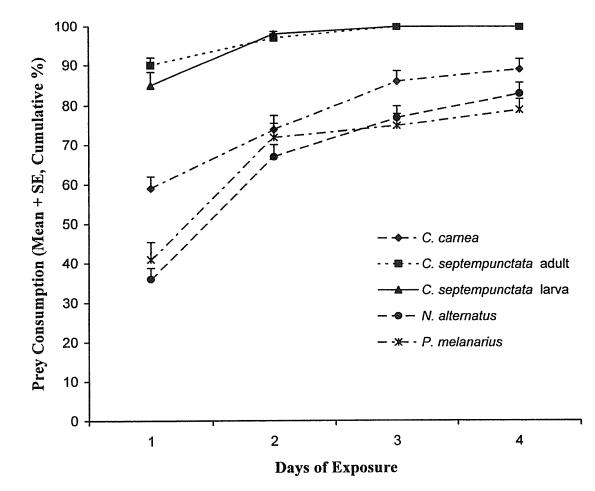
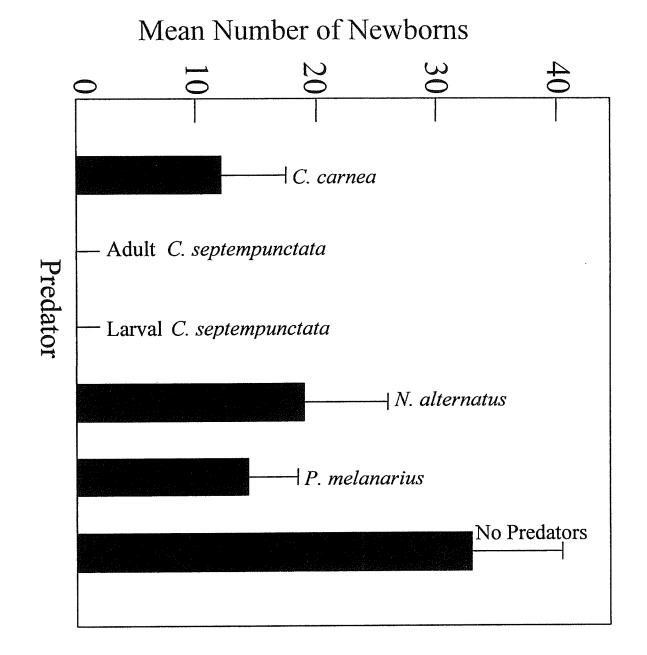


Fig. 3.4.4 Mean number ( $\pm$  SE) of newborn *A. pisum* in laboratory cages four days after introduction of predators.



# CHAPTER 3.5

Response of insect natural enemies to nepetalactone in fields of alfalfa in the

**Canadian** Prairies

### Abstract

The response of insect pests and their natural enemies to nepetalactone, a component of aphid sex pheromone, was examined in fields of alfalfa, *Medicago sativa* Linnaeus (Leguminosae), in Manitoba. Two treatments, control and nepetalactone, were applied in a nested split plot design, in which the distance of treatment application from the field margin was the sub-plot factor. Males of the lacewing, *Chrysopa oculata* Say (Neuroptera: Chrysopidae), were consistently attracted to the compound, but the response of the aphid parasitoid, *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae), was not consistent. The presence of host plants modified the pattern of lacewings' response to the compound across the field: lacewings did not penetrate 50 m or further deep in the field until regrown plants regrew considerably. The attraction of natural enemies did not result in significant reduction of pest populations in fields of alfalfa, although there was a consistent but nonsignificant reduction in numbers of pea aphids, *Acyrthosiphon pisum* (Harris). Placement of lures more effectively in terms of the time of placement, doses of lures and the techniques of lure placement may improve the results.

## Introduction

Alfalfa, Medicago sativa Linnaeus (Leguminosae), is an important forage crop, which is grown for hay and seed production in Canada. Fields of alfalfa for hay production are frequently grown next to seed fields of the same crop. The crop has a perennial growth habit and complex plant structure, which favour diverse assemblages of insect pests and their natural enemies (Summers 1998). Insects causing major damage to alfalfa in the Canadian Prairies include Lygus bugs, Lygus spp., alfalfa plant bug, Adelphocoris lineolatus (Goeze) (Hemiptera: Miridae) and pea aphid, Acyrthosiphon pisum (Harris) (Homoptera: Aphididae) (Harper 1978, 1988; Schaber and Entz 1988; Soroka and Murrell 1993; Timlick et al. 1993). In Manitoba these insect pests cause economic damage to alfalfa grown for seed but not to that for hay, probably because the economics of the crop types differ and because mowing removes insect pests from the hay field (Khattat and Stewart 1980; Schaber et al. 1990b). Mowing may also displace natural enemies from the mown field (Harper et al. 1990; Shcaber et al. 1990b). Attraction of these displaced natural enemies into adjacent seed fields would be expected to increase natural suppression of insect pests. Nepetalactone, which is a component of aphid sex attractant pheromones (Dawson et al. 1988), might be useful in attracting the displaced natural enemies. Nepetalactone has been shown to attract aphid parasitoids and lacewings in Europe and Korea (Abdel-Kareim and Kozár 1988; Dawson et al. 1988; 1990; Hardie et al. 1991, 1993, 1994; Gabryś et al. 1997; Boo et al. 1998).

In this paper, responses of insects to nepetalactone were examined in fields of alfalfa in order to determine whether the compound can be used to attract natural enemies and augment natural biological control in seed fields. Large- and small-scale trials were conducted in the context of commercial alfalfa production in Manitoba, whereas the previous work has been done on relatively small-scale in cereals, crucifers, orchards and woodlands (Hardie *et al.* 1994; Gabryś *et al.* 1997; Boo *et al.* 1998) in Europe and Asia.

## **Materials and Methods**

Large- and small-scale field trials were conducted during the summers of 2000 and 2001. The large-scale trials were conducted in two alfalfa seed fields in each year and in one alfalfa hay field in 2001. The small-scale trial was conducted in an alfalfa seed field in 2001.

## Large-scale trials in seed fields

**Field set up and nepetalactone release**. The study was conducted at two locations in Manitoba, Arborg (Latitude 50° 56' N; Longitude 97° 5' W) and Riverton (Latitude 50° 59' N; Longitude 96° 59' W). The fields were more or less square and ranged from 18 to 44 ha. At each location, the selected seed field shared a common margin with a hay field. In accordance with normal production practice, the seed field was sprayed with insecticide in late June. A week after spraying, the seed field was divided into four strata that were perpendicular to the shared margin and two treatments, control and nepetalactone, were applied to the strata alternately (Fig. 3.5.1). A 40 x 7 mm (l x w) piece of 3 mm thick plastic strip impregnated with nepetalactone was used as a dispenser. In the nepetalactone strata, three 5 x 5 m plots were established with their closest edges at 5, 50, and 95 m from the shared margin. In each plot five lures were placed at the height of 1–2 cm above the top of the plant canopy, one on each corner and one in the middle. Immediately after lure placement, the adjacent hay field was mown.

Insect sampling. The fields were sampled once a week. In 2000 and 2001 water trap samples were taken for three weeks after lure deployment. A water trap consisted of a 16  $x 16 \times 5$  cm (1 x w x d) transparent polyethylene dish three quarters filled with saturated salt solution. The traps were placed below the middle lure of each nepetalactone plot so that the lure was suspended centrally above the water surface. One drop of liquid soap was added to each 4 l of salt solution. Water traps without lures were placed in corresponding positions in the control strata. At weekly intervals, traps were emptied, and the salt solution was replaced with fresh solution. In addition to these water trap samples, sweep net samples, each of 15 sweeps, were taken each week from lure deployment until the end of August. Sweep net samples were taken along lines parallel to the shared field margin outward from the nepetalactone plots and corresponding plots in the control strata. In successive weeks, the side of the plot for sweeping was alternated. Sweep net samples were also taken at 10 and 20 m from the nepetalactone plots and at corresponding positions in the control strata (Fig. 3.5.1). Preliminary analyses showed that distance to the plot did not significantly affect insect catch. Therefore, sweep net data recorded at 10 and 20 m intervals were pooled with data from their adjacent plots. In 2001 vacuum samples were also taken at weekly intervals for three weeks. Vacuum samples were taken covering the entire  $5 \ge 5$  m area in nepetalactone plots and in corresponding plots in the control strata.

Data were transformed  $\{\log_{10} (X+1)\}$  when needed and repeated measures split plot analysis of variance was performed. After preliminary analysis of each field separately, it was determined that a pooled analysis was appropriate. In the pooled analysis, field and year were considered as components of blocks. Therefore, two blocks

in two fields in two seasons resulted in a total of eight replicate blocks. Repeated measures were weeks. Terms in the among-subject repeated-measures analysis were nepetalactone treatment, replicate blocks, distance to the field margin, and nepetalactone treatment x distance interaction. Nepetalactone treatment was tested as a main plot effect, and distance and its interaction as subplot effects. In the within-subject analysis, week since nepetalactone deployment, and its interactions with all the above were included. Throughout the study, an  $\alpha$  level of 0.05 was used. In reporting results, interaction terms are presented only if they were significant.

# Large-scale trial in the hay field

The trial was conducted in a square shaped 24 ha hay field of alfalfa near Arborg, Manitoba. The field was separated from another alfalfa hay field by an 8 m wide gravel road running north-south, on which vehicles ran occasionally. On either side of the road were ditches, which were usually dry and full of grassy vegetation. The total distance between the two hay fields was about 20 m. The study field was mown on 12 June 2001. Ten days after mowing, strata were laid out, and lures and water traps were deployed in the field as described for the seed field trial (Fig. 3.5.1). Sweep net samples, vacuum samples and water trap samples were taken for three weeks as described for the seed field trials. In addition, walking from one side to the other of the plots (5 x 5 m), two people checked alfalfa plants for aphid mummies in nepetalactone treated plots and in corresponding areas in the control strata and counted the mummies in the third week. Data were transformed  $\{log_{10} (X+1)\}$  when required and repeated measures split plot analysis of variance was performed as described above.

# Small-scale trial in the seed field

This trial was conducted in 2001 in a seed field that was separated by a 5 m wide ditch from a hay field of alfalfa near Teulon (Latitude 50° 25' N; Longitude 97° 15' W), Manitoba. Treatments were applied on 4 July 2001, a week after the seed field was sprayed with insecticide. Two treatments, control and nepetalactone, were applied in a 2 x 2 Latin square design with the Latin square replicated twice, and the squares separated by 50 m. The plot size in the Latin squares was 6 x 6 m, and the plots were separated from each other by 6 m. The closest plots to the field margin were 5 m from it. Nepetalactone lures were placed in the central 1 x 1 m areas of plots assigned to the nepetalactone treatment. Placement of lures and water traps in the 1x1 m areas was as described for large-scale trials. The plots were sampled as before by taking water trap samples, vacuum samples, and sweep net samples once a week for three weeks. Aphid mummies were counted in each plot in the third week after nepetalactone deployment. Data were transformed  $(\log_{10} (X+1))$  when needed and analyzed as a modified Latin square repeated measures analysis; because between squares and columns effects were never significant, these were pooled with error (Sokal and Rohlf 1995) to increase analytical sensitivity.

#### Results

# Large-scale trials in seed fields

Water traps in nepetalactone treated strata caught significantly more lacewing adults than those in the control strata (Table 3.5.1). The lacewings were males of *Chrysopa oculuta* Say (Neuroptera: Chrysopidae). Lacewing captures did not differ across weeks, though catches in the third week were considerably higher than preceding

weeks. In the nepetalactone-treated strata, distance of traps from the field margin did not influence lacewing captures (Table 3.5.2).

Despite being attracted to nepetalactone, the number of lacewing adults collected by sweep net from the nepetalactone-treated strata did not differ from that in the control strata (Table 3.5.1). The lacewings collected in sweep-net samples in the trials in 2000 were not identified to species, but in 2001 both sexes of the lacewings, *C. oculata* and *Chrysoperla carnea* (Stephens), were found, and 55 and 62% of these adults were females, respectively. Of the two species, *C. carnea* was the dominant species representing 79% of the total adults, while *C. oculata* constituted 21% of adult chrysopids in sweep net samples. The number of lacewing larvae in nepetalactone treated strata was 20% higher (8–39% in individual fields) than in the control strata, although this difference was not significant. Distance to the field margin did not influence adult and larval lacewing catches (Table 3.5.2) nor did week since nepetalactone deployment.

There were occasional captures of the aphid parasitoid *Aphidius ervi* Haliday (Hymenoptera: Aphidiidae) in water traps, and in sweep net and vacuum samples. No *Praon* spp. individuals were found in water traps, however they appeared in low numbers in other samples. Neither nepetalactone treatment, nor the distance from the field margin produced any significant effect on the numbers of *A. ervi* and *Praon* spp. captured (Tables 3.5.1 and 3.5.2), except that catches of *A. ervi* in vacuum samples were marginally greater in nepetalactone-treated plots (Table 3.5.1).

Coccinellid adults and larvae were usually found in sweep net samples in the seed fields. The larvae were not identified to species; but adults were mostly *Coccinella septempunctata* (Linnaeus) (Coleoptera: Coccinellidae). There was no effect of nepetalactone or of the distance to the field margin on larval and adult ladybird beetle catches. Numbers of adult and larval coccinellids were low, and for simplicity, they are pooled in Tables 3.5.1 and 3.5.2.

There were also low numbers of the damsel bug, *Nabis alternatus* (Parshley) (Hemiptera: Nabidae), caught in sweep net samples in the seed fields. Nepetalactone deployment did not significantly influence nabid catches (Table 3.5.1). There was significant effect of the distance from the field margin (Table 3.5.2), although there was no significant distance x treatment interaction.

*Lygus* bugs were rarely found in water traps and vacuum samples, but they were usually present in sweep net samples. The species were predominantly *L. lineolaris* (Palisot de Beauvois) and *L. borealis* (Kelton), with the former species dominant in most samples. Neither nepetalactone, nor distance from the field margin significantly influenced *Lygus* bug catches (Tables 3.5.1 and 3.5.2). Numbers of *A. lineolatus*— which were found occasionally in water traps and vacuum samples, but regularly in sweep net samples – were not significantly influenced by nepetalactone deployment (Table 3.5.1). However, catches of *A. lineolatus* were significantly influenced by the distance from field margin: catches gradually declined with increased distances from the margin (Table 3.5.2). There was no significant treatment x distance interaction for *A. lineolatus* catches. The result was similar for *A. pisum*, except for there being no significant effect of distance from field margin on *A. pisum* catches (Tables 3.5.1 and 3.5.2).

#### Large-scale trial in the hay field

Results from water traps in the hay field trial were similar to those in the seed field trials for chrysopid adults, *A. ervi* and *Praon* spp. (Tables 3.5.1 and 3.5.2), except

that catches of chrysopid adults were related to distance from the field margin. In nepetalactone strata, no lacewings were captured in traps placed beyond 5 m in the hay field during the first two weeks of the trial. In the third week in nepetalactone strata, traps 5 m from the field margin captured more lacewings than did those at 50 and 95 m, but catches at 50 and 95 m did not differ (Fig. 3.5.2).

In sweep net and vacuum samples, catches of most insects were lower than in the trials in seed fields. However, both *A. ervi* and *Praon* spp. were found. For *A ervi* in sweep net samples in nepetalactone strata, catches at 5 and 50 m were marginally significantly greater than at 95 m from the field margin (Table 3.5.2), and there was no significant effect of week since lure deployment on this trend. Unlike seed field trials, vacuum samples from the nepetalactone treated strata in the hay field trial did not contain more *A. ervi* than from the control strata (Table 3.5.1), although the distance trend in vacuum samples was similar to that in sweep net samples (Table 3.5.2). Neither nepetalactone, nor the distance from the margin in nepetalactone treated strata influenced the number of aphid mummies in the hay field (Tables 3.5.1 and 3.5.2). However, aphid mummies were more abundant in plots 5 m from the field margin, and the trend matched with the general pattern of distribution of *A. ervi* in foliage samples (Table 3.5.2).

### Small-scale trial in the seed field

The only significant effect was that of nepetalactone treatment on adults of the chrysopid, *C. oculata* (Table 3.5.3). In the baited traps, lacewing catches in different weeks did not differ significantly, however, the number increased considerably in the third week. Individuals of *A. ervi* and *Praon* spp. were rarely caught in these traps. No individuals of *Praon* spp. were collected in sweep net samples. The distance trend for *A.* 

*ervi* was similar, although not significant, to that in the large-scale trials. The numbers of *Lygus* spp. and *A. lineolatus* in vacuum samples, and in sweep net samples were too low to be analyzed.

# Discussion

There were more *C. oculata* males in water traps in nepetalactone-treated strata. In trials in 2001, three females of *C. oculata* were obtained in nepetalactone-baited water traps, whereas no females of *C. oculata* were trapped in non-baited water traps. In seed fields, sweep net samples tended to contain nonsignificantly more of both sexes of *C. oculata* and *C. carnea* in nepetalactone strata. For *C. oculata*, although females can call, it is usually males that produce mating calls in the form of substrate-borne vibrations created by vigorous stereotyped jerking motions of the abdomen. These calls provoke females to search for mates (Henry 1984). There is little quantified account of distances over which these calls are effective, though Henry (1984) suggested them to be effective over short distances. Even if effective over short distances, courtship calls produced by males attracted to nepetalactone strata could in turn result in increased numbers of females.

Chrysopids are predominantly generalist predators, and they feed on a variety of soft-bodied arthropod prey, including aphids (Garland 1985; New 1988). Many aphid species use the same pheromone compounds (Dawson *et al.* 1990), which would allow their use as cues to locate aphid prey (Hooper *et al.* 2002). The attraction of chrysopids to nepetalactone is apparently analogous to that observed for the generalist predator, *Chrysopa cognata* Wesmael and aphid parasitoids in the genus *Praon* (Hardie *et al.* 1991, 1993, 1994; Boo *et al.* 1998). For predators, both males and females need to locate prey,

whereas for parasitoids, only females need to locate hosts (Boo et al. 1998). Boo et al. (1998) examined the attractiveness of nepetalactone to lacewings in both laboratory and field conditions in Korea. In olfactometer studies in the laboratory, they found attraction of both male and female C. cognata to nepetalactone. They also found attraction of C. cognata to nepetalactone-baited water traps in peach orchards, but did not mention the sex of the trapped lacewings. If both sexes of C. oculata respond to nepetalactone, as is the case for C. cognata, it is likely that nepetalactone is either an aggregation pheromone compound for them or is a cue associated with food. For predaceous species, both sexes use the same prey items, so it is likely that they would respond to the same chemical cues for food. However, Hooper et al. (2002) found that nepetalactone and its derivative compounds elicited responses from both predatory and non-predatory species of chrysopids, which suggest that the response was not related to food location. Furthermore, if these compounds were used by chrysopids as cues to locate oviposition sites, females should be the responsive group. Therefore, the compounds are probably used by chrysopids as cues related to intraspecific aggregation or copulation. Irrespective of feeding habit, both sexes would be expected to respond to an aggregation pheromone, although one sex could be responsive to sex-specific aggregation pheromone or sex attractant pheromone. There is evidence for the existence of pheromones in chrysopids (Szentkirályi 2001). Results in the present study that C. oculata males were attracted to nepetalactone suggests that nepetalactone by itself or compounds associated with nepetalactone is probably involved in male-specific chemical communication among C. oculata individuals. Chemical communication in different chrysopid species has been previously found (Boo et al. 1998; Hooper et al. 2002; Chauhan et al. 2004). Chauhan et

*al.* (2004) reported that iridodial is a male-specific aggregation pheromone in *C. oculata*, and the compound has been found as impurities (5–8%) in nepetalactol, which is a reduced product of nepetalactone.

The seed field trials did not reveal any significant effect of distance of nepetalactone placement from the field margin, but in hay fields, there were effects of distance and these changed over time (Fig. 3.5.2). I believe that lacewing adults were probably not present in the seed and hay fields immediately after the insecticide application and mowing, respectively. Thus, the difference between the distance relationships is unlikely to be due to different residual populations. Rather, it is likely that both nepetalactone and adequate vegetation cover are required to induce lacewings to enter a field. The alfalfa hay field was mown leaving about 10 cm stems but no foliage, and adequate vegetation cover was apparently not present until the third week of the trial, which was  $\geq 24-31$  days after mowing. The catches at the edge of the hay field during the first two weeks probably represent lacewing adults making exploratory flights into an area producing attractant cues but failing to provide suitable habitat. I have evidence for this only for C. oculata males as females of these species, and both sexes of C. carnea did not frequently enter water traps and few specimens were collected in foliage samples in the hay field.

In Manitoba, the pea aphid parasitoid guild comprises predominantly *A. ervi* (Matheson 1988). Glinwood *et al.* (1999) found that *A. ervi* responded to nepetalactone in wind tunnels. I did not find any evidence for the attraction of *A. ervi* to nepetalactone-baited water traps. *Aphidius* spp. are thought not to be attracted to water traps (Powell, personal communication). However, vacuum sampling tended to catch more *A. ervi* in

nepetalactone strata than in control strata in seed field trials, although, sweep net sampling failed to reveal any such trend. Vacuum samples were taken from a restricted area,  $\leq 3.5$  m from the nearest nepetalactone dispenser, whereas sweep net samples were taken from areas 2.5 to >20 m from the nearest nepetalactone dispensers. If A. ervi are attracted to nepetalactone and aggregate within a short distance of the attractant the observed pattern of sweep net and vacuum samples would result. Glinwood (1998) found that aphid sex pheromone compounds increased parasitization by the generalist parasitoids Praon volucre (Haliday) only adjacent to the pheromone dispensers, whereas the compounds increased parasitization by the specialist parasitoid A. rhopalosihpi De Stefani Perez even at distances 1 m away from dispensers. This suggests that generalist parasitoids are more likely to remain active close to the attractant. Aphidius ervi is a generalist parasitoid (Glinwood et al. 1999), and hence detection of the attraction in vacuum samples, but not in sweep net samples is more likely. But in the hay field no such trend was found, which was probably due to low occurrence of aphid hosts, as in areas with no or few hosts, parasitoids attracted to aphid sex pheromones may disperse away quickly in absence of other cues (Glinwood et al. 1999). Until the third week of the trial, aphids were hard to find in the hay field, but they were commonly found in seed fields. In the hay field, A. ervi numbers in vacuum samples tended to decline as distance from the field margin increased; the trend was numerically similar for both A. ervi in sweep net and vacuum samples, and aphid mummies. This pattern may be related to the unsuitability of the newly-mown hay field as a habitat. However, unlike the pattern for C. oculata, A. ervi penetrated more into the field. An A. ervi female lays up to 600 eggs in its lifetime (Kambhampati and Mackauer 1989). Schwörer and Völkl (2001) reported that

even when 10 aphids were available in a colony, less than two aphids were parasitized by an *A. ervi* female. This suggests that *A. ervi* females disperse their eggs widely, and the penetration of *A. ervi* is probably related to this oviposition behaviour of dispersing their eggs.

No evidence that nepetalactone attracted aphid parasitoids in the genus *Praon* was found. In Europe, *P. dorsale* Haliday, *P. volucre* Haliday, *P. abjectum* Haliday and *Aphytis myliaspidis* (Le Baron) are attracted to nepetalactone (Hardie *et al.* 1991, 1994; Gabryś *et al.* 1997). The difference between the present study's results and those from Europe may be because the parasitoid species are different, or because most of the present trials were at a much larger spatial scale than the European studies. In Manitoba, the dominant *Praon* that attack pea aphids are *P. pequodorum* Viereck and *P. occidentale* Baker (Matheson 1988), species for which response to nepetalactone has not been reported. The present small-scale trial used a spatial scale similar to some European studies in which *Aphidius* and *Praon* spp. responded to nepetalactone (W. Powell, pers. comm.). The absence of significant attraction of *Praon* spp. to nepetalactone in this trial suggests that scale as the sole cause of these different results can be eliminated.

Among other insects, although their numbers were not greater in nepetalactonetreated strata, numbers of *N. alternatus* and *A. lineolatus* declined greatly with increased distances from field margins. This could be the result of hay-cutting, which probably displaced insects causing them to migrate into adjacent seed fields of alfalfa that were used in this study. Migration of pest and predator species of insects from mown fields into adjacent seed fields of alfalfa has been noted previously (Schaber *et al.* 1990*b*; Chapter 3.2, this thesis). The detection of such migration is dependent upon the mobility,

physiological stages of arthropods and host plants, and the time of the season (Hughes 1943; Khattat and Stewart 1980; Schaber *et al.* 1990*b*). *Adelphocoris lineolatus* is a relatively less mobile species and they usually move less than 3m at a time (Hughes 1943). In contrast, *Lygus* species, which were commonly present in fields, are very mobile insects capable of penetrating deep into fields in a short span of time (Khattat and Stewart 1980; Schaber *et al.* 1990*b*), which probably made the distance effect inconspicuous for *Lygus* spp.

The present study indicates that nepetalactone is a potential attractant for insect natural enemies including predatory chrysopids and aphidiids, which parasitize *A. pisum* on legume crops (Starý 1976; Kambhampati and Mackauer 1989). However, pest insects were not significantly reduced in nepetalactone strata. There was a consistent but not significant trend of reduction in aphid numbers. Glinwood *et al.* (1999) concluded that careful timing of aphid semiochemical deployment based on knowledge of both pest and parasitoid local ecology is important. The present study found evidence that deploying nepetalactone had some benefits and it may be that exploring different methods and times of lure deployment could increase these benefits.

			Seed fi	elds		Hay f	ield <sup>1</sup>
		<u></u>	Nepeta-			Nepeta-	
Samples	Insects	Control	lactone	Statistics	Control	lactone	Statistics
Water trap	Chrysopid adults	$0.1 \pm 0.1$	$7.8 \pm 1.6$	$F_{1,7} = 97.6; P < 0.01$	0	$3.4 \pm 0.9$	$F_{1,2} = 60.1; P < 0.05$
(number/	Aphidius ervi	$0.1 \pm 0.0$	$0.1 \pm 0.0$	$F_{1,7} = 1.34$ ; ns	$0.3 \pm 0.1$	$0.2\pm0.1$	$F_{1,2} = 1.8$ ; ns
trap/week)	Praon spp.	0	0		0	0	
Sweep net	Chrysopid adults	$0.2 \pm 0.1$	$0.3 \pm 0.1$	$F_{1,7} = 0.95$ ; ns	0	0	
(number/15	Chrysopid larvae	$1.0 \pm 0.1$	$1.2 \pm 0.2$	$F_{1,7} = 3.12$ ; ns	0	0	
sweeps)	Coccinellids	$1.2 \pm 0.4$	$1.2 \pm 0.4$	$F_{1,7} = 0.62$ ; ns	0	0	
	Nabis alternatus	$0.4 \pm 0.1$	$0.3 \pm 0.1$	$F_{1,7} = 1.25$ ; ns	0	0	
	Aphidius ervi	$0.9 \pm 0.1$	$0.8 \pm 0.1$	$F_{1,7} = 0.44$ ; ns	$0.8\pm0.3$	$0.6\pm0.1$	$F_{1,2} = 0.9$ ; ns
	Praon spp.	$0.2\pm0.0$	$0.1 \pm 0.0$	$F_{1,7} = 0.28$ ; ns	0	$0.1 \pm 0.1$	$F_{1,2} = 1.0$ ; ns
	Lygus spp.	$8.2 \pm 1.2$	$8.6 \pm 1.3$	$F_{1,7} = 4.26$ ; ns			
	Adelphocoris lineolatus	$18.0 \pm 3.2$	$17.1 \pm 2.6$	$F_{1,7} = 0.11$ ; ns			
	Acyrthosiphon pisum	$66.9\pm21.2$	$63.6\pm20.1$	$F_{1,7} = 0.59$ ; ns			
Vacuum samples	Aphidius ervi	$0.5 \pm 0.1$	$0.8 \pm 0.3$	$F_{1,3} = 5.86; P < 0.10$	$0.8 \pm 0.2$	$0.8 \pm 0.3$	$F_{1,2} = 0; \text{ ns}$
(number/plot)	Praon spp.	0	$0.1 \pm 0.0$	$F_{1,3} = 2.95$ ; ns	0	0	
Aphid mummies (number/plot)	Acyrthosiphon pisum				9.7 ± 1.6	12.8 ± 5.0	$F_{1,2} = 0.9; \text{ ns}$

Table 3.5.1. Numbers (mean  $\pm$  s.e.) of insects in relation to treatments in large-scale trials in four seed fields and one hay field.

Numbers (means) are average of captures over the weeks.

ns = not significant. <sup>1</sup> Data missing indicates that no records of corresponding insects were kept. Block effect was not significant, so block effect was pooled with error to increase the sensitivity of the test.

				eed field				Hay field <sup>1</sup>	
a 1	_	Distance	e from field m	nargin (m)	_	Distance	from field n		
Samples	Insects	5	50	95	Statistics	5	50	95	Statistics
Water trap (number/trap	Chrysopid adults	7.6 ± 1.4	$7.5 \pm 1.4$	8.3 ± 2.4	$F_{2,28} = 0.13$ ; ns	$6.5 \pm 0.2$	1.7 ± 1.3	2.2 ± 1.5	$F_{2,4} = 52.89; P < 0.01$
/week)	Aphidius ervi	$0.1 \pm 0.0$	$0.1 \pm 0.0$	$0.1 \pm 0.0$	$F_{2.28} = 0.45$ ; ns	$0.2 \pm 0.2$	$0.2 \pm 0.2$	$0.2 \pm 0.2$	$F_{2,4} = 1.40$ ; ns
	Praon spp.	0	0	0	_,,	0	0	0.2 2 0.2	- 2,4
Sweep net	Chrysopid adults	$0.3\pm0.1$	$0.3 \pm 0.1$	$0.4 \pm 0.1$	$F_{2,28} = 0.81$ ; ns	0	0	0	
(number/15	Chrysopid larvae	$1.1 \pm 0.2$	$1.2 \pm 0.2$	$1.2 \pm 0.3$	$F_{2,28} = 1.96$ ; ns	0	0	0	
sweeps)	Coccinellids	$1.5 \pm 0.5$	$1.2 \pm 0.6$	$1.1 \pm 0.3$	$F_{2.28} = 0.13$ ; ns				
	Nabis alternatus	$0.5\pm0.1$	0.3 ± 0.1	$0.2\pm0.1$	$F_{2,28} = 4.32; P < 0.05$				
	Aphidius ervi	$0.7 \pm 0.2$	$0.7 \pm 0.2$	$0.9\pm0.2$	$F_{2,28} = 1.12$ ; ns	$0.7 \pm 0.3$	$0.8 \pm 0.2$	$0.2 \pm 0.2$	$F_{2,4} = 6.20; P \approx 0.06$
	Praon spp.	$0.1 \pm 0.1$	$0.2 \pm 0.1$	$0.1 \pm 0.1$	$F_{2,28} = 0.87$ ; ns	$0.2 \pm 0.2$	0.0 ± 0.2	0.2 ± 0.2	*
	Lygus spp.**	$8.5 \pm 1.4$	$9.5 \pm 1.7$	$7.3 \pm 0.8$	$F_{2,24} = 0.26$ ; ns		-	Ť	
	Adelphocoris lineolatus	$19.1 \pm 4.0$	$17.5 \pm 2.2$	$14.0 \pm 2.0$	$F_{2,28} = 2.18$ ; ns				
	Acyrthosiphon pisum	67.5 ± 13.7	$58.8\pm20.2$	$66.0 \pm 18.8$	$F_{2,28} = 0.66$ ; ns				
Vacuum samples	Aphidius ervi	$0.9 \pm 0.1$	$1.1 \pm 0.1$	$0.5 \pm 0$	$F_{2,12} = 1.58$ ; ns	$1.3 \pm 0.7$	$0.8 \pm 0.2$	$0.2 \pm 0.2$	$F_{2,4} = 5.01; P \approx 0.08$
(number/plot)	Praon spp.	$0.1 \pm 0.1$	$0.1 \pm 0.1$	0	*	0	0.0 ± 0.2	0.2 ± 0.2	1 2,4 5.01, 1 ~ 0.08
Aphid mummies (number/plot)	Acyrthosiphon pisum					22.5 ± 14.5	8.5 ± 2.5	7.5 ± 4.5	$F_{2,4} = 3.62$ ; ns

Table 3.5.2. Numbers (mean ± s.e.) of insects at different distances from the field margin in the nepetalactone treated strata in large-scale trials in four seed fields and one hay field.

Numbers (means) are average of captures over the weeks. ns = not significant. \* = Analysis was not possible, because data had little variability to be analyzed. \*\* Four (4) values were missing and df was adjusted to 24

<sup>1</sup>Data missing indicates that no records of corresponding insects were kept because of their low occurrence.

There was no significant interaction between treatments and distances. Therefore, data from control were excluded to increase sensitivity of the analysis for distance effect.

Table 3.5.3. Numbers (mean  $\pm$  s.e.) of insects in relation to different treatments and distances of nepetalactone deployment from the field margin in the nepetalactone treated strata in the small-scale trial.

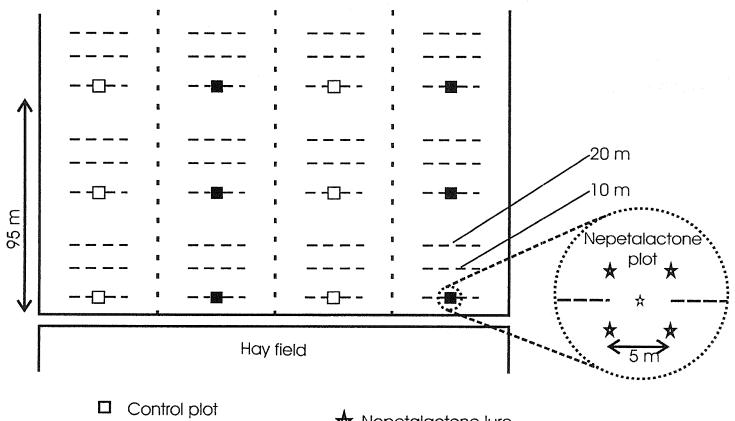
						ance	
		Tre	atments		(nepetalac	tone plots)	
Samples	Insects	Control	Nepetalactone	Statistics	8 m	20 m	Statistics
Water trap (number/trap/week)	Chrysopid adults	0	6.9 ± 1.1	$F_{1,3} = 73.59; P < 0.01$	5.8 ± 1.3	8.0 ± 1.9	$F_{1,2} = 0.10;  \text{ns}$
Sweep net (number/15 sweeps)	Aphidius ervi Praon spp.	$0.2 \pm 0.1 \\ 0$	$\begin{array}{c} 0.3 \pm 0.2 \\ 0 \end{array}$	*	$\begin{array}{c} 0.3\pm0.2\\0\end{array}$	$0.2 \pm 0.1$ 0	*
	Acyrthosiphon pisum	$62.9\pm8.5$	$62.2 \pm 8.2$	$F_{1,3} = 0$ ; ns	$60.0 \pm 11.1$	$64.5 \pm 13.8$	$F_{1,2} = 0.13$ ; ns
Vacuum samples (number/plot)	Aphidius ervi Praon spp.	$1.0 \pm 0.3$ $0.1 \pm 0.1$	$\begin{array}{c} 1.3 \pm 0.6 \\ 0 \end{array}$	$F_{1,3} = 0.60; \text{ ns}$	$1.5 \pm 0.6$ $0.1 \pm 0.1$	$\begin{array}{c} 0.8\pm0.8\\0\end{array}$	$F_{1,2} = 0.22$ ; ns *
Aphid mummies (number/plot)	Acyrthosiphon pisum	$13.6 \pm 4.7$	13.5 ± 3.3	$F_{1,3} = 0.07$ ; ns	15.0 ± 5.3	12.0 ± 4.7	$F_{1,2} = 0.17$ ; ns

Numbers (means) are average of captures over the weeks.

\* = Analysis was not possible, because data had little variability to be analyzed.

Square and column effects were not significant. Therefore, square and column effects were merged with error term to increase error df and sensitivity of the test for placement of nepetalactone (distance) effect.

Fig. 3.5.1. Layout of the large-scale trials.

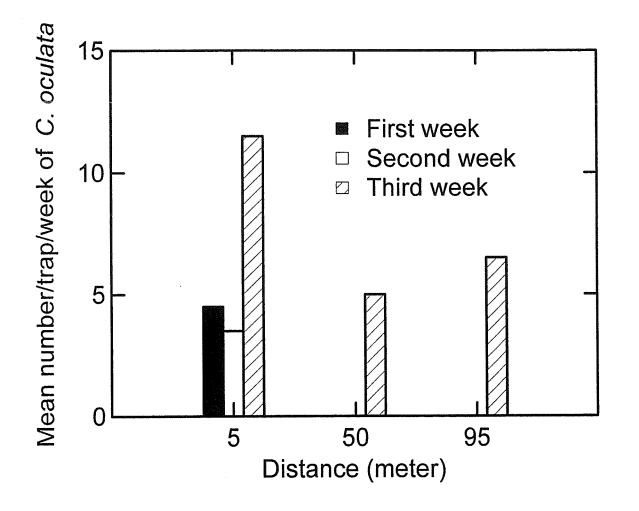


- Nepetalactone plot
- Stratum boundary
- ·Sweep net track

- $\bigstar$  Nepetalactone lure
- $\star$  Nepetalactone lure with water trap



Fig. 3.5.2. Catches of *Chrysopa oculata* in water traps in nepetalactone strata in relation to the distance from the field margin in the alfalfa hay field.



# CHAPTER 3.6

Rearing Lacewings, *Chrysoperla carnea* and *Chrysopa oculata* (Neuroptera: Chrysopidae), on prepupae of Alfalfa Leafcutting Bee, *Megachile rotundata* 

(Hymenoptera: Megachilidae)

# Abstract

Lacewings are an important group of insect biological control agents. Protocols for rearing lacewings often require rearing of an additional insect species to be used as a diet, and this is costly. Prepupae of alfalfa leafcutting bee, *Megachile rotundata* (F.), which are commercially available, inexpensive and can be stored, were evaluated as a larval diet of the lacewings, *Chrysoperla carnea* (Stephens) and *Chrysopa oculata* Say. The leafcutting bee prepupae were suitable for rearing lacewings: 90% of *Chrysoperla carnea* eggs reached adulthood. For *Chrysopa oculata* eggs, survival to adulthood was 66%, but this increased to 91% when pupating larvae had access to empty cocoons of leafcutting bee prepupae. The diet allowed us to establish colonies of the lacewings in the laboratory.

**KEY WORDS** 

Larval diet, fecundity, oviposition, culture

# Introduction

The green lacewings (Neuroptera: Chrysopidae) are an important group of insect predators (Dean and Satasook 1983). Natural populations of chrysopids can be augmented by inoculative or inundative releases (Ridgway and Jones 1969, Nordlund *et al.* 2001). While small numbers are often useful for laboratory studies and inoculative releases, large numbers of cultured chrysopids are required for inundative releases (Nordlund *et al.* 2001). Protocols for culturing of chrysopids using natural, subnatural artificial and artificial diets are available (Finney, 1948; 1950; Ridgway *et al.* 1970; Morrison *et al.* 1975; Morrison 1985; Nordlund *et al.* 2001; Yazlovetsky 2001).

Suitable larval diets include eggs of the lepidopterans *Phthorimaea operculella* (Zeller) (Finney 1948, 1950) and *Sitotroga cerealella* (Olivier) (Ridgway *et al.* 1970, Morrison *et al.* 1975, Morrison 1977, 1985) (Gelechiidae), and *Anagasta kuehniella* (Zeller) (Pyralidae) (Zheng *et al.* 1993a, 1993b), larvae of *P. operculella* (Finney 1948, 1950) and honeybee (Ferran *et al.* 1981, cited in Yazlovetsky 2001; Matsuka and Niijima 1985), aphids (Tauber and Tauber 1974) and artificial diets (Hagen and Tassan 1965, 1966, Vanderzant 1969). Finney (1948; 1950) reared *Chrysoperla* larvae on paper sheets covered with *P. operculella* (Zeller) eggs and honey. Ridgway *et al.* (1970) used *S. cerealella* (Olivier) eggs to feed larvae in Hexcel<sup>®</sup> (Hexcel Products Inc., Dublin CA), which has honey-comb like chambers that separate individual larva, whereas, Morrison *et al.* (1975) dispensed the eggs in sheets of ornamental Masonite<sup>®</sup> separated by organdy cloth. Masonite<sup>®</sup> consisted of 0.64 cm<sup>2</sup> cells removed from solid material in a regular pattern leaving a  $0.32 \text{ cm}^2$  border between cells. When Masonite<sup>®</sup> was discontinued, Morrison (1977) suggested Verticel<sup>®</sup> (Hexcacmb, University Park IL), which contains

triangular cells. Several other materials including plastic light diffusing grids and shredded paper have also been suggested.

Finney (1948) processed *P. operculella* larvae with sodium hypochloride, hot water and paraffin, and fed them to the chrysopid larvae being reared. Ferran *et al.* (1981, cited in Yazlovetsky 2001) used a mixture of larval worker honeybee powder and honey, whereas Matsuka and Niijima (1985) used larval honeybee drone powder and water. However, due to its hygroscopic nature, diet made from larval honeybee powder turns into a syrup, and develops mould rapidly. Similarly, several other natural insect materials including powdered *S. cerealella* moths, aphids, and crickets have been used, though these diets failed to give any advantage (Yazlovetsky 2001).

To reduce costs, searches for artificial larval diets have been made. Hagen and Tassan (1965, 1966, 1970), and Vanderzant (1969) developed liquid diets made primarily from enzymatic hydrolysate of yeast and casein, sugar, vitamins and water, but they were not satisfactory: an artificial diet for *Chrysoperla carnea* (Stephens) which contained wax-coated yeast hydrolysate droplets required costly preparation and also resulted in high mortality (Hagen and Tassan 1965; Vanderzant 1969). However, Vanderzant (1969) had better success by adding vitamins and minerals. Today, several diets improved from Vanderzant's are available, though not all larvae reared on them reach adulthood (Yazlovetsky 2001). Cohen and Smith (1998) developed a semi-solid larval diet containing protein, lipid, carbohydrate, cholesterol, and water. This diet resembles the inside of insect prey in both texture and composition. Combinations of honey, chicken eggs, dried cow's milk, beef liver, bacto-agar, pig fat and butter have improved performance of artificial diets further (Yazlovetsky 2001). Although, progress has been

made with artificial larval diets, chemically defined diets are often more expensive, and to make them more economical further improvements are required (Nordlund *et al.* 2001).

Most protocols are suitable for culturing chrysopid larvae on a large scale operation. These protocols can often be inconvenient for small-scale operations. Natural diets require rearing of one insect, which is usually complex and expensive, to feed another (Finney 1950; Morrison 1985). Artificial diets are even less satisfactory. For example, an artificial diet of wax-coated yeast hydrolysate requires costly preparation, may cause high mortality of lacewings (Hagen and Tassan 1965, Vanderzant 1969), the required ingredients may be expensive (Nordlund *et al.* 2001) and are often difficult to obtain locally. Hence, it is desirable to have alternative protocols that do not require additional rearing of insects and that rely on materials available locally.

This paper describes rearing techniques and bionomics of *Chrysoperla carnea* (Stephens) and *Chrysopa oculata* Say fed as larvae on prepupae of the alfalfa leafcutting bee, *Megachile rotundata* (F.) (Hymenoptera: Megachilidae).

# **Materials and Methods**

# Specimen collection

Using a sweep net, five to eight adult females of *C. carnea* and *C. oculata* were collected from alfalfa fields in Manitoba in early August of 2001. Each female was placed individually in a 118 ml screw-cap specimen container with a small piece of alfalfa shoot and transferred to the laboratory in a picnic cooler. In the laboratory, females were held in the specimen containers overnight at  $20 \pm 2^{\circ}$ C and 12:12 (L:D) h.

The next day, eggs laid in the containers were carefully harvested with forceps. A colony of each species was established from these eggs using alfalfa leafcutting bee prepupae, and yeast plus sucrose paste as larval and adult diets, respectively. Eggs obtained from these colonies were used in the present study.

# Diets

Alfalfa leafcutting bee prepupae were used as the larval diet. The prepupal cocoons, which were in the second year of storage at 5°C, were transferred into a 15–18°C room one or two days before use. Cocoons were cut open at one end with a scalpel and the prepupae were pulled out of cocoons with forceps. Care was taken to avoid injury to prepupae.

Adult lacewings were fed on a paste of yeast flakes (52% protein) and sucrose prepared following Morrison (1985). The food was prepared once a week and was kept frozen until use.

#### **Rearing methods**

 $F_2$  and  $F_3$  generation eggs were collected from each colony. Sixty to 78 eggs of each species were placed in conspecific batches of 8–10 eggs per larval rearing unit made of a 60 x 15 mm covered Petri dish. Two leafcutting bee prepupae were added to each unit which was labeled and placed at 25 ± 2°C, 18:6 (L:D) h and 70 ± 5% relative humidity, the conditions at which all rearing took place.

On the day of hatching, newly emerged lacewing larvae were transferred to fresh units, in batches of 5–10 larvae per unit. Two to three alfalfa leafcutting bee prepupae were provided in each unit. Fresh food was provided every second day during the first six

days. After six days, the number of lacewing larvae was reduced to one per unit to avoid cannibalism, and from then until pupation fresh food was provided every day. The lacewing pupae were left undisturbed.

Newly emerged adults, both males and females, were transferred in conspecific batches of 8–10 into a pre-oviposition unit made of a transparent 1 liter plastic container 14 cm high, 11 and 8.5 cm diameter at the top and bottom, respectively. The lid of the container had a 1 mm hole drilled near the edge for aeration. A small piece of distilled-water-saturated cotton wad in a  $35 \times 10$  mm Petri dish was placed on the floor of the container. Food was presented by placing 2–3 drops of the yeast and sucrose paste on a 10.5 x 4 cm strip of brown cardboard, which was placed on the upper rim of the container so that the food faced down. The food and the cardboard was changed every day and every third day, respectively to avoid mold development. On the day eggs were first seen, females were separated and individual females were placed in oviposition units, which were similar to pre-oviposition units. Eggs laid inside oviposition units were collected and reared for colony development.

### Performance of culture

The time needed for egg hatching, the number of eggs hatched, the number of larvae and pupae, the duration of larval and pupal periods, the number and sex of emerged adults and the pre-oviposition period were recorded. To develop a fecundity schedule, 15 females of *C. carnea* and 10 of *C. oculata* were followed for 30 days from initial oviposition in oviposition units.

We also examined whether the provision of empty leafcutting bee cocoons for pupating *C. oculata* larvae improved survival. Two treatments, no cocoon (control) and

with cocoon, were compared. In the latter treatment, an empty leafcutting bee cocoon was provided in each larval rearing unit on the ninth or 10th day after hatch. All other operations remained as described above. Chi-square test were used to conduct the statistical analysis (Sokal and Rohlf, 1995).

#### **Results and Discussion**

Alfalfa leafcutting bee prepupae permitted rearing of C. carnea and C. oculata larvae (Table 3.6.1). About 2–3% eggs did not hatch because they were eaten or damaged by the larvae that hatched earlier. The total time required from oviposition to adult emergence of C. carnea averaged 24.7 (23-29) d. The growth and development of C. carnea is influenced by the type and amount of food fed to their larvae (Zheng et al. 1993b, Obrycki et al. 1989). The time required from oviposition to adult emergence in the present study is similar to previous reports of developmental duration (Hagen and Tassan 1965, Zheng et al. 1993a). The leafcutting bee prepupae allowed 90% of C. carnea eggs to survive to adulthood, and this rate is higher than those obtained on natural or artificial diets in previous studies (Hagen and Tassan 1965, Ridgway et al. 1970, Morrison et al. 1975, Obrycki et al. 1989). Zheng et al. (1993a) obtained 75-100% and 65–73% survival from egg to adult on high and intermediate amounts of larval food, respectively. In this study about 52% of emerged adults were females. Zheng et al. (1993a) obtained a similar sex ratio on low to intermediate food quantities, and 67% females on a high amount of larval diet.

The total time required from oviposition to adult emergence of *C. oculata* averaged 34.7 (31-38) d, which is comparable to the time required on natural diets (Obrycki *et al.* 1989). Twelve percent of larvae failed to pupate, and 66% of eggs

survived to adulthood. Provision of empty leafcutting bee cocoons significantly increased the survival through the pupal stage ( $\chi^2 = 4.02$ ; df = 1; P < 0.05), probably by providing a better site for anchoring cocoon-webs (Table 3.6.2). Egg to adult survival increased to 91% in the presence of leafcutting bee cocoons for pupation. Sixty five percent of emerged adults in this study were females. Obrycki *et al.* (1989) reared up to 81% of larvae to adults on various natural diets, and less than 57% of those adults was females.

The lacewing species differed in pupation sites. *Chrysoperla carnea* pupated at both the floor-sidewall and lid-sidewall junctions. Cues leading to this site selection are unclear as little is known about pupation of *C. carnea* (Canard and Volkovich 2001). *Chrysopa oculata* usually pupated on the floor-sidewall junctions perhaps because this species pupates in the ground (Burke and Martin 1956).

Four individuals of each species had problems in mating or oviposition and were dropped from the fecundity analysis to avoid bias (Hagen and Tassan 1970). The fecundity schedule of the lacewing species differed (Fig. 3.6.1). Females of *C. carnea* laid on an average a total of 679 eggs in 30 d, and oviposition was high until 16 d then declined gradually. Females of *C. oculata* oviposited more or less uniformly and females laid an average of 424 eggs during the period (Fig. 3.6.1). Longevity of adult lacewings depends on climatic conditions and resources (Canard and Volkovich 2001). McEwen and Kidd (1995) found *C. carnea* females fed sugar solution survived about 33 d, but the oviposition was non-existent. Unfortunately this study does not indicate how long the females would survive and oviposit. But it is clearly indicated that they could potentially survive for over 34 days.

The intrinsic rate of natural increase  $(r_m)$  of *C. carnea*, calculated following Southwood (1978), in this study was 0.646 per wk. From Zheng *et al.* (1993*a*) I calculated  $r_m = 0.665$  per wk in 1984, and  $r_m = 0.781$  per wk in their 1986 trial. The higher value in the 1986 trial was thought to be due to the higher survival of fresh field-collected eggs used that year and the higher fecundity of the first generation adults (Zheng *et al.* 1993*a*). In this study, the  $r_m$  of *C. oculata* was 0.463 per wk and 0.505 per wk in the absence and presence of leafcutting bee cocoons, respectively. Population increase is influenced by insect fecundity, which depends on the protein concentration of adult diets (Morrison 1985). The protein concentration in adult diet (< 52%) used in this study was less than the recommended level of 65% (Morrison 1985), which may have reduced the  $r_m$  value.

It is indicated in this study that *C. carnea* is more fecund and develops faster than *C. oculata*, resultantly the rate of increase is higher for *C. carnea*. Therefore, populations of *C. carnea* are expected to be greater than those of *C. oculata* in field crops. This is exactly the case in alfalfa fields of Manitoba. Chrysopid populations were studied in alfalfa fields of Manitoba, where it was found that *C. carnea* predominates over *C. oculata* (Chapter 3.5).

Diets in this study allowed satisfactory rearing of lacewings. Results in this study suggest that alfalfa leafcutting bee prepupae can be used as a larval diet for *C. carnea* and *C. oculata*. The bee prepupae are commercially available, cheap (the food required for rearing a larva cost less than 1.3 and 1.95 Canadian cents for *C. carnea* and *C. oculata*, respectively) and can be preserved for at least a year at 5°C. The method described allows small-scale production and maintenance of lacewing colonies at reasonable cost. The

possibility of rearing multiple larvae per unit with the same amount of the diet needs to be studied, as it could reduce per capita diet requirement and costs.

	Dura	tion (d) (M	$ean \pm SE)$	Survival (%)			
Species	Egg	Larva	Pupa	Egg	Larva	Pupa	Overall
Chrysoperla carnea	4	$10.9 \pm 0.1$	9.8 ± 0.1	98 (60) <sup>a</sup>	100 (50)	92 (50)	90
Chrysopa oculata	5.4	$13.2 \pm 0.1$	$16.5 \pm 0.2$	97 (78)	88 (50)	77 (44)	66

Table 3.6.1. Development of two lacewing species fed alfalfa leafcutting bee prepupae as a larval diet

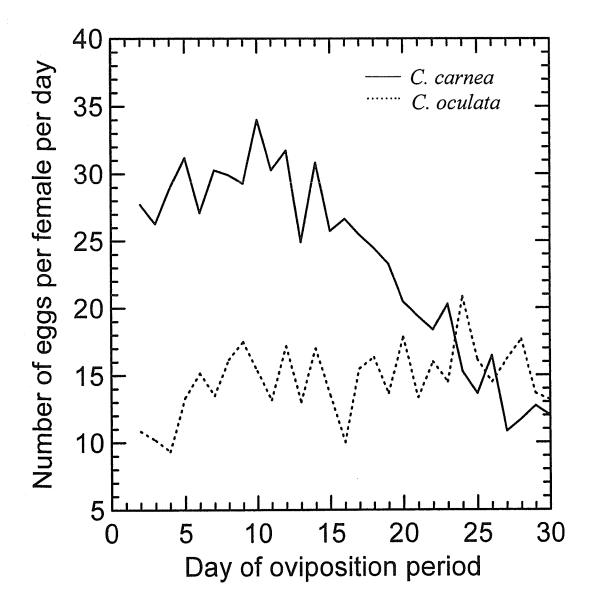
<sup>a</sup> Values in parentheses refer to the number of individuals at the beginning of stages.

Treatments	Percent survival (Mean ± SE)						
	Larvae to pupa	Pupa to adult	Overall				
Control (65) <sup>a</sup>	88 ± 4	77 ± 6	68 ± 6				
Leafcutting bee cocoons (15)	$100 \pm 0$	93 ± 7	93 ± 7				

 Table 3.6.2. Influence of empty cocoons of alfalfa leafcutting bees on developmental success of C. oculata

<sup>a</sup> Values in parentheses refer to the number of individuals at the beginning.

Fig. 3.6.1 Fecundity of *C. carnea* and *C. oculata* females in the laboratory in the observed 30 days of the oviposition period (results are based on 15 *C. carnea* and 10 *C. oculata* females).



#### **CHAPTER 4**

### **General Discussion**

Depending on the kind of pests, sampling programs for insect pests attempt to determine the presence of a pest, to estimate numbers and distribution of a pest population, or to assess the population dynamics of a pest over time (Pedigo 1999). Several sampling devices and techniques are available for monitoring insect pests in field crops, although, no single method is free from error (Harper et al. 1993). The extent of error can vary among sampling methods. Therefore, selection of appropriate sampling devices and techniques is very important, and forms the basis for successful and economical pest management programs (Pedigo 1999). The method chosen should be sufficiently precise to identify pest population fluctuations, but also affordable, simple and quick enough so that it can be done frequently to facilitate timely management decisions. Sweep-net sampling usually fulfills these requirements, and has been the most frequently used tool for estimating insect populations from vegetation (Southwood 1978). Although sweep-net sampling collects only a portion of individuals present on alfalfa plants, it has been a reliable method for monitoring Lygus bug populations on various crops including alfalfa (Fleischer et al. 1985; Schotzko and O'Keeffe 1986, and references therein; Harper et al. 1993; Snodgrass 1993). The present study provides evidence that sweep-net sampling, which is an easy and cheap method of sampling arthropods (Southwood 1978), is the most precise and efficient method of assessing insect pest populations on alfalfa. Sweep-net sampling can cover a relatively larger area in a shorter span of time than other methods, and therefore, is effective in detecting the presence of pests and their population fluctuations. In situations where alternative

sampling methods are used, the calibration equations developed in the present study (Chapter 3.1) would be useful in pest management decision making where the economic injury levels were originally derived by using sweep-net sampling.

The present study was conducted, in part, to determine the insect pest complex and assess their populations in relation to alfalfa production in Manitoba. Based on results comparing sampling methods, sweep-net sampling was used for assessing arthropod populations on alfalfa plants. Of the various species of insect pests found, *Lygus* spp., *Adelphocoris lineolatus*, and *Acyrthosiphon pisum* were the most prevalent species. These pests were also of prime concern to alfalfa growers in the province. These pests were found to occur throughout the season, and their populations were high for a considerable period in the season (Figs. 3.2.4–3.2.6), sometimes reaching the nominal thresholds levels (Table 3.2.20).

Among the other pests, *Agromyza frontella* and *Bruchophagus roddi* might be potentially damaging pests of the crop in the province. Growers, however, are little concerned about these pests, probably because of the inconspicuous nature of these pests and the injury they cause. Although *A. frontella* were found to infest alfalfa plants, the levels of damage were inconsistent, a trend that has been found throughout the distribution of the pest. In Canada and in the United States, only 25% of the fields studied suffered economic damage due to *A. frontella* attack (Thompson 1981; Hendrickson and Day 1986). However, Thompson (1981) noted that infestation on leaves reduce the quality of hay.

Comparison of this study with previous reports from elsewhere in the Canadian Prairies (Murrell 1987; Harper *et al.* 1990; Schaber *et al.* 1990*b*; Schaber 1992; Soroka

and Murrell 1993) reveals that the damaging pest spectrum is similar in all Canadian Prairie Provinces. However, Adelphocoris superbus (Lilly and Hobbs 1962) and Hypera postica, which were reported to damage alfalfa in Alberta (Harper et al. 1990; Schaber et al. 1990b), were not found in the present study. Although no H. postica was caught in the present study, the insect occurs elsewhere in Manitoba: it has been reported in Brandon and Killarney areas by extension personnel (Gavloski 2001). In 2002, one grower collected a large sample of insects by using a mechanically operated sweeping device in his organic field of alfalfa at McCreary near Dauphin. The grower froze the sample in a plastic bag and upon examination one adult of H. postica was found in that sample. Adelphocoris rapidus, which occurs in eastern Canada and United States (Hughes 1943), was also not found in the present study. Therioaphis maculata did not appear frequently in samples in the present study, although it is a common pest of alfalfa in the United States (Radcliffe et al. 1976) but of little importance in Canada (Goplen et al. 1987). Acyrthosiphon kondoi, which is a relatively new pest of alfalfa in North America (Soroka 1991), was seldom found in this study.

In Manitoba, it is not uncommon that a hay field is grown adjacent to a seed field of alfalfa. Hay fields in this study were usually harvested twice, which is also the practice in Alberta (Harper *et al.* 1990). The hay-cut caused surviving plant bug adults to leave the mown fields, and many of them migrated to nearby seed fields. However, hay-cut appeared to be relatively less detrimental to some predators including nabids, spiders and opilionids than was the insecticide application applied in seed fields. All these probably contributed to the fact that there were lower numbers of most insect pests in hay fields than in seed fields, and no pest control measures were required in hay fields.

Alfalfa fields for seed production usually received pest control measures. There are several pest control methods available, which include but are not limited to cultural and chemical methods. As cultural methods of pest control, burning of alfalfa stubble in spring and planting of resistant cultivars have been frequently used. Although its effects are dependent on insect species, the life stages at which the pest overwinters, plant stages and the time of the season (Schaber and Entz 1988; 1991; 1994), burning increases the availability of plant nutrients and organic matter in soil (Lilly and Hobbs 1962; Dormaar and Schaber 1985) and increases hay and seed yield, and may reduce pest populations (Bolton and Peck 1946; Lilly and Hobbs 1962; Tippens 1964; Schaber and Entz 1988) to such low levels that a spring burning at 10 cm plant height would eliminate the application of insecticides in alfalfa fields for seed production (Schaber and Entz 1994). In addition, burning is less detrimental than insecticides to natural enemies and pollinators (Schaber and Entz 1988; 1994). However, burning is not a practice commonly used by growers in Manitoba, probably because it is often hazardous, and there are operational and legal problems associated with the practice. The lack of time between the time when fields become dry enough to burn and time when growth resumes may also discourage burning.

There are no recommended alfalfa cultivars available that are resistant to *Lygus* spp. and *A. lineolatus* in this region. Resistant cultivars could be advantageous, particularly for managing *Lygus* spp. which occurs in many other crops and is highly mobile, meaning that control of *Lygus* spp. in alfalfa by using chemicals will normally provide short term benefits. In North America, resistant cultivars have provided successful control of *A. pisum* and diseases (Summers 1998). There are numerous

registered cultivars available, which are resistant to *A. pisum* and various diseases (Petrowski 1999). The majority of the cultivars (at least five of nine) used in the present study are resistant to *A. pisum* and diseases, and this indicates that growers often use pest resistant cultivars. However, most resistant cultivars usually lack effectiveness against more than one species of insect pests (Mueke *et al.* 1978). Thus where, as in the present study, more than one species of damaging or potentially damaging pests occur simultaneously in the fields, cultivars that are resistant to multiple pest species are required. Until multiply resistant cultivars that are equally effective against all of the damaging species are developed, the use of resistant cultivars may reduce, but will probably not eliminate the need for application of other pest control measures (Mueke *et al.* 1978). Since alfalfa seeds produced are mostly used for seeding hay crops, such multiple resistant cultivars must also satisfy the requirements for successful and economical hay production.

Chemical control had been the primary method used by growers for controlling insect pests in alfalfa fields for seed production. Insecticides are applied mainly to control *Lygus* spp. and *A. lineolatus*. Although some thresholds are available (Soroka 1991; Harris 1992, cited in Soroka and Murrell 1993; Schaber 1992; Soroka and Murrell 1993), it is unclear whether these thresholds were scientifically developed. Although growers claimed that they use economic thresholds in making pest control decisions, it appears in the present study that appropriate economic thresholds are seldom used (Table 3.2.20). In the process of pest control decision making, there has also been a lack of co-ordination among hay and seed alfalfa growers, and the present study provides evidence that such co-ordination would benefit alfalfa seed growers.

Schaber *et al.* (1990*a*) considered late May to late June as the early season, and the beginning of August as the late season for insecticide application in alfalfa fields in Alberta. Schaber and Entz (1994) consider that late season starts after mid-July in Alberta. In Manitoba, mid-August or later seems to be considered as the late season. All seed growers in this study made the early season (usually in June) application of insecticide usually 7–10 days before the projected date of leafcutting bee release. Although the insecticide application affected some natural enemies including some species of carabid beetles (Chapter 3.3), most of the carabid species including *Pterostichus melanarius*, which made up 65% of the total carabid catches, remained unaffected. The tolerance of *P. melanarius* to several insecticides has been reported previously, and the species is moderately tolerant to dimethoate (Hagley *et al.* 1980), which was frequently used in this study. The insecticide application also did not affect spiders, the group of predators that were prevalent in the early part of the season.

There were instances where a few growers did not make the second application of insecticides late in the season. In the Pacific Northwestern United States (Johansen *et al.* 1977) and in Saskatchewan (Craig 1973), control of *Lygus* spp. and *A. lineolatus* during the bud and early flowering stages appeared to be more important than controlling later in the season. Johansen *et al.* (1977) considers that control of mirids by insecticides early in the season allows time for predators to build up, and inflict natural suppression on pest populations so that no control measures would be required late in the season. However, this may not always be the case. Generally, an early season treatment would protect the crop in northern areas of Canada where mirids have only one generation per year (Craig 1961, 1973; Schaber *et al.* 1990*a*). One well timed early insecticide application would

also protect the crop in places where there are two generations of plant bugs, but the second generation is relatively small (Craig 1961). In the southern Canadian Prairies, where there is often a second generation of mirids, the early season treatments may not adequately protect the crop for the entire season if the second generation develops rapidly and abundantly (Craig 1983; Schaber et al. 1990a). In such a case, the second spray would benefit the growers provided the late season pest population is high enough that the insecticide application is justified. In the present study, mirids often had two generations and compared with populations in the first generation, populations in the second generation were larger, but probably not large enough to justify insecticide application (Table 3.2.20). This could be, at least partly, due to the activities of natural control agents. In the present study, relatively low numbers of common predators including coccinellids (Fig. 3.2.12 b, d, f), Orius spp. (Fig. 3.2.14 b, d, f), Nabis spp. (Fig. 3.2.15 b, d, f) and chrysopids (Fig. 3.2.17 b, d, f) were present early in the season when the first insecticide application was made. The trend was similar for their combined numbers (Fig. 3.2.8 b, d, f). After the first insecticide application, numbers of those predators started to increase from early to mid-July to reach peak levels around mid-August. Increases of these predator populations often coincided with those of pest insects, as explained previously (Chapter 3.2). Evidence that these predators contribute toward suppression of pest insects on alfalfa has been found (Chapters 3.2 and 3.4). In addition, parasitoids also contribute to pest suppression (chapter 3.2).

Generally, alfalfa requires at least six weeks after pollination in order to mature seeds (Richards 1989). Therefore, in normal seasons, the second insecticidal treatment, which usually occurs in late August, seems to be too late to influence seed development

and yield. In this study, the greatest pest populations occurred when most plants had passed the late seedpod stage and seedpods were at ripening stage when the second application of insecticides occurred (Figs. 3.2.4 f, 3.2.5 f and 3.2.6 f). The growers who did not make the second application of insecticides indicated that it was unlikely that they lost a significant amount of seed leaving the fields unsprayed during the late season. This is because population trends in this study suggest that pest numbers were not high enough to cause economic damage. In addition, many Lygus adults of the late-season pest population were immigrants, suggesting that they did not feed on developing seeds in the alfalfa fields. In previous studies, there have been mixed results for seed yields when pests were controlled late in the season. In Saskatchewan, Soroka and Murrell (1993) did not find any yield increase in plots that were treated with insecticides early and late in the season (30.09 g per  $0.5 \text{ m}^2$ ) compared with plots that were treated only early in the season (32.19 g per 0.5 m<sup>2</sup>). In southern Alberta, Schaber et al. (1990a) controlled mirids with the insecticide trichlorfon late in the season (late July or early August). They obtained a significantly greater amount of seed in insecticide-treated plots in one year when very few A. lineolatus were present, but no significant yield advantage in another year when densities of A. lineolatus were high in check plots late in the season. They attributed the lack of significant yield increase in the latter year to high wind, which probably drifted the applied insecticides away (Schaber et al. 1990a). However, it was not clear whether yield benefits obtained by the late season insecticide application resulted in any economic benefits. Charnetski (1983a, 1983b) did not obtain any significant yield benefit when insect pests were controlled with insecticides late in the season. In Washington State, where different Lygus spp. infest alfalfa in climatic conditions that differ from those in

Manitoba, high densities of Lygus spp. (30 individuals/sweep) for two weeks in late July damaged 20% more seeds compared with low densities (2-3 individuals/sweep) of Lygus bugs infesting alfalfa throughout the season (Johansen and Eves 1973). Craig (1973) suggested that a high density of *Lygus* spp. (5/sweep) late in the season (late July to early August) would require control measures to be applied in order to protect the developing alfalfa seeds. Soroka and Murrell (1993) did not find a significant yield reduction when they allowed A. lineolatus at a low density (one bug/two stems in a sleeve-cage) to feed upon alfalfa plants late in the season (from late July or early August until harvest). However, at higher densities ( $\geq 2$  bugs/two stems of alfalfa), there were significant yield reductions. Therefore, a large population of pests during the late season could be damaging particularly where there is a prolonged summer (Soroka and Murrell 1993). To tolerate winter and persist, alfalfa stands require sufficient photosynthate (carbohydrate) to be reserved in roots (Goplen et al. 1987). Intense pest infestation may affect the reserves if many leaves and buds are killed by the pest feeding. For alfalfa pests, little is known about the late season thresholds for decreased stand persistence of alfalfa. However, the numbers would probably be greater than those affecting seed yields, because killing a bud is likely to require more intensive feeding compared with damaging a seed. In such cases, it is unlikely that at pest populations late in the season in this study stand persistence would be affected.

There have been growing concern about unnecessary and excessive use of insecticides, and increasing demands for improved pest management practices which ensure the justified use of insecticides (Debach and Rosen 1991). For example, an integrated pest management (IPM) program that promoted non-chemical methods of

insect control on alfalfa was introduced in Alberta in the late 1970s (Schaber and Richards 1979). Increased use of non-chemical methods would lead toward decreased use of chemical insecticides and increased benefits from natural enemies (Debach and Rosen 1991). Alfalfa seems to be a good candidate crop for improved pest management practices where the use of insecticides can be reduced. This is because the crop is relatively persistent and has the capability of withstanding some pest attack (Strong 1968). Strong (1968) documented, in a manipulative study, that alfalfa plants respond to a reduction of seedpods by producing more buds and florets. In addition, there are different methods including cultural and biological methods of pest control, which are compatible with each other and can be integrated into a package (Schaber and Richards 1979; Schaber and Entz 1991), known to be effective elsewhere in the Canadian Prairies.

The presence of various natural enemies forms the basis of increased natural control of pests. In the present study, natural biocontrol agents were found to abound in fields. Both field and laboratory studies in the thesis indicates that many of these natural control agents reduce pest populations in alfalfa fields. Although the current practices affected natural enemies, alfalfa growers in Manitoba seldom adopted any measures to conserve and augment the natural enemy populations. For a pest management program to be effective and sustainable, the decisions relating to pest management need to consider ecological parameters including plant growth stage and the presence and activities of natural biocontrol agents (Debach and Rosen 1991). An environmentally friendly and sustainable pest control approach must minimize the use of chemical insecticides and maximize the use of beneficial natural enemies through conservation and augmentation (Debach and Rosen 1991; Pedigo 1999). It seems that the present seed alfalfa pest control

method with application of insecticides early and late in the season needs to be revised, as this practice does not usually allow natural enemy populations to be conserved and augmented (Craig 1973; Johansen *et al.* 1977). There are some insecticides, which kill insect pests selectively, for example, Thuringiensin<sup>®</sup>, selectively kills nymphs and affect fecundity of *Lygus hesperus* (Tanigoshi *et al.* 1990) and trichlorfon selectively kills insect pests while causing little mortality to predators and parasitoids (Johansen and Eves 1973), and leafcutting bees (Tanigoshi *et al.* 1990). However not many insecticides are selective, and *Lygus* spp. has become resistant to the commonly used insecticides in the United States including trichlorfon (Xu and Brindley 1993; Snodgrass 1996). Availability and use of selective insecticides would help conserve and augment natural enemies (Debach and Rosen 1991). Conservation is often a relatively slow process, and practices that augment insect natural enemy populations may be required to supplement and boost conservation processes (Debach and Rosen 1991).

There are no measures adopted deliberately by Manitoban growers that would augment natural biocontrol agents in alfalfa fields. Manipulation of the crop field environment such that it favours natural enemies is an option for augmenting natural enemy populations (Debach and Rosen 1991). Several methods and techniques of augmentation have been used (Stern *et al.* 1964; van den Bosch and Stern 1969; Abdel-Kareim and Kozár 1988; Dawson *et al.* 1990; Hardie *et al.* 1991, 1994; Boo *et al.* 1998), although not in seed alfalfa fields. The present study, which examined responses of insect natural enemies to aphid sex pheromone compounds, provided evidence that nepetalactone, an aphid sex pheromone compound, could help augment populations of natural enemies. However, timing is very important in the process of manipulation of

insect habitat (Debach and Rosen 1991; Schaber and Entz 1991) and insect behaviour (Debach and Rosen 1991), and appropriate methods and timing of lure deployment could provide better results.

Releases for augmentation are another option, which can increase the presence and activities of insect natural enemies (Debach and Rosen 1991; Pedigo 1999). There are several instances where parasitoids were redistributed or introduced and established through mass releases in Canada (Broadbent *et al.* 1999, 2002; Wylie *et al.* 2005) and in the United States (Mackauer and Finlayson 1967; Angalet and Fuester 1977; Day 1987, 1996; Day *et al.* 1990, 1992). Although parasitoids could also be augmented by periodic releases, commercialized parasitoid releases for large scale alfalfa production are not known, possibly due to the cost and technological obstacles to be overcome for mass rearing of parasitoids.

Augmentation of predators, however, has more frequently been practiced in commercial crop fields. For example, augmentation through inundative or inoculative releases of cultured chrysopids, a commonly occurring group of natural enemies in the Canadian Prairies, has been frequently used for different field crops in North America (Nordlund *et al.* 2001, and references therein). Although some natural enemies including coccinellids and chrysopids being commercially available for augmentative releases, growers in the Canadian Prairies usually do not adopt this technique, possibly because there are problems in retaining the released agents in the target areas. In addition, the associated costs and technical problems are also discouraging (Nordlund *et al.* 2001): released predator eggs are often destroyed by other predators and parasitoids, whereas, retention of chrysopid adults and larvae in target areas have frequently been found to be

difficult, as they tend to leave the fields in which they were released (Duelli 1988; New 1988; Nordlund *et al.* 2001). Similarly, retention of released coccinellids in the fields also appears to be difficult (Cartwright *et al.* 1979; Deristadt and Flint 1996). Deployment of something that are attractive to these natural enemies would retain them in the fields where they were released. Although the present study provides evidence that nepetalactone is an attractant to some natural enemies, the practical use of this compound depends upon further studies in order to determine the effective times, distances, doses and methods of deployment.

Releases for augmentation also require cultures of the predaceous agents that are to be released. Several methods are available for mass culture of different groups of predators including chrysopids (Nordlund *et al.* 2001, and references therein). These techniques often require rearing of one insect to be fed to another insect (Nordlund *et al.* 2001, and references therein). Alternatively, commercial foods are usually less efficient, are often not locally available, and are costly (Nordlund *et al.* 2001, and references therein). The present study has also provided a foundation for the development of a technique that is free from most of the above problems of rearing lacewing larvae. However, with the present protocol, the technique is suitable only for small-scale operations, and technological automation and improvement would be necessary to make it suitable for mass rearing of chrysopids.

## **Future Research**

The occurrence and seasonal distribution of the most common insect pests and their natural enemies in alfalfa fields of Manitoba have been elucidated in the present studies. Growers' reliance on chemical insecticides for controlling insect pests has also been documented, although little is known about where, when and how the threshold values that growers use were developed. Thus, research on development of economic thresholds for pest management decision making is required.

In the present study, parasitoids were found to attack nymphs of *Lygus* spp., however what species of parasitoids are involved in killing *Lygus* nymphs in Manitoba remains to be studied. Nothing is known about parasitism of *Lygus* eggs, and also of *A*. *lineolatus* in Manitoba. There is also a lack of information about parasitism of pests, particularly of mirids, by nematodes in Manitoba. Future research in this area would help to complete the picture of pest-natural-enemy interactions.

During sampling, some dead *A. pisum* individuals, which seemed to have been killed by fungal infections were found, although no attempts were made to determine the causal organism(s) and the extent of resulting mortality. Little is known about pathogeninduced mortality of pests on alfalfa in the Canadian Prairie Provinces. A thorough investigation of these interaction is an avenue for future research. This could also provide a baseline for development of microbial pesticides for alfalfa production.

Nonchemical methods of pest control in alfalfa fields frequently involve burning, although burning is hazardous and may face legal constraints. The use of resistant varieties is usually free from such problems. The tremendous success of resistant cultivars against aphids including *A. pisum* and the popularity of such cultivars are

encouraging. Surprisingly, plant resistance to *Lygus* spp. has received little attention (Tingey and Pillemer 1977), although alfalfa germplasm with resistance to mirid pests is available in the United States (Sorensen *et al.* 1988). Such germplasm could provide the basis for further research and improvement. Research on the development of varieties resistant to multiple insect pests including *Lygus* spp. and *A. lineolatus* would help promote non-chemical control of insect pests.

Until practicable alternative means that provide adequate control of damaging insect pests are available, growers are likely to continue using chemical insecticides. The currently recommended insecticides are broad spectrum, and they are unsafe for beneficial organisms including pollinators. Further research is required in order to develop insecticides that are safe for beneficial organisms including pollinators.

The present study provides evidence that aphid semiochemicals attract natural enemies. This finding generates a basis for extensive research in order to develop better timing and deployment of the compounds. The result also indicates the need for research on the presence and use of semiochemicals in other organisms, both beneficial and harmful species.

The present study provides a foundation for using leafcutting bee prepupae in rearing chrysopid larvae. Successful large-scale culture of chrysopid larvae by feeding leafcutting bee prepupae requires research on technological automation and further improvement.

## Conclusions

The present study indicates that *Lygus* spp., *Adelphocoris lineolatus* and *Acyrthosiphon pisum* are the most prevalent insect pests that attack commercial alfalfa crops in Manitoba. These pests are of primary concern to growers in seed fields but not in hay fields of alfalfa. Although growers often use aphid resistant cultivars, alfalfa seed growers rely heavily on chemical methods for controlling insect pests, primarily the plant bugs, on alfalfa. Growers indicated their awareness and use of economic thresholds, however, it is doubtful whether growers consider appropriate thresholds in making treatment decisions (Table 3.2.20).

The presence and activities of parasitoids (Fig. 3.2.4 and 3.2.6, and Table 3.2.3) and predators (Fig. 3.2.8; chapter 3.4) of commonly occurring pest species in alfalfa have been determined. It was found that parasitoids cause a substantial amount of mortality to *Lygus* spp. (Fig. 3.2.4 and Table 3.2.3) and *Acyrthosiphon pisum* (Fig. 3.2.6 and Table 3.2.3), however, parasitoids were not found to greatly affect populations of *Adelphocoris lineolatus* in Manitoba.

Different species of predators appears to be influential on populations of different pest species at different times. For example, coccinellids appears to exert most influence on post-first hay-cut or insecticide application population build up of *Lygus* spp., *A. lineolatus* and *A. pisum*. Considering the results in the laboratory trial, *Nabis alternatus* also seems to be an important predator.

The present study provides evidence that insecticide application inflicts a set back on natural enemies in seed fields, particularly on the predator species. Although further studies are needed, particularly with regard to the deployment technique and timing of

placement, it seems that the deployment of the aphid sex pheromone compound, nepetalactone, could enhance recolonization by natural enemies with resulting benefits of pest suppression (Chapter 3.5). The present study provides baseline information for an easy and inexpensive method of culturing chrysopids by using leafcutting bee prepupae that are produced simultaneously with alfalfa seed production (Chapter 3.5). Technological automation and development of the method could promote economic augmentation of natural enemies in fields of alfalfa.

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Appendix I. Weather conditions and cumulative degree-days (CDD) (for Lygus spp.
and A. lineolatus: CDD above 10.6°C, and for A. pisum: CDD above 4.7°C) at
different locations of Manitoba during April to September, 1999–2001.

	Temperature (°C)/	199	9	2000 <sup>1</sup>	20011
Months	rainfall (mm)	Steinbach*	Teulon	Arborg	Arborg
April	Maximum temperature	26.0	27.0	23.5	26.5
	Minimum temperature	- 4.5	- 6.0	- 12.0	- 9.5
	Mean temperature	7.2	7.2	2.7	4.3
	CDD above 10.6°C	31.0	34.5	5.3	17.6
	CDD above 4.7°C	118.5	121.9	59.9	66.5
	Total rainfall	17.9	38.0	16.0	21.6
May	Maximum temperature	30.0	32.0	27.0	27.0
	Minimum temperature	- 3.0	- 2.0	- 5.0	- 2.5
	Mean temperature	12.5	12.6	9.5	12.0
	CDD above 10.6°C	95.4	95.8	27.1	76.2
	CDD above 4.7°C	244.7	249.2	162.2	226.1
	Total rainfall	110.8	107.3	51.0	92.4
June	Maximum temperature	31.0	32.0	27.0	28.0
	Minimum temperature	- 1.0	3.0	- 1.5	1.5
	Mean temperature	16.5	16.9	13.7	15.5
	CDD above 10.6°C	180.5	189.4	99.4	147.5
	CDD above 4.7°C	353.8	365.8	268.4	323.5
	Total rainfall	95.1	78.1	127.4	122.6
July	Maximum temperature	32.0	33.0	31.5	29.5
-	Minimum temperature	7.0	6.5	2.5	3.5
	Mean temperature	19.7	19.8	18.9	19.6
	CDD above 10.6°C	282.6	286.3	258.1	278.4
	CDD above 4.7°C	465.5	469.2	441.0	461.3
	Total rainfall	85.2	75.8	22.0	68.8
August	Maximum temperature	29.0	31.0	29.5	34.0
-	Minimum temperature	5.0	5.0	4.5	3.5
	Mean temperature	18.3	19.0	17.6	19.4
	CDD above 10.6°C	238.1	261.4	216.5	273.9
	CDD above 4.7°C	421.0	444.3	399.4	456.8
	Total rainfall	73.1	57.8	42.0	52.8
September	Maximum temperature	27.0	26.0	24.0	28.0
	Minimum temperature	- 4.5	-2.0	- 3.5	- 3.0
	Mean temperature	11.6	12.5	10.8	13.4
	CDD above 10.6°C	59.8	65.4	42.0	98.
	CDD above 4.7°C	207.9	220.5	190.6	263.5
	Total rainfall	59.5	64.2	74.6	29.4

\* No data from Dugald were available. Therefore, data from Steinbach, which is close to Dugald (< 30 km) were considered.</li>
 <sup>1</sup> No data from Riverton were available, however, fields in Arborg and Riverton were within about 15 km.

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Appendix II. Species of spiders collected in sweep-net and pitfall trap samples from<br/>alfalfa fields in Manitoba, 2001.Sampling methodFamilySpecies

Sampling method	Family	Species
Pitfall trap	Agelenidae	Agelenopsis actuosa (Gertsch & Ivie)
Sweep-net	Araneidae	Argiope trifasciata (Forskål)
Sweep-net		Hypsosinga pygmaea (Sundevall)
Sweep-net		Neoscona arabesca (Walckenaer)
Sweep-net		Singa keyserlingi McCook
Sweep-net, pitfall trap	Clubionidae	Clubiona abboti L. Koch
Pitfall trap		Clubiona byrantae Gertsch
Pitfall trap		Clubiona Canadensis Emerton
Pitfall trap		Clubiona johnsoni Gertsch
Sweep-net		Clubiona obesa Hentz
Pitfall trap		<i>Clubiona riparia</i> L. Koch
Pitfall trap	Dictyniidae	Argenna obesa Emerton
Sweep-net		Dictyna foliacea (Hentz)
Sweep-net		Dictyna minuta Emerton
Sweep-net		Dictyna volucripes Keyserling
Sweep-net		<i>Emblyna maxima</i> (Banks)
Pitfall trap	Gnaphosidae	Gnaphosa parvula Banks
Pitfall trap	-	Micaria pulicaria (Sundevall)
Sweep-net		Micaria rossica Thorell
Pitfall trap		Zelotes fratris Chamberlin
Pitfall trap	Hahniidae	Hahnia cinerea Emerton
Pitfall trap		Neoantistea magna (Keyserling)
Pitfall trap	Linyphiidae	Agyneta allosubtilis Loksa
Pitfall trap		Allomengea dentisetis (Grube)
Sweep-net		Aphileta misera (O.PCambridge)
Sweep-net		Baryphyma trifrons (O.PCambridge)
Sweep-net		Bathyphantes brevis (Emerton)
Sweep-net		Bathyphantes canadensis (Emerton)
Pitfall trap		Ceraticelus laetus (O.PCambridge)
Pitfall trap		Ceraticelus minutus (Emerton)
Pitfall trap		Ceratinella brunnea Emerton
Sweep-net, pitfall trap		Collinsia plumosa (Emerton)
Sweep-net		Eperigone undulata (Emerton)
Pitfall trap		Eridantes utibilis Crosby & Bishop
Sweep-net		<i>Erigone atra</i> Blackwall
Sweep-net		Erigone blaesa Crosby & Bishop
Sweep-net		Erigone dentigera O.PCambridge
Pitfall trap		Grammonota gentiles Banks
Pitfall trap		Islandiana princeps Braendegaard
Pitfall trap		Meioneta fabra (Keyserling)

Continued (Appendix II)

Sampling method	Family	Species
Pitfall trap		Meioneta simplex (Emerton)
Sweep-net		Microlinyphia mandibulata (Emerton)
Sweep-net		Neriene clathrata (Sundevall)
Pitfall trap		Pocadicnemis americana Millidge
Sweep-net		Porrhomma terrestre (Emerton)
Sweep-net		Tenuiphantes zebra (Emerton)
Pitfall trap	Liocranidae	Castianeira descripta (Hentz)
Pitfall trap	Lycosidae	Alopecosa aculeata (Clerck)
Pitfall trap	·	Hogna frondicola (Emerton)
Pitfall trap		Pardosa distincta (Blackwall)
Pitfall trap		Pardosa dromaea (Thorell)
Pitfall trap		Pardosa modica (Blackwall)
Pitfall trap		Pardosa moesta Banks
Sweep-net, pitfall trap		Pirata insularis Emerton
Pitfall trap		Pirata minutus Emerton
Sweep-net		Pirata piraticus (Clerck)
Pitfall trap		Trochosa terricola Thorell
Sweep-net	Philodromidae	Philodromus histrio (Latreille)
Sweep-net		Philodromus rufus quartus Dondale & Redner
Pitfall trap		Thanatus striatus C.L. Koch
Sweep-net		Tibellus maritimus (Menge)
Pitfall trap	Pisauridae	Dolomedes striatus Giebel
Sweep-net	Salticidae	Habronattus decorus (Blackwall)
Sweep-net		Phidippus whitmani Peckham & Peckham
Pitfall trap		Sitticus striatus Emerton
Sweep-net		Tutelina similis (Banks)
Sweep-net	Tetragnathidae	Tetragnatha laboriosa Hentz
Pitfall trap	Theridiidae	Enoplognatha marmorata (Hentz)
Sweep-net		Theridion murarium Emerton
Sweep-net	Thomisidae	Bassaniana utahensis (Gertsch)
Sweep-net		Misumenops asperatus (Hentz)
Sweep-net		Misumenops celer (Hentz)
Pitfall trap		<i>Ozyptila gertschi</i> Kurata
Pitfall trap		Xysticus discursans Keyserling
Pitfall trap		Xysticus emertoni Keyserling
Pitfall trap		<i>Xysticus ferox</i> (Hentz)
Sweep-net		Xysticus luctuousus (Blackwall)
Pitfall trap	Titanoecidae	Titanoeca nivalis Simon

					N	umbers in	different	t trials				
	1999					20	00		2001			
	H	[ay	S	eed	ŀ	łay	Se	eed	Н	ay	S	eed
Species		Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle
Agonum corvus Leconte	22	46	8	4	2		5	1				
A. cuprepenne Say		1	4								l	
A. cupreum Dejean	571	683	206	368	972	510	185	104	158	37	61	51
A. decentis Say	1		1				1					
A. dilutipenne Motschulsky	2										2	
A. gratiosum Mannerheim	7	1	21	6	3	2	10				1	
A. obsoletum Say								1			1	
A. placidum Say	29	26	108	27	2		1		15	4	4	11
A. retractum Leconte					1							
A. sordens Kirby				1							1	
A. thoreyi Dejean		1	1		1					1	1	1
Agonum sp.		1										
Amara apricaria Paykull			1	1							2	
<i>A. avida</i> Say	1						1		1			1
A. bokori Csiki							1					
A. carinata Leconte				7			2			1		
A. coelebs Hayward		1										
A. cupreolata Putzeys	13	7	23	69	8	1	25	49	5	3	9	13
A. ellipsis Casey									1		1	1
A. farcta Leconte		3	5	3		1	9	3				
A. impuncticollis Say			1	1					1			
A. lacustris Leconte							2	4		1	3	3
A. littoralis Mannerheim	7	3	49	73	96	25	19	9	61	14	95	17
A. obesa Say		-	6		33	23	3	17	4	. 1		1

Appendix III. Carabid beetle species and their numbers captured in pitfall traps in alfalfa fields in Arborg, Dugald, Riverton, and Teulon, Manitoba during 1999–2001.

Continued (Appendix III)					N	umbers in	different	t trials				
	1999					20	000		2001			
	E	[ay	S	Seed		Hay		eed	Hay		Seed	
Species	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle
A. patruelis Dejean		1							1			
A. quenseli Schönherr									1	1		
A. sinuosa Casey				1								
A. torrida Panzer	9		56	82	27	3	112	38	2		3	7
Amara sp.							-			4	1	3
Anisodactylus harrissi Leconte	1		16				2					
A. sanctaecrusis Fabricius		1	2				4					
Bembidion canadium Casey				1			1					
B. cordatum Leconte	1		3	3								
B. fortestriatum Motschulsky											1	
B. frontale Leconte	1											
B. lampros Herbst		13										
B. nigripes Kirby	1		1									
B. nitidum Kirby	1		2									
B. obscurellum Motschulsky	10	13	8	6								
B. patruele Dejean	1						_		_			10
B. quadrimaculatum L.	39	34	39	28			8	4	3	1	21	10
B. rapidum Leconte			2									
B. rupicola Kirby	12	23										
B. salinarium Casey	1											
B. transparens Gebler	1											
B. viridicolli La Ferté			1							1		
Bembidion sp.		1									-	
Broscodera sp.											1	
Calathus ingratus Dejean			1									

## **Continued (Appendix III)**

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					N	umbers in	differen	t trials				
	1999					20	000		2001			
	H	lay	S	eed	ŀ	Hay		eed	H	lay	Seed	
Species	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle
Calosoma calidum Fabricius	2	2	6	2	5		4	1	8	1	5	3
Carabus granulatus L.		1										
C. serratus Say			1									
C. taedatus Fabricius					2		15	21	3	1	9	1
Chlaenius alternatus Horn	1	1			4		4	3				
C. pensylvanicus Say			4	1			1					
C. sericeus Forster	1	1	3	1	2		3				1	
Cymindis borealis Leconte			2	2								
Cymindis sp.				1								
Dichaelus sculptilis Say			1				1					
Diplocheila obtusa Leconte				1			1		1		2	
Dyschirius globulosus Say			2									
D. politus Dejean				1								
Elaphrus sp.											1	
Geopinus incruvus (Say)											1	
Harpalus affinis Schrank									1			
H. amputatus Say			1	1	2	2	1	1	1			
H. egregius Casey							1					
H. erraticus Say			1									
H. erythropus Dejean										1	1	1
<i>H. faunus</i> Say		1										1
H. fulvilabris Mannerheim			1				5				4	
H. funerarius Csiki			3									
H. herbivagus Say	6	4	13	17	2	1	16	2			3	2

## **Continued (Appendix III)**

Continued (Appendix III)					N	umbers in	different	trials		·····		
	1999						000		2001			
	E	lay		eed	ł	lay		eed	H	[ay		eed
Species		Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle
H. lewisi Leconte	1		2									
H. megacephalus Leconte			1									
H. opacipennis Haldeman						5			1			
H. pensylvanicus De Geer	14	4	54		1	1	7	5			2	5
H. sommulentus Dejean	3		16	15								
H. uteanus Casey						1	3		2		1	1
Lebia moesta Leconte			5	2								
L. pumila Dejean			1	1								
Lebia sp.										1		
Notiophilus aquaticus L.	1	3	10		6	1	7	6	1		2	6
N. borealis Harris				2								
N. intermedius Lindroth			1									
N. semiatriatus Say											1	
Oxypsellaphus pusillus (Casey)	32	28	59	26	4	1	61	30	1		2	7
Patrobus stygicus Chaudoir			1									
Pterostichus adstrictus Escholtz			1		1		79	43	1	2	2	
P. caudicalis Say			1									
P. corvus Leconte	129	73	491	337	387	250	90	57	6	9	3	22
P. femoralis Kirby	10	7	73	77	6	5	25	15	14	. 5	14	12
P. leconteianus Lutshnik			1									
P. luctusosus Dejean											1	1
P. lucublandus Say	107	84	284	318	373	183	121	78	40	) 7	16	13
P. melanarius Illiger	1537	1694	1282	1214	2922	780	5666	2759	476	193	860	612
P. pensylvanicus Leconte			2	2	1		6	4		1	1	<u></u>

## **Continued (Appendix III)**

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Continued (Appendix III)												
	Numbers in different trials											
		19	99	<u></u>		20	000			20	01	
	Hay			eed	F	Hay		Seed		lay	Seed	
Species	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle	Edge	Middle
P. serutator Leconte												1
Stenolophus comma Fabricius	49				1							
Synuchus impunctatus Say			8									
Trichocellus cognatus Gyllenhal			1									
Total	2624	2762	2897	2791	4864	1795	6508	3255	809	293	1141	807