EFFECT OF *LYGUS* BUGS (HEMIPTERA: MIRIDAE) ON FIELD BEANS IN MANITOBA

A Thesis

Submitted to

The Faculty of Graduate Studies

The University of Manitoba

By

Tharshinidevy Nagalingam

In Partial Fulfillment of the Requirements for

The Degree of

DOCTOR OF PHILOSOPHY

Department of Entomology

Winnipeg, Manitoba, CANADA

ABSTRACT

Tharshinidevy Nagalingam. The University of Manitoba, 2016. EFFECT OF *LYGUS* BUGS (HEMIPTERA: MIRIDAE) ON FIELD BEANS IN MANITOBA

Supervisor: Prof. Neil J. Holliday

Lygus lineolaris (Palisot de Beauvois), *L. elisus* (Van Duzee), *L. borealis* (Knight) and *Adelphocoris lineolatus* (Goeze) were the major species of plant bugs present in commercial field bean and soybean fields in 2008–2010. *Lygus lineolaris* comprised 78–95% of the mirid adults and <10% were *A. lineolatus*. *Lygus lineolaris* reproduced in field beans and completed a single generation. In field beans, adults entered the crop in late July, corresponding to growth stages from late vegetative to pod initiation, and females laid eggs in the crop. Nymphs hatched and developed and were most numerous at the seed development and seed filling stage. At seed maturity, late instar nymphs and adults were present. In soybeans, *L. lineolaris* reproduced but nymphs had poorer survival than in field beans. Late in the season, adult numbers greatly increased in field beans and soybeans, partly due to immigration of adult *Lygus* bugs from early-maturing crops. Field beans and soybeans appeared to be a transient host for *A. lineolatus*. There were no effects on yield quality or quantity associated with the numbers of plant bugs seen in field surveys.

In laboratory and field cages, the type of injury from *L. lineolaris* feeding differed among plant growth stages but not between nymphs and adults, although nymphs generally were more injurious. At flowering to pod initiation, abortion of buds, flowers or pods was the most common response to feeding injury; pod abortion did not occur when injury occurred at later growth stages. Sometimes abortions resulted in reduced yield quantity, but sometimes plants

compensated for the injury. No loss of seed quality occurred from feeding at this stage. During seed development and filling, feeding injury most frequently affected the vascular supply to filling seeds, resulting in shriveled seeds and pods at harvest, and consequent reduced total harvested seed weight. At seed maturity, direct seed injury, involving penetration of the testa and loss of cotyledon tissue, was the most frequent injury and resulted in pits in the seed coat at harvest. There was no loss in yield quantity when feeding occurred at seed maturity, but seed pitting reduced yield quality.

DEDICATION

To my aunties Vijidevi (Peri) and Jeyanthini (Mummy)

To my dear sister Thulaja

To my dear friend Kajal

To my brothers Piraba and Kannan

To my husband Ananthan and my dear son Hrishi

For their unconditional love, encouragement and understanding which always motivated me towards my career path

ACKNOWLEDGEMENTS

Firstly, I would like to offer my sincere thanks to my advisor, Dr. N. Holliday for his excellent guidance throughout the course of my program and for his critical commentary on the thesis and manuscripts. Thank you very much Dr. Holliday for your great support and care in various ways which helped me succeed in my program. My thanks are due to the members of my advisory committee Drs. R. Lamb, R. Currie, S. Arntfield and R. Gulden for their comments and suggestions. Special thanks go to Dr. Lamb for his advice on my experiments and for reviewing my manuscripts. I thank Drs. T. Galloway, the late R. Roughley, A. Costamagna, B. Sharonowski, K. Rochon, P. Mackay, E. Huebner, and M. Sumner and Mr. J. Bannerman for all their support throughout my study period.

Special thanks go to Bruce Brolly for connecting me to Manitoba's pulse industry and for input on experimental design. Thanks are due to pulse growers in Manitoba for access to their fields, and to Parent Seed Farms and Viterra Seed Farms for grading seed samples. Special thanks go to Dennis Lange for grading and other assistance. Dr. A. Hou and his technicians assisted with threshing, and also advised on growing navy beans in environmental chambers. Dr. P. Balasubramanian advised on bean production.

Technical support from D. Holder, L. Babey, I. Brown, M. Malesko, R. Terhorst, A. Dufresne and A. Iverson is greatly appreciated. Thanks are due to A. Leroux, C. Babel, A. Sharma, A. Rios, B. Rogala, T. Harms, C. To, L. Peixoto, J. Crowe, J. Mcleod, T. Dickson, T. Sharanowski, S. Ibara, S. Surowiec, R. Duarte, J. Veilleux and T. Sharma for field and laboratory help. I am grateful to K. Graham and M.A. Baker for their help. I thank all the graduate students in the Department of Entomology, especially L. Andreassen, J. Veilleux, J. Du, S. Oghiakhe, R. Bahreini, S. Desai, M. Zhang, A. Rios and A. Leroux for their moral support, motivation and

encouragement. Special thanks go to Dr. L. Holliday for her love and care, which encouraged and motivated me at difficult times.

Essential financial support for the successful completion of this study was provided by The University of Manitoba, Manitoba's Agri-Food Research and Development Initiative, and the Manitoba Pulse Growers Association.

The support and encouragement of my husband Yogananthan Thambimuthu and my son Hrishikesh Yogananthan is highly appreciated. Special thanks to the Sel Paranjothy family for accommodating me in their home when I first came to Canada and for their kind support and encouragement during my study. I thank all my friends in Winnipeg for their help throughout my study. Last but not least, I wish to offer my gratitude to my family members for their understanding and unwavering support throughout my career path.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF COPYRIGHTED MATERIALS

CHAPTER 3 – Manuscript I: Plant bugs (Hemiptera: Miridae) on field beans and soybeans in Manitoba: species composition and seasonal occurrence. A revised form of this article is published in the Canadian Entomologist published by Cambridge University Press as Nagalingam, T. and Holliday, N.J. 2015. Plant bugs (Hemiptera: Miridae) on field beans and soybeans in Manitoba: species composition and seasonal occurrence. Canadian Entomologist **147**: 766–775. An adapted version of the article is used in this thesis with the permission of Cambridge University Press.

CHAPTER 1: General Introduction

Introduction

In Manitoba, *Lygus* bugs are pests of several economically important crops, including canola, alfalfa, buckwheat and seed alfalfa (Timlick *et al*. 1993; Turnock *et al*. 1995; Mostafa 2007; Uddin 2005; Wise *et al*. 2005). In Manitoba, *Lygus* bugs usually have one or two generations per growing season (Gerber and Wise 1995; Mostafa 2007). *Lygus lineolaris* (Palisot de Beauvois), *L. borealis* (Knight) and *L. elisus* (Van Duzee) are the three major species present in Manitoba. The most abundant among them is *L. lineolaris* (Gerber and Wise 1995; Mostafa 2007). This species has been reported to be a dominant species in Manitoba for more than two decades (Timlick *et al*. 1993; Gerber and Wise 1995; Mostafa 2007).

To feed continuously on young reproductive tissues, *Lygus* bugs in Manitoba move from crop to crop in synchrony with the development of buds or flowers of the target hosts (Gerber and Wise 1995; Leferink and Gerber 1997; Mostafa 2007). *Lygus* bugs on canola, seed and hay alfalfa, and buckwheat are well studied in Manitoba (Timlick *et al*. 1993; Turnock *et al*. 1995; Mostafa 2007; Uddin 2005; Wise *et al*. 2005). Influxes of adult *Lygus* bugs are found in buckwheat and seed alfalfa fields late in the season (Uddin 2005; Wise *et al*. 2005; Mostafa 2007). Based on studies carried out in commercial fields of buckwheat in Manitoba, Mostafa (2007) suggests that the source of these adults could be canola fields adjacent to them. When there are no physical barriers in between fields, *Lygus* bugs also can disperse to adjacent crops after alfalfa fields are mowed for hay (Schaber *et al*. 1990.). These situations may result, late in the growing season, in large numbers of adult *Lygus* bugs moving into crops that are still green, and bearing flowers or seeds.

Field beans are one of the late season crops in Manitoba; they are planted in May and harvested during mid- to late-September (Manitoba Agriculture Food and Rural Development

2011a). In Manitoba, *Lygus* bugs have been observed on field beans, in some years in large numbers (Gavloski 2001–2010), and spraying for plant bugs occurred in some of those years (Gavloski 2001–2010). Field beans may suffer yield loss from *Lygus* bug feeding. The species composition and seasonal patterns of occurrence of plant bugs on field beans in Manitoba are unknown, and the nature of damage to field beans in Manitoba is poorly understood. Also there is no information on the relationship between insect numbers and loss of field bean yield quality and quantity. The research reported in the following chapters was designed to provide the missing information. This research was partly funded by The Manitoba Pulse Growers' Association, whose membership includes both field bean and soybean producers. Soybean growers requested information about the pest status of plant bugs in soybeans and so, although most of my research was performed on field beans, field surveys included both crops.

Objectives of the research

1. Assessment of seasonal patterns and species composition of mirid bugs in field beans and soybeans in Manitoba.
2. Document the types of injury caused by different life stages of *Lygus* bugs feeding on different growth stages of field beans.
3. Examine in controlled conditions and in field cages the effect of feeding of *Lygus* bugs on different growth stages of field beans.

Thesis organization

This thesis is organized into four main sections: General Introduction, Literature Review, Research and the General Discussion. The general introduction provides the background and the rationale for the research. The literature review covers the importance of field bean and soybean growing in Canada and aspects of their production in Manitoba, plant bug biology with particular

reference to species of importance in Manitoba, the feeding mechanisms of *Lygus* bugs and interactions with their host plants, effects of injury on different plants, and sampling methods for *Lygus* bugs. The research section is written in the style of a series of manuscripts of scientific papers. In each manuscript, one of the three objectives of the new research is addressed. Each research manuscript comprises an abstract, introduction, materials and methods, results and discussion specific to that manuscript. In the general discussion the findings from these manuscripts are related to each other, and conclusions from the research are derived.

The first manuscript of this thesis has been published by The Canadian Entomologist (2015, Volume 147, pages 766–775). The contents of my second manuscript will be combined with the laboratory studies of the third manuscript and will be submitted for publication to the Canadian Entomologist. The field cage component of the third manuscript is unlikely to be publishable as there is great variability among the experimental results.

CHAPTER 2: Literature Review

CHAPTER 2

Literature Review

Lygus and other plant bugs (Hemiptera: Miridae) are among the most well-studied insects on a wide range of crops in North America, and there is a vast amount of literature on these pests. I have focussed my literature review on the major species of plant bugs in Manitoba with greatest focus on the commonest species, *Lygus lineolaris* (Palisot de Beauvois). Further, the treatment of literature on field beans (*Phaseolus vulgaris* L.) (Fabaceae) is focused on production in Canada and more specifically Manitoba. A small part of my study concerns soybeans (*Glycine max* (L) Merr.) and so a brief treatment of soybean production is included in the literature review.

1a. Field beans in Canada

Field beans are an important leguminous crop in Canada (Park 1985). Cultivated *P. vulgaris* grown as a grain legume are referred to by several names including "field beans", "dry beans" "edible beans", and "dry edible beans" (Manitoba Agriculture Food and Rural Development 2011a). Navy (white pea), pinto, red kidney, great northern, black, small red, pink, and cranberry (Romano) are the important market classes of field beans produced in Canada (Agriculture and Agri-Food Canada 2012). In Manitoba, navy and pinto beans cover the largest area of bean cultivation; other bean types are grown to a limited extent (Pulse and Soybean Growers Association 2015).

In Canada, growing of field beans as a commercial crop began in the mid-19th century in Ontario (Park 1985), where they have been grown commercially ever since (Pulse Canada 2003). Field bean production has extended to other regions of Canada during the last three decades. Since the 1970s, most of Canadian field beans have come from southern Alberta, southern Manitoba and Ontario, with small amounts produced in Quebec and Saskatchewan (Pulse

Canada 2003). During the last two decades, the highest area seeded to field beans in Manitoba was 126,000 ha in 2002. There has been a reduction in the production area in recent years, and in 2014 the planted area was about 58,000 ha (Manitoba Agriculture Food and Rural Development 2014a). In 2010, Manitoba's field bean crop value was $43.49 million (Pulse and Soybean Growers Association 2015). Canada is one of the largest exporters of pulse crops to the world market (Pulse Canada 2013), and about 70% of Canada's bean crop is exported (Pulse Canada 2007). The main markets for Canadian field beans are the United States of America, United Kingdom, Italy, Japan, Dominion Republic, Angola and Greece (Agriculture and Agri-Food Canada 2012).

Field beans are mostly used for human consumption and they are canned or dried or are processed into food products such as tortillas; damaged and low-grade beans are used as livestock feed (Agriculture and Agri-Food Canada 2012). Field beans are nutritious: they are rich in protein and in dietary fibre (carbohydrates with low glycemic index); they are a good source of iron, folate and manganese; they are low in fat; free of saturated fat and cholesterol; and contain potassium, magnesium and phosphorus. Small red, red kidney and pinto beans are rich in antioxidants (Agriculture and Agri-Food Canada 2012).

Two types of production system are adopted by Manitoban field bean growers, one is cultivation of the crop in rows spaced at 76–91 cm, which is referred to as a row seeded crop; the other is a solid seeded crop, which has a lower row spacing that suits the farmer's available equipment (Manitoba Agriculture Food and Rural Development 2011a). Row seeded cultivation is most common in Manitoba, with a seed rate of 52–72 kg/ha, depending on the seed size and variety of beans planted. Field beans are a warm season crop and prone to frost damage (Manitoba Agriculture Food and Rural Development 2011a), and so are seeded later than more

frost-tolerant crops. Field beans are planted when the soil is moist; the planting depth is 3–5 cm (Manitoba Agriculture Food and Rural Development 2011a).

Field beans seeds are sensitive to placement of fertilizers, and seed burn occurs if seed is too close to fertilizer (Manitoba Agriculture Food and Rural Development 2011a). Band application, in which fertilizer is applied in 5 cm bands to the side and 5 cm below the seeds, is normally practised (Manitoba Agriculture Food and Rural Development 2011a). *Rhizobium phaseoli* Dangeard inoculants are used to facilitate establishment of symbiotic nitrogen fixation in the root system of field beans (Manitoba Agriculture Food and Rural Development 2011a). About 17 kg/ha of elemental nitrogen is needed by field beans from the time of seeding to the onset of nitrogen fixation (Manitoba Agriculture Food and Rural Development 2011a). Supplementary nitrogen applications of 22.4–44.8 kg/ha are necessary if the soil nitrogen is less than 39 kg/ha, spring temperatures are cool, higher seed yields are anticipated, or root rot disease impairs root efficiency (Manitoba Agriculture Food and Rural Development 2011a). Field bean yields are not responsive to applied phosphorus, but are associated with available soil phosphorus. For solid seeded field beans with a row spacing of 15–18 cm, the P_2O_5 fertilizer recommendation ranges between 0 and 22.4 kg/ha, inversely related to soil P_2O_5 of 25–0 ppm (Manitoba Agriculture Food and Rural Development 2011a). Fertilizer recommendations for K_2O vary from 0 to 73 kg/ha, inversely related to soil K_2O of 225–0 ppm (Manitoba Agriculture Food and Rural Development 2011a).

Even very low weed populations may cause severe yield loss in field beans (Manitoba Agriculture Food and Rural Development 2011a).The most common broadleaved weeds are wild buckwheat (*Polygonum convolvulus* L.*)*, ragweed (*Ambrosia* L.), nightshades (*Solanum* spp.), redroot pigweed (*Amaranthus retroflexus* L.), wild mustard (*Sisymbrium officinale* (L.)) and

lamb's quarters (*Chenopodium album* L.); common grassy weeds are green foxtail (*Setaria viridis* (L.)) and wild oats (*Avena sativa* L.) (Pulse Canada 2003). Perennial weeds include Canada thistle (*Cirsium arvense* L.), sow thistle (*Sonchus* spp.) and quack grass (*Elytrigia repens* (L.) Nevski) (Pulse Canada 2003). Weed management may be achieved by planting in a clean field and by inter-row cultivation in row-seeded crops. The weed species present in fields are determined by crop rotation; cereals in the rotation before beans can offer clean fields before cultivation. Effective herbicides for annual or perennial weed control in field beans are limited (Manitoba Agriculture Food and Rural Development 2014b).

Rust, white mould, bacterial blight, root rot and bean common mosaic are the most common diseases of field beans in Manitoba (Manitoba Agriculture Food and Rural Development 2011a). There are also several abiotic disorders that are common in field beans, such as sunscald, bronzing, baldhead, and zinc deficiency (Manitoba Agriculture Food and Rural Initiative 2011a).

Before harvesting the crop, the desiccant Reglone® (Diquat) is applied to the crop when the crop has lost 80–90% of its leaves. This application hastens the drying of leaves and also reduces seed staining from contact with green leaves (Manitoba Agriculture Food and Rural Development 2011a). To harvest row crop field beans, they are pulled, windrowed, and then combined using a pick-up header. Pulling occurs by cutting the bean stem just below the soil surface with a fixed blade, rod weeder or rotary disk-type puller. Pulling and windrowing can also be done as one operation. Windrows are then picked up using a Sund or Rake-up pick-up (Manitoba Agriculture Food and Rural Development 2011a). Pulling is not performed in solid-seeded crops, which may be windrowed and then combined, or direct combined. Precautions, such as use of a lifter, must be taken to collect low-growing pods; despite these, yield losses at

harvest of solid seeded field beans are higher than for the row-cropped fields (Manitoba Agriculture Food and Rural Development 2011a).

The size and moisture content of the seeds are the key factors that need to be considered during the harvest and handling of beans in order to maintain seed quality. Field beans are threshed when the seed moisture content is 16–22% (Manitoba Agriculture Food and Rural Development 2011a). Threshing below a moisture content of 16% increases seed damage (Manitoba Agriculture Food and Rural Development 2011a). Wet and immature beans are prone to staining from leaves, stems and dirt. The best moisture level for storage of field beans is 16%; below this, there may be splitting or cracking of the seed coat, resulting in reduced quality. The structures used to store beans are elevated bins, hopper bottom bins and flat surfaces (Manitoba Agriculture Food and Rural Development 2011a).

Quality standards of bean seeds vary with market classes (Ontario Ministry of Agriculture Food and Rural Affairs 2009a). The general quality and grade of bean seeds are determined by the colour, odour, and uniformity of the bean seeds and the presence of contaminants. Damaged seeds, frozen seeds, fire-burnt seeds, immature seeds, stained seeds and contaminants such as soil particles, fungi (ergot and sclerotinia), excreta, fertilizer pellets, off-type seeds and insect particles are commonly seen factors that reduce bean seed quality (Pulse Canada 2003; Canadian Grain Commission 2014).

Before grading, percentage of "dockage" is determined; dockage is that component that can be removed by seed cleaning (Canadian Grain Commission 2014). After seed cleaning, a representative portion of the sample is taken for grading (Canadian Grain Commission 2014). Not all foreign material is removed during cleaning and the presence of such material results in rejection or lowering of the grade; "percent pick" is the term used to indicate the percentage by

weight of undesirable material in samples for grading, and includes defective beans, such as those with splits, pits, checked seed coats or discolouration, as well as misshapen beans, or those of contrasting types, and foreign material.

Field bean yield quality and quantity may be affected by insect pests attacking the growing crop. In Canada, seed corn maggot (*Delia platura* Meigen), wireworms (Elateridae), green clover cutworms (*Hypena scabra* (Fabricius)), army cutworms (*Euxoa auxiliaris* (Grote)), red backed cutworms (*Euxoa ochrogaster* (Guenee)), pale western cutworms (*Agrotis orthogonia* Morris), grasshoppers (*Melanoplus* spp.), bean leaf beetle (*Certoma trifurcata* (Forster)), potato leafhopper (*Empoasca fabae* Harris), *Lygus* bugs (*Lygus* spp.) and alfalfa plant bugs (*Adelphocoris lineolatus* (Goeze)) are the insect pests recorded on field beans (Pulse Canada 2003; Manitoba Agriculture Food and Rural Development 2011a, 2014b; Gavloski 2001–2010).

According to Gavloski (2001–2010), the following insects damage field beans in Manitoba. Seed corn maggots and wireworms attack the roots and germinating seeds of field beans, resulting in death or stunting of plants. Green clover worms, grasshoppers and bean leaf beetles defoliate plants. Potato leafhopper is a sucking pest and causes hopper burn symptoms, in which leaves curl and pucker, and eventually the leaf edges become scorched.

Plant bugs such as *Lygus* bugs (*Lygus* spp.) and alfalfa plant bug (*A. lineolatus*) occur in Manitoba (Gavloski 2001–2010). In 2007, several fields in Manitoba were sprayed with insecticide for controlling plant bugs, and seed damage consistent with *Lygus* damage was found (Gavloski 2001–2010). Before the completion of my research no information was available on the effect of plant bug injury on field bean yield quantity and quality in Manitoba.

1b. Reproductive structures of *Phaseolus vulgaris*

The major feeding sites of *Lygus* bugs are the reproductive structures of plants and therefore in this section the reproductive structures of beans are described. The reproductive structures of bean plants are compound racemes that arise from the base of trifoliate leaves (Sage and Webster 1987). Each compound raceme consists of a peduncle with an axillary node at its base and up to five other nodes along its length (Sage and Webster 1987). At each node there are three buds produced (Sage and Webster 1987). The buds in the basal part of the raceme flower first and flowering proceeds acropetally (Adams 1967). *Phaseolus vulgaris* beans are self-pollinated (Peksen 2007) and pollination in *P. vulgaris* occurs at the bud stage prior to flower opening (Sage and Webster 1990). Hence there is a little opportunity for the flowers to be cross-pollinated by insect pollinators.

In natural conditions, there is abortion of some buds or the structures that arise from them, and the probability of abortion varies with the position of the bud in the compound raceme (Sage and Webster 1987). Frequency of abortions is less for the basal buds than for the other nodes of the raceme (Tamas *et al*. 1979; Sage and Webster 1987; Mauk *et al*. 1987). As a result of the pattern of abortions, numbers of flowers formed, numbers of pods formed and numbers of pods harvested decrease towards the tip of an inflorescence (Mauk *et al*. 1987). Most abortions occur within 5 days of anthesis (flowering) in *P. vulgaris* (Sage and Webster 1987) and the different buds in a raceme initiate the process at different times (Sage and Webster 1987). The critical development time for most abortion to occur is at the green bud stage and in the early stages of fruits and embryo development (Sage and Webster 1987).

As well as natural levels of abortion, several factors may trigger additional abortion of buds, flowers and pods in beans. Water stress (Mauk *et al*. 1984) and high temperatures promote

higher levels of abortion (Baker *et al*. 1946). Feeding by insects such as *Lygus* bugs also causes abortion in several types of beans such as Lima beans (Baker *et al*. 1946), snap beans (Fisher *et al*. 1946), and green beans (Khattat and Stewart 1975).

Pods are the fruits of *P. vulgaris*; pods are modified leaves in which the mid vein runs dorsally along the pod and provides the vascular supply for the carpel region of the pod (Woodcock 1935). Individual fruit of beans are monocarpellary and unilocular, with several seeds attached along a long placental region on the ventral edge of the pod (Woodcock 1935). The placenta consists of two vascular bundles, each providing the vascular supply to alternate ovules or seed loci along the pod's length; the vascular bundle supplies nutrients to the seed through an S-shaped funiculus (Woodcock 1935). In healthy pods, some bean seeds fail to develop and the weight of the seeds in different positions along the pod varies (Harris 1915). The percentage of seeds that abort is reduced towards the distal (stigmatic) end of a pod (Harris 1915; Nakamura 1988). Seed weight at the proximal end of the pod is lower than the weight of the seeds in other positions (Harris 1915; Nakamura 1988).

1c. Soybeans in Manitoba

Since 2005, there has been a steady increase in soybean production in Manitoba, and in 2013 the area of soybean cultivation in the Province was 52, 609 ha (Statistics Canada 2014). About 10 to 18% of Canadian soybean production comes from Manitoba. The major destinations for Manitoba's soybean production are the United States, Japan and several other countries in Asia, and Europe (Manitoba Agriculture Food and Rural Development 2012). Canadian soybean exports during 2011–2012 totalled 2.6×10^6 tons (Agriculture and Agri-Food Canada 2012; Statistics Canada 2014). The Manitoba contribution to exports in 2011 was worth more than $160 million (Manitoba Agriculture Food and Rural Development 2012). Soybeans are used for

human consumption and for livestock feed (Manitoba Agriculture Food and Rural Development 2012). Soybeans are also a source of printing ink, biodiesel, waxes, oils and solvents (Manitoba Agriculture Food and Rural Development 2012).

Soybeans are grown in southern Manitoba, in a wide range of soil conditions (Manitoba Agriculture Food and Rural Development 2011b). Seeds are sensitive to seed rot and seedlings are prone to frost damage (Manitoba Agriculture Food and Rural Development 2011b). Best results for soybean establishment are from seeding at 40 seeds/m^2 at a depth of 1.9–3.8 cm (Manitoba Agriculture Food and Rural Development 2011b). Soybeans are very sensitive to weed pressure, and the primary weed control method is herbicide application (Manitoba Agriculture Food and Rural Development 2011b).

Soybeans are attacked by a wide range of insects (Zeiss and Klubertanz 1993). In Manitoba, wireworms and seed corn maggots (*D. platura*) attack the roots or germinating seeds early in the season; red backed cutworm (*E. ochrogaster*) feeds on stems so that they break off; green clover cutworm (*H. scabra*), alfalfa caterpillar (*Colias eurytheme* (Boisduval)), thistle caterpillar (*Vanessa cardui* L.), alfalfa looper (*Autographa californica* (Speyer)), grasshoppers (*Melanoplus* spp.) and alfalfa weevils (*Hypera postica* Gyllenhal) are defoliators of soybeans, and soybean aphids (*Aphis glycines* Matsumura) are sucking pests (Gavloski 2001–2010; Manitoba Agriculture Food and Rural Development 2011b). There have been no reports of the presence of plant bugs or their effect on soybean yield and quality in Manitoban soybeans.

2. Plant bug classification, distribution and importance

Plant bugs belong to the order Hemiptera, family Miridae, subfamily Mirinae and tribe Mirini (Knight 1930; Schwartz and Foottit 1992b). In North America, *Lygus* Hahn and *Adelphocoris* Reuter are the two major pest genera of plant bugs. There are 34 species of *Lygus* present in

North America (Kelton 1975) and 22 of these are found in the Prairie Provinces of Canada (Kelton 1980). In the Prairie Provinces, *L. lineolaris, L. elisus* (Van Duzee) and *L. borealis* (Knight) are the important pest *Lygus* species (Kelton 1980).

Lygus lineolaris is a trans-American species that occurs from southern Mexico, north to Great Slave Lake and the Mackenzie Delta. *Lygus elisus* is found west of the Mississippi valley in the US, and in Canada occurs in the Prairie Provinces and north to the Yukon (Kelton 1975). *Lygus borealis* occurs predominantly in the Great Plains and is a northern species in the western part of North America extending south to Iowa (Kelton 1975).

In crops in the Prairie Provinces, *Lygus* species composition and relative dominance vary with region, year, date of collection and the type of crop grown (Schwartz and Foottit 1992b; Timlick *et al.* 1993; Gerber and Wise 1995; Braun *et al.* 2001; Cárcamo *et al.* 2002). *Lygus borealis* and *L. elisus* are the dominant species in Alberta and Saskatchewan, whereas *L. lineolaris* is the dominant species in Manitoba (Craig 1983; Butts and Lamb 1991a; Schwartz and Foottit 1992a). In Saskatchewan, *L. lineolaris* and *L. borealis* are both abundant in some growth stages of alfalfa, but in canola *L. lineolaris* is the dominant species (Braun *et al*. 2001). In Alberta, *L. lineolaris* is the dominant species in canola grown in the boreal region, but *L. keltoni* Schwartz and Foottit is the dominant species in the canola grown in the grassland region (Cárcamo *et al*. 2002).

The alfalfa plant bug, *Adelphocoris lineolatus*, is an introduced species from Europe (Kelton 1980). In Canada, *A. lineolatus* was first collected in Nova Scotia in 1917 (Knight 1921), and it has been recorded in Manitoba from 1941, and in Saskatchewan from 1947 (Craig 1963). By 1992 it was widespread in alfalfa throughout Canada (Schaber 1992). There are two species of *Adelphocoris* in Manitoba, *A. lineolatus* (Goeze) and *A. rapidus* (Say) (Kelton 1980).

3a. Biology of *Lygus lineolaris*

Lygus lineolaris overwinter as diapausing adults. In the southern U.S.A, the critical photoperiod is 12.5:11.5 h (L:D), and at shorter day lengths the adults undergo diapause (Bariola 1969; Snodgrass 2003). Alfalfa fields (Khattat and Stewart 1980) and plant debris (Snodgrass 2003) are potential overwintering sites. In early spring, adult *L. lineolaris* emerge from hibernation and feed on suitable host plants (Kelton 1975). Table 1 shows some of the biological parameters of *L. lineolaris* during the growing season. Reproductive activity starts immediately after spring emergence, and adults mate several times during their life time (Bariola 1969). There is a pre-oviposition period of 7 to 12 days, depending on temperature (Table 1). Females insert their ovipositor into plant tissues and eggs are laid into the tissues; the operculum region of the egg can be seen at the surface of the plant tissue (Painter 1929). Eggs are laid singly or in loose aggregations. There are five nymphal instars (Kelton 1975; Schwartz and Foottit 1992b). The duration of nymphal development varies greatly, depending on growth conditions and food material (Table 1). First and fifth instar nymphs have longer developmental periods than the other nymphal instars (Table 1).

The number of generations *Lygus* bugs produces per year varies greatly, and depends mainly on the length of the growing season, mean temperature (Craig 1983; Leferink 1992), accumulated degree days (Champlain and Butler 1967), other climatic conditions and host plants (Kelton 1975). *Lygus lineolaris* produces one generation in northern Alberta (Butts and Lamb 1991a) and northern Saskatchewan (Craig 1983). Two generations occur in southern Manitoba (Timlick *et al*. 1993; Gerber and Wise 1995), southern Saskatchewan (Craig 1983), eastern Ontario (Painter 1929; Craig 1983) and southern Alberta (Salt 1945). Three generations of *L. lineolaris* occur in New York (Ridgway and Gyrisco 1960; Stewart and Khoury 1976). *Lygus*

borealis in Manitoba produces two generations in alfalfa (Gerber and Wise 1995) and the first generation adults of both *L. lineolaris* and *L. borealis* have the same egg-laying period (Gerber and Wise 1995)

Table 1. Major biological parameters of Lygus lineolaris

Parameter	Conditions	Measure	References
Pre-oviposition period (days)	27 °C	7.6 ± 1.7	Slaymaker and Tugwell 1982
	32–21 °C	6.8 – 11.5	Bariola 1969
Egg laying period (weeks)	Field collected overwintering adults	5–7	Bariola 1969
Total number of eggs	Laboratory reared adults	31–183 (Average 98.7)	Bariola 1969
	Field collected overwintering adults	303 ± 44 –334 ± 33	Gerber 1995
	Field collected first generation adults	250 ± 27	Gerber 1995
Peak oviposition rate (per day)	27 °C Potato sprouts	4.8	Slaymaker and Tugwell 1982
Peak oviposition period	27 °C	18 days after emergence	Slaymaker and Tugwell 1982
Incubation period (days)	27 °C Potato sprouts	8.7 ± 1.0	Slaymaker and Tugwell 1982
	27 °C Green beans	5–9	Bariola 1969
	27 °C Cotton	10–13	Bariola 1969
	20 °C Green beans	12–17	Ridgway and Gyrisco 1960
	25 °C Green beans	7–9	Ridgway and Gyrisco 1960
Average nymph developmental time (first to fifth instars) (days)	27 °C Green beans	2.8, 2.1, 2.4, 2.6 and 4.2	Bariola 1969
	21 °C Cotton	3.5, 3,3.5,4,5.9	Bariola 1969
	20 °C Green beans	7, 4.8, 5.5, 6, 7.9	Ridgway and Gyrisco 1960
	25 °C Green beans	4.8, 3, 3.3,3.3,5.2	Ridgway and Gyrisco 1960
	22.1 °C Oil seed rape	5.1, 3.4, 3.0, 3.3, 3.5	Leferink 1992
	15.6 °C Oil seed rape	8.8, 6.5, 6.4, 7.9, 12.3	Leferink 1992
	27 °C Cotton	3.5, 3.0, 3.5, 4.0, 5.9	Bariola 1969
	25 to 27 °C Potato sprouts	2–3 (first to fourth instar) 4 (fifth instar)	Slaymaker and Tugwell 1982
Longevity female (days)	27 °C	39.9 ± 18.9	Slaymaker and Tugwell 1982
Longevity male (days)	27 °C	38.1 ± 15	Slaymaker and Tugwell 1982
Egg to adult development period (days)	27 °C	23.9 ± 1.9	Slaymaker and Tugwell 1982
Egg to adult survival rate (%)	27 °C, Potato sprouts	76.0	Slaymaker and Tugwell 1982

3b. Biology of alfalfa plant bug

Alfalfa plant bugs, *Adelphocoris lineolatus*, overwinter as eggs in plant stems (Hughes 1943), commonly in the perennial stems of alfalfa. In spring, eggs hatch and nymphs develop through five instars (Craig 1963). Development time from egg to adult ranges from 33.5 to 46 days (Hughes 1943). *Adelphocoris lineolatus* produces one generation to the north of 53° N (Murrell 1987) and two or more generations in the areas south of 51° N (Craig and Loan 1984; Murrell 1987). In Manitoba, there are probably two generations in alfalfa (Uddin 2005). Wise *et al*. (2005) report that *A. lineolatus* produces a single generation in buckwheat in experimental plots, but according to Mostafa (2007) buckwheat acts only as a transient host and there is no strong evidence of reproduction and nymphal development on buckwheat.

4. Feeding of *Lygus* bugs

Lygus bugs possess piercing and sucking mouth parts as in other Hemiptera (Triplehorn and Johnson 2005). The mouthparts are modified to form a proboscis. The labium forms the outer cover of the proboscis and within that cover there are four stylets; two outer stylets are modified mandibles and the two inner stylets are modified maxillae (Triplehorn and Johnson 2005). These stylets are connected to retractor and protractor muscles and are used to probe the plant tissue for food (Triplehorn and Johnson 2005).

In 1954, Flemion *et al.* stated that the feeding injury *Lygus* bugs inflict on plants is caused only by mechanical rupturing of cells so that they lose their contents. However, the role of salivary enzymes in causing the injury has since been elucidated. Strong (1970) and Strong and Kruitwagen (1968) demonstrated that enzyme activity is primarily responsible for the injury, and polygalacturonase is the enzyme primarily responsible for digestion of plant tissues. Recent studies have provided supporting evidence for this and that *Lygus* bugs use a “laceration and

maceration" action to cause injury: plant tissues are drilled or lacerated and then saliva is injected into the tissue followed by enzymatic action (maceration) and subsequent ingestion of cell contents (Shackel *et al*. 2005; Backus *et al.* 2007).

In *Lygus* bug saliva, polygalacturonase (Strong and Kruitwagen 1968; Strong 1970; Laurema *et al*. 1985; Cooper *et al*. 2013), α-amylases (Agblor *et al*. 1994; Agusti and Cohen 2000) and alkaline proteases are the major enzymes involved in digestion (Laurema *et al*. 1985; Cooper *et al*. 2013). Acid proteinase, phosphatase, trehalase, invertase, phenoloxidase, laccase, alkyl hydro peroxide reductase, glucose dehydrogenase, and xanthine dehydrogenase are minor components (Laurema *et al*. 1985; Cooper *et al*. 2013).

Polygalacturonase in *Lygus* bug saliva is a pectinase that enzymatically digests plant cell walls by solubilizing the middle lamella of the cell wall (Strong and Kruitwagen 1968; Agblor *et al*. 1994; Cohen and Wheeler 1998). Polygalacturonase hydrolyses glycosidic linkages between adjacent, substituted galacturonic acid residues in the α-1, 4 linked homogalacturonin backbones of simple pectin polymers (Cook *et al*. 1999). Endo-polygalacturonases degrade polygalacturonic acid in plant cell walls and produce oligogalacturonides while exo-polygalacturonases hydrolyse the non-reducing ends of the polygalacturonic acid polymer and produce the monosaccharide galacturonic acid. Some polygalacturonases exhibit both endo- and exo-activity, producing galacturonic acid oligomers with a degree of polymerization of seven or smaller (Cook *et al*. 1999). Saliva of *Lygus* bugs contains more than one polygalacturonase with endo, exo or both hydrolytic modes of action (Agblor *et al.* 1994; Celorio-Mancera *et al*. 2009).

Once cell walls are broken, α-amylases and proteases help in extra-oral digestion of cell contents (Cooper *et al*. 2013). Phosphatase acts on ester bonds and helps in dephosphorylation of

proteins (Hori 2000). Trehalases detoxify allelochemicals of plants, suppressing plant-defence mechanisms (Hori 2000). Laccase, alkyl hydroperoxide reductase, glucose dehydrogenase, and xanthine dehydrogenase also function in the detoxification of plant defensive chemicals (Cooper *et al*. 2013).

In earlier days, the role of non-enzymatic secretions in injury was investigated, particularly the mechanism resulting in growth distortion or abortions of reproductive organs. Auxins are produced in meristematic tissues of the terminal or lateral buds, fruiting structures and seeds, and are associated with numerous plant processes including cell enlargement, apical dominance and regulation of abscission of vegetative and fruiting organs (Thimann 1972). In some mirids the presence of the auxin, indole acetic acid (IAA), in the salivary glands (Miles and Lloyd 1967) or the transportation of IAA to the salivary gland by ingestion through its diet (Nuorteva 1956) has been shown. No IAA is found in the saliva of *L. hesperus* Knight and *L. rugulipennis* Popp. (Strong 1970). Findings of Laurema and Varis (1991) also provide supporting evidence for this; only very small amounts of tryptophan, a precursor of IAA, occur in the salivary glands of *L. rugulipennis* and no IAA is found in salivary gland extracts. Therefore there is no or little contribution of IAA from *Lygus* bugs saliva in the injury of plant tissues.

The preferential feeding of *Lygus* bugs on meristematic tissues could lead to hormonal imbalances resulting from localized injury at the feeding site. Hormonal imbalance leads to altered vegetative growth such as reduction in stem growth and increased growth of lateral branches (Jeppson and Macleod 1946), and morphological deformation of fruits or seeds (Rice 1938; Allen and Gaede 1963). Because of the major role auxins play in regulating abscission of fruiting structures, abscission of these structures can occur as a result of destruction of the auxin-

producing tissues in the meristematic tissues during *Lygus* bug feeding (Davis *et al*. 1963; Khattat and Stewart 1975). Fisher *et al*. (1946) found that *L. lineolaris* feeding produced less flower abscission in beans when plants were treated with a foliar application of auxin and naphthalene acetic acid (NAA). Thus plant growth distortions and abortions resulting from *Lygus* bug feeding can be attributed to feeding destroying auxin-producing plant tissues, rather than, as originally suggested, to the injection of auxins from the insects' saliva.

5a. Host plants of *Lygus* bugs and alfalfa plant bugs

Young (1986) recorded 385 host plants of *L. lineolaris* in the United States and Canada. *Lygus lineolaris* feeds on 55 plant families including both monocotyledons and dicotyledons. The plant families containing most host plant species are Asteraceae, Fabaceae, Brassicaceae, Rosaceae, Graminae, Polygonaceae, Umbelliferae, Chenopodiaceae, Solanaceae, and Malvaceae (Young 1986). Young (1986) reported 40 taxa of hosts from the family Fabaceae (=Leguminosae) including alfalfa (*Medicago sativa* L.), snap beans or green beans (*P. vulgaris*), several clover species (*Trifolium* L.), soybeans, American potato bean (*Apios americana* Medic.), peanut (*Arachis hypogaea* L.), milk-vetch (*Astragalus diphysus* Gray), partridge pea (*Cassia fasciculate* Michx.), crown vetch (*Coronilla varia* L.), begger's tick (*Desmodium perplexum* Schub.), sweet peas (*Lathyrus* L.), lespedeza (*Lespedeza cuneata* (Dumont) G. Don), bird's-foot trefoil (*Lotus corniculatus* L.), spotted burclover (*Medicago arabica* (L.) Huds.), sweet clovers (*Melilotus* spp. L.), sour clover (*Melilotus indica* (L.) All., Lima beans (*Phaseolus lunatus* L.), garden pea (*Pisum sativum* L.), kudzu (*Puerarai lobata* (Willd.) Ohwi), black locust (*Robinia pseudoacacia* L.), hemp sesbania (*Sesbania exaltata* (Raf.)), wild bean (*Strophostyles helvola* (L.) Ell.), vetches (*Vicia* spp. L.) and cowpea (*Vigna sinensis* Endl.).

In Canada, *Lygus* bugs affect several economically important crops including canola (*Brassica rapa* subsp. *oleifera* (DC) *Metzger*, *B. napus* L.) (Butts 1989; Butts and Lamb 1990a, 1990b, 1991b; Leferink and Gerber 1997), alfalfa (*M. sativa*) (Timlick *et al*. 1993; Mostafa 2007; Uddin 2005), strawberries (*Fragaria* × *ananassa* Duchesne) (Mailloux and Bostanian 1988; Bostanian *et al*. 1990), grapevines (*Vitis vinifera* L.) (Fleury *et al*. 2006), sunflowers (*Helianthus annuus* L.) (Gavloski 2001–2010), wheat (*Triticum* L.) (Wise *et al*. 2000), green beans (Khattat and Stewart 1975, 1980; Stewart and Khattat 1980), and buckwheat (*Fagopyrum esculentum* Moench) (Wise *et al*. 2005).

Adelphocoris lineolatus has a narrow host range, and feeds mainly on cultivated legumes such as alfalfa, *M. sativa*, red clover, *Trifolium pratense* (L.) (Craig 1983) and alsike clover *T. hybridum* L. (Hughes 1943). It has also been reported on other crops such as potatoes, *Solanum tuberosum* L. (Hughes 1943) and buckwheat (Hughes 1943; Wise *et al*. 2005; Mostafa 2007).

5b. Nature and impact of *Lygus* bug damage in different plants

The damage resulting from *Lygus* bug feeding is variable and depends on the species of the host plant and the type of plant part affected. Not all the damage caused by *Lygus* bugs is easily categorized. Five generalized types of plant damage have been reported from feeding of *Lygus* bugs: necrosis, “cat facing” or “deformation of young fruits”, “abscission of fruiting structures”, “production of embryo-less or shrivelled seeds” and “reduction of vegetative growth” (Strong 1970). In this section, symptoms of injury occurring in a wide variety of plants are summarized in Table 2. I provide a more detailed review of the nature and impact of *Lygus* bug damage in various types of leguminous crops.

Table 2. Damage symptoms caused by feeding of mirid bugs (Information for this table came from Strong 1970, Tingey and Pillemer 1977, Butts 1989 and the references cited therein).

Damage category	Symptoms	Crop	Reference
Necrosis	Brown discoloured anthers	Cotton	Pack and Tugwell 1976
		Cotton	Williams *et al*. 1987
	Sunken lesions	Cashew	Hill 1983
	Shrunken anthers and leaf ragging	Cotton	Pearson and Maxwell-Darling 1958
	Water soaked areas on fruits	Peaches	Phillips and De Ronde, 1965
		Tea	Calnaido 1959
		Cotton	Hill 1983
	Blackened buds and flowers	Coffee	Hill 1983
	Black spots on pods	Cocoa	Hill 1983
	"Bleached" areas on leaves	Grasses	Haws 1978
	Bleached areas on seeds	Wheat	Wise *et al*. 2000
	Seed pitting	Green beans	Khattat and Stewart 1975
		Lima beans	Baker *et al*. 1946
		Lima beans	Elmore 1955
		Lima beans	Bushing *et al*. 1974
		Cowpea	Baker *et al*. 1946
	Kernel brown spot	Sunflowers	Charlet 2003
	Brown raised areas on stems and pods	Canola	Butts and Lamb 1990b
"Cat facing" or deformation of young fruits	Apical seediness	Strawberry	Allen and Gaede, 1963
	Cat facing	Peach	Rice 1938; Rings 1958
		Peach	Phillips and Deronde 1965
		Apple	Boivin and Stewart 1982
	Apical seediness/cat facing/button berries	Strawberries	Handley and Pollard 1993a
		Strawberries	Easterbrook 2000
	Misshapen fruit	Cotton,	Pack and Tugwell 1976
		Apple	Boivin and Stewart 1982
Production of embryo-less or shrivelled seeds	Shrivelled seed/increased percentage of malformed seeds/ Reduced seed weight	Alfalfa	Sorensen 1939
		Carrot	Flemion and Olson 1950
		Carrot	Scott 1969
		Green beans	Khattat and Stewart 1975
		Lima beans	Elmore 1955
		Lentils	Summerfield *et al*. 1982
		Buckwheat	Mostafa 2007
		Wheat	Wise *et al*. 2000
		Soybean	Broersma and Luckmann 1970
		Canola	Butts and Lamb 1990b

Damage category	Symptoms	Crop	Reference
Abscission of fruiting forms	Premature drop of buds, flowers, fruits	Cotton	McKinlay and Geering 1957
		Cotton	Williams *et al*. 1987
		Cotton	Strong 1968
		Cotton	Strong 1970
		Tomato	Davis *et al*. 1963
		Snap bean	Fisher *et al*. 1946
		Alfalfa	Sorensen 1939
		Tea	Calnaido 1959
		Apple	Prokopy and Hubbell 1981
		Cabbage	Getzin 1983
		Green bean	Khattat and Stewart 1975
		Soybean	Broersma and Luckmann 1970
		Canola	Butts and Lamb 1990b
	Silver top heads with aborted seeds	Grasses	Arnott and Bergis 1967
		Grasses	Haws 1978
Reduction of vegetative growth	Decreased biomass	Alfalfa	Shull *et al*. 1934
		Sugar beet	Varis 1972
	Stunted plants	Cotton	Hill 1983
	Decreased root weight	Cotton	Tingey *et al*. 1975
	Tip die back		
Other injuries which are not classified under Strong (1970).	Split stem lesions	Cotton	King and Cook 1932
		Poplar	Sapio *et al*. 1982
	Rupturing of seed coat	Canola	Butts and Lamb 1990b
	Swollen nodes	Cotton	Dale and Coaker 1958
		Sugar beet	Hori 1967
	Leaf crinkling	Sugar beet	Hori 1967
	Leaf roll	Sugar beet	Hori 1967
	Exudation of gum	Cotton	Hill 1983
		Apple	Michaud *et al*. 1990
	Increased number of vegetative branches	Alfalfa	Jeppson and Macleod 1946
		Guayule	Addicott and Romney 1950
		Cotton	Hill 1983
	Multiple crowns	Sugarbeet	Varis 1972
	Bunched terminal growth	Cashew	Hill 1983
	Elongation of internodes	Cotton	Dale and Coaker 1958
	Elongation of styles	Coffee	Hill 1983
	Increased plant height	Carrot	Scott 1969
		Cotton	Tugwell *et al*. 1976
	Increased root weight	Sugar beet	Varis 1972
	Increased number of fruits	Asparagus	Grafius and Morrow 1982
	Reduce seed viability	Sunflower	Charlet 2003
	Reduce oil content	Sunflower	Charlet 2003
	Reduced germination percentage	Wheat	Wise *et al*. 2000
		Canola	Butts and Lamb 1990b
	Reduced number of seeds	Buckwheat	Mostafa 2007

In leguminous crops several of the categories of injury reported in Table 2 are produced. Seed pitting is one type of localized necrotic symptom produced on bean seeds. Seed pitting results from feeding by *Lygus* bugs after seed has been formed and has begun to fill; feeding on very small seeds causes them to fail to develop (Elmore 1955). Usually smooth firm intumescences (swellings) of pods are associated with the pitted areas of seeds (Baker *et al*. 1946). The injured areas tend to enlarge in proportion to seed growth, resulting in tearing of the seed coat (Elmore 1955). Economic loss results from discarding of the beans with conspicuous pits, or lowering of grade (Baker *et al*. 1946). Losses from seed pitting are reported for several types of beans such as Lima beans (Baker *et al*. 1946; Elmore 1955; Bushing *et al*. 1974), black eye cowpea (Baker *et al*. 1946) and green beans (Baker *et al*. 1946; Khattat and Stewart, 1975).

Reproductive organs injured by *Lygus* bugs turn brown, shrivel and abscise; this is referred as "blasting" (Strong, 1970). In different types of beans and soybeans, this occurs due to the weakening of stems by *Lygus* bug feeding and oviposition (Baker *et al*. 1946; Elmore 1955; Broersma and Luckman 1970; Khattat and Stewart 1975). In Lima beans, up to 42% of pod abortion has been reported (Elmore 1955). Young bean pods that are less than 5 cm long are susceptible to pod abscission following *Lygus* bug feeding (Baker *et al*. 1946; Elmore 1955).

Feeding on alfalfa buds, flowers, green pods and seeds by late nymphs and adults can result in increased numbers of damaged seeds, reduced numbers of healthy seeds and reduced seed germination (Mostafa 2007). Alfalfa seed production can be reduced by 15–100% depending on the *Lygus* bug density (McMahon and Arrand 1955). Twenty two percent of alfalfa seeds were damaged by *Lygus* bugs in Saskatchewan (Bolton and Peck 1946).

6. Sampling methods for *Lygus* bugs

Absolute and relative sampling methods are used to sample *Lygus* bugs. Relative estimates allow comparisons of numbers between locations and between times but are not related to a constant sampling unit (Southwood 1978). Relative estimates can sometimes be calibrated to provide accurate estimates of the population (Southwood 1978). The major relative sampling methods used in the *Lygus* spp. population estimates are sweep net sampling, drop cloth sampling, beat tray methods and sticky traps.

Sweep net sampling is efficient in capturing adults and older nymphs of *Lygus* bugs (Zink and Rosenheim 2004; Sharp and Bagwell 2006; Musser *et al*. 2007). Sweep net sampling usually samples the upper plant parts (Sedivy and Kocourek 1988) and the stages present on the upper plant parts are caught most efficiently. Adult *Lygus* bugs are more active on leaves and on meristematic terminals (Snodgrass 1998) and older nymphs frequently visit leaves and upper terminals (Wise and Lamb 1998) so they are caught more efficiently than young nymphs. Males are sampled more efficiently than females in cotton (Zink and Rosenheim 2004) while in Pima cotton, sweep net sampling is most effective for reproductive males, intermediate in efficiency for reproductive females and pre-reproductive males and least effective for pre-reproductive females (Spurgeon and Cooper 2013). Compared to visual sampling, sweep net sampling underestimates *L. lineolaris* numbers in cotton (Snodgrass and Scott 1997).

Drop cloth sampling involves placing a piece of cloth between plant rows, shaking the plants vigorously over the cloth, and collecting the insects that fall on the cloth. White and black (Musser *et al.* 2007) cloths have been used in different studies. In flowering cotton, drop cloth sampling can be calibrated to give more accurate estimates of *L. lineolaris* nymphal populations than sweep net samples provide (Snodgrass 1993). Drop cloth sampling is suitable for collection

of nymphs (Sharp and Bagwell 2006; Musser *et al.* 2007). In flowering cotton, treatment thresholds are based on drop cloth sampling (Musser *et al*. 2009).

The white pan beat method is widely used in strawberries (Mailloux and Bostanian 1988; Gleason and Lewis 1992; Kovach *et al*. 1993; Jay *et al.* 2004) and alfalfa (Uddin 2005) to monitor *Lygus* nymphs. In cotton, Rosenheim *et al*. (2004) used a rectangular washing bowl and insects were dislodged into it by vigorously tapping plants five times while holding the bowl beneath the plants; nymphs falling into the bowl were counted. Nymphs are collected more efficiently than adults by this method (Rosenheim *et al*. 2004).

Sticky traps are used to monitor adult *Lygus* bugs in strawberries (Rosenheim *et al*. 2004; Word and Hutchison 2003). Rosenheim *et al*. (2004) found that white sticky traps are more effective for monitoring *L. rugulipennis* populations in strawberries than yellow or blue sticky traps, but Word and Hutchison (2003) found that yellow traps are more effective than white traps for detecting adults of *L. lineolaris* in June-bearing strawberries.

Absolute methods relate numbers to a fixed unit of habitat (Southwood 1978), and for *Lygus* bugs involve enclosing plants in bags or cages and counting the numbers of *Lygus* bug stages captured. One method is whole plant bag sampling in which a plant is enclosed in a cloth bag and its stem cut, all the insects on the plant are removed by vacuuming and by shaking or beating the plant inside a large container (Byerly *et al*. 1978; Garcia *et al*. 1982). In cotton, whole plant bag sampling is more efficient for capturing *L. hesperus* nymphs than D-vac and sweep sampling (Byerly *et al*. 1978). Instead of bags, large cages (4 x 2 x 2 m) may also be used to enclose plants (Zink and Rosenheim 2004). Smaller cages do not provide accurate numbers for adults as the disturbed adults fly away. While using the larger cages, the time required for a

single collection limits the number of samples, but fewer disturbances to the adult population occur (Zink and Rosenheim 2004).

Mechanical suction devices also can provide absolute samples (Race 1960; Byerly *et al.* 1978; Garcia *et al*. 1982; Fleischer *et al.* 1985). D-vac sampling and sweep net sampling underestimate the immature population of *Lygus* spp. in cotton but they are able to detect the same population trend as whole plant bag sampling (Byerly *et al*. 1978). Garcia *et al.* (1982) used a Berlese method, in which plants were randomly selected and then cut into pieces and the pieces placed in a Berlese funnel for 24 h to extract insects (Garcia *et al*. 1982). Garcia *et al.* (1982) also performed visual counts in the field by randomly selecting a plant and then, beginning at the top, cutting the plant into pieces and counting the insects (Garcia *et al*. 1982).Visual sampling detects more adults than the Berlese funnel method and bag sampling methods (Garcia *et al*. 1982). The visual sampling method has the disadvantage of sampler variation **(**Morris 1960). For nymphs, visual and Berlese sampling detected larger numbers than the bag sampling method (Garcia *et al*. 1982). Suction sampling and beat sampling are effective methods to catch nymph and adult of *L. rugulipennis* in strawberries. (Jay *et al*. 2004).

The sampling efficiency of different sampling methods depends on factors such as the type of crop sampled, plant size, time of sampling, gender or stage of the *Lygus* bugs, and the person conducting sampling. The commercial sampling methods used in different crops vary and sweep net sampling in field crops (Sevacherian and Stern 1972), drop-cloth methods in cotton (Snodgrass 1993) and beat tray methods in strawberries (Mailloux and Bostanian 1988; Gleason and Lewis 1992; Kovach *et al*. 1993; Jay *et al.* 2004) are recommended based on their sampling efficiencies in those particular crops. In canola, sweep net sampling at the edges of the fields and at various distances into the fields gave the same density of plant bugs and therefore sampling of

edges is recommended (Wise and Lamb 1998). Plant size also influences the sampling efficiency: sweep net samples are less efficient when plants are tall whereas the drop cloth method is more efficient on larger plants (Snodgrass 1998).

Sampling efficiency of sweep net sampling, drop cloth sampling and whole plant sampling in cotton varies with different samplers (Musser *et al*. 2007). Wise and Lamb (1998) found that the experienced samplers caught more plant bugs in canola than inexperienced samplers. However Spurgeon and Cooper (2011) found that in cotton there were no differences in the population estimates from different samplers. No sampling method is suitable when plant material is wet during rain or when there is dew on the foliage (Jay *et al*. 2004). Leaf wetness and wind speed reduce sweep net efficiency (Cherry *et al.* 1977). Suction and beat sampling are more effective early in the morning and later in the day when air temperatures are lower and adults are less active (Jay *et al*. 2004).

Despite the amount of work published on different aspects of *Lygus* bugs all over the world, there is little information to provide a basis for managing plant bugs on field beans in Manitoba. The research reported in the following chapters is intended to add the missing information to aid in managing this insect in field beans and soybeans in Manitoba.

CHAPTER 3 – Manuscript I

Plant bugs (Hemiptera: Miridae) on field beans and soybeans in Manitoba: species composition and seasonal occurrence.

This manuscript describes the results from surveys conducted in commercial field bean and soybean growers' fields to determine the species composition, seasonal pattern of occurrence and the pest status of plant bugs in field beans and soybeans. The information from the surveys was the basis for the selection of insects and plants for the studies reported in the following manuscripts.

Abstract

In Manitoba, commercial soybeans and two types of field beans, navy and pinto, were sampled for plant bugs. At the centre and margins of 36 fields, sweep net and tap tray samples were taken weekly. In all three crops, *Lygus lineolaris* (Palisot de Beauvois) comprised >78% and *Adelphocoris lineolatus* (Goeze) <10% of adult mirids. Species composition varied among years but not among crops. For *Lygus*, nymphs were 46% of the catch in trays and 23% in sweeps, but total *Lygus* catch in trays was <2% of that in sweeps. Catch/sample effort was similar at field centres and margins for both *Lygus* bug adults and nymphs. *Lygus lineolaris* reproduced and developed in all three crops. In field beans, *Lygus* bug adults were first collected in late vegetative and early pod set stages and late instar nymphs and adults were present from pod elongation until maturity — results consistent with immigration of first summer generation reproductive adults and development of the second generation in the crops. There was evidence of late season immigration of *Lygus* bugs into all crops. There was no loss of seed quantity attributable to mirids in any of the crops; in field beans there was no evidence that mirids reduced seed quality.

Introduction

Production of field beans (*Phaseolus vulgaris* L.), including white pea (navy), pinto, cranberry, kidney, black and small red beans, and of soybeans (*Glycine max* L.), has increased in Canada in the last decade (Pulse Canada 2014). Manitoba is the largest producer of field beans in Canada (Pulse and Soybean Growers Association 2015) and they and soybeans together contributed $207 million to the provincial economy in 2011 (Manitoba Agriculture Food and Rural Development 2014a). In Manitoba, field beans and soybeans are planted in mid- to late-May. Field beans are harvested from late-August to early-September (Manitoba Agriculture Food and Rural Development 2011a) and soybeans are harvested in September and October (Manitoba Agriculture Food and Rural Development 2011b).

Plant bugs (Heteroptera: Miridae) of the genus *Lygus* and *Adelphocoris* are important crop pests in the Canadian Prairie Provinces (Kelton 1980), where they attack a wide range of crops including canola (Butts and Lamb 1990b; Gerber and Wise 1995), alfalfa (Craig 1983; Soroka and Murrell 1993; Gerber and Wise 1995; Mostafa and Holliday 2008) and buckwheat (Wise *et al*. 2005). The most common *Lygus* species present in Manitoba crops are *Lygus lineolaris* (Palisot de Beauvois), *Lygus borealis* (Knight), and *Lygus elisus* (Van Duzee) (Timlick *et al*. 1993; Gerber and Wise 1995; Mostafa 2007). Adults of *Lygus* spp. overwinter in litter and crop residues (Kelton 1975) and nymphs develop through five instars (Schwartz and Foottit 1992b). In southern Manitoba, *L. lineolaris* is bivoltine; in spring, offspring of overwintered adults develop on early-flowering plants. Beginning in July, as these first summer generation insects become reproductive adults, they disperse to later-flowering plants and produce a second generation there (Gerber and Wise 1995). *Adelphocoris lineolatus* (Goeze) overwinters as eggs

in plant stems (Hughes 1943), develops through five nymphal instars (Craig 1963) and is largely bivoltine in the study area (Craig and Loan 1984).

Plant bugs use piercing and sucking mouth parts (Tingey and Pillemer 1977) to inject salivary enzymes that macerate host plant tissues, and ingest the resulting fluids (Miles 1972; Cooper *et al*. 2013). In early growth stages of legumes, feeding can result in shedding of buds, flowers and pods and consequent reduced quantity of yield (Elmore 1955). Feeding on later growth stages can result in seed pitting (Baker *et al*. 1946; Elmore 1955) that reduces seed quality.

Producers of field beans and soybeans in Manitoba are concerned about the presence of plant bugs in their crops and the implications for yield quantity and quality; in 2002 and 2007 insecticides were applied to field beans for plant bug control (Gavloski 2001–2010). In addition, processors have reported blemishes on field beans that they attribute to insect damage, and it is reported that 5–20% seed damage may occur in severe *Lygus* bug infestations to field beans in Canada (Agriculture and Agri-Food Canada 2005). Nevertheless, little is known about plant bugs and their effects on these crops in Manitoba. Therefore, the objective of this study was to determine the species composition and seasonal pattern of occurrence of plant bugs in field beans and soybeans in Manitoba, and to seek evidence of crop loss resulting from these insects.

Materials and methods

Field surveys were conducted from 2008 to 2010 in commercial fields of field beans (navy or pinto) and soybeans in, and to the west of, the Red River Valley in southern Manitoba. A total of 17 navy bean, 10 pinto bean and 9 soybean fields were sampled from areas close to Carman (49.50° N, 98.00° W), Portage la Prairie (50.14° N, 98.25° W) and Letellier (49.35° N, 97.37°

W). Twenty nine fields were square and 64 ha in area and the rest were rectangular and 128 ha. Field beans were planted as row crops with a row spacing of 56 cm. Soybeans were solid seeded with a row spacing of 15 cm. Except on rainy days, samples were taken at weekly intervals from the late vegetative stage of the crop until harvest. On each sampling date, the crop growth stage was recorded, as well as harvesting activities in adjacent crops.

In each field, five permanent sampling locations were marked with flags: one near the centre of the field, and four at about the mid-points of each side, at least 5 m in from the field margin and avoiding headland rows. On each sample day, at each of the five locations, 40, 180° sweeps across two rows (Kogan and Herzog 1980) were made with a 38 cm-diameter sweep net. In addition, one tray sample was taken at each location, using a 30 x 25 cm white tray. The foliage of a 30 cm length of a single row of plants was tapped by hand vigorously five times over the tray. The foliage and the insects that fell into the tray were collected. The sampling direction from the marker flag was alternated to avoid the same plants being sampled in successive weeks. Sweep and tray samples were put individually into Ziploc® bags and brought to the laboratory in an insulated cooler with freezer packs. The samples were stored in a 5 $^{\circ}$C room for up to a week before plant bugs were separated. Plant bug adults were identified to species and the nymphs of *Lygus* bugs and *Adelphocoris* were identified to genus using the keys of Kelton (1975, 1980), Schwartz and Foottit (1992b) and Mueller *et al*. (2003). In 2009 and 2010, the instar of all *Lygus* bugs and *Adelphocoris* nymphs was recorded, but this was not done in 2008.

For both field beans and soybeans, at crop maturity, 4 m^2 of crop from each sample location was harvested by hand. The harvested samples were placed in burlap sacks, air dried indoors, and then threshed using a bean plot combine (Wintersteiger Classic 1540-44). After threshing, samples were sieved and the bean seeds were weighed to estimate yield. Quality of

field beans was assessed by a commercial grader who reported insect damage and seed grade based on a sub-sample of 250 ml of seeds from each sample. Soybean seeds were not graded because a grader was not available.

The relationship between plant bug numbers and yield, quality and quantity, was assessed by simple Pearson correlations. Analysis of contingency tables was used to compare species and stage composition among years and crops, catches among sampling locations, and catches of sweep and tray samples. All analyses were performed using Systat 13 (Systat 2009), with an α level for significance of ≤ 0.05.

Results

Crop growth stages are defined in Table 3. The R1–R3 stage of field beans occurred on 14–28 July 2008; 1–7 August 2009 and in late July 2010. Field beans were harvested during the third and fourth week of September in 2008 and 2009, and in the second and third week of September in 2010. Soybeans were not sampled in 2008. In soybeans, the R1–R2 stage occurred during the first and second week of August in 2009 and during the last week of July and first week of August in 2010. Soybeans were harvested during the last week of September and first week of October in 2009 and 2010.

Three species of *Lygus* bugs and one species of *Adelphocoris* were commonly collected during the study (Table 4): *L. lineolaris*, *L. borealis* and *L. elisus* and *A. lineolatus*. *Lygus lineolaris* was the dominant *Lygus* species in all the bean fields and represented 78–95% of the adults, with none of the remaining common *Lygus* species exceeding 13% of the total annual collection of adults (Table 4). The total number of *A. lineolatus* never exceeded 10% of the adult mirid collection in any year or crop. In field beans, the relative frequency of *L. lineolaris*, *L.*

borealis, *L. elisus* and *A. lineolatus* differed among years (Likelihood ratio (LR)χ^2 = 53.1, df = 6, $P < 0.001$), but not between navy and pinto beans (LRχ^2 = 3.8, df = 3, $P = 0.3$). Soybeans were sampled in 2009 and 2010 and frequency of *L. lineolaris*, other *Lygus* species pooled together, and *A. lineolatus* differed between the two years (LRχ^2 = 226.2, df = 2, $P < 0.001$), but not between field beans (navy and pinto together) and soybeans (LRχ^2 = 1.4, df = 2, $P = 0.5$).

In 2009 and 2010, a total of 1700 *Lygus* bugs were collected in sweep samples of which 77% were adults, 5% were instars 1–3, and 18% were instars 4–5. In tray samples, the total catch was 31, of which 55% were adults, and about 23% were in each nymphal category. Sampling method significantly influenced the relative frequencies of the three stages (LRχ^2 = 12.6, df = 2, $P = 0.002$). Although tray samples yielded a higher proportion of nymphs, the total catch of nymphs in tray samples was less than from sweep sampling.

As there were four samples near the field margin and one in the middle of the field, the frequencies in edge and middle samples were compared with the expectation that the total in edge samples would be four times that in the middle sample. Frequencies in edge and middle did not differ from this expectation for *Lygus* bug adults (LRχ^2 = 2.4, df = 1, $P = 0.1$) or nymphs (LRχ^2 = 2.8, df = 1, $P = 0.1$). Numbers of *Adelphocoris* adults and nymphs were too small for reliable assessments of effects of sample position.

In field beans, adults of *L. lineolaris* were usually first collected in mid-July to early August and were found until the crop matured. In several fields, adults were present on the first day of sampling, so it is unknown when they were first present. In fields where sampling occurred before adult detection, that detection occurred from 21 July to 5 August at stages from late V to R3 of the navy and pinto beans. In 2008 and 2010, *Lygus* bug nymphs were first

collected 1–3 weeks after the first adults (Fig. 1). In 2009, numbers of nymphs were very low (Table 4); nymphs were collected only late in the season and only third to fifth instars were detected. In 2010, nymphs of instars 1–3 were collected both at the edge and in the middle of the fields in field beans, 2–3 weeks after the first adults; later instars were collected until the penultimate week of sampling. In all years, the numbers of *Lygus* nymphs peaked in mid-August to mid-September, in most cases 1–2 weeks before the peak of adult numbers from the last week of August to mid-September (Fig.1). Except for two fields in 2008 where numbers were very low and the peak occurred at stage R2, the peaks of nymphs and adults occurred at R6 or later.

In soybeans, the first *Lygus* adults were found in stage R1–R6, in late July to mid-August. The nymphs appeared 1–5 weeks after the first appearance of adults. Adult numbers peaked between 7 and 26 September (Fig. 2), when the crop stage was R5–R6. In the majority of fields, the peak for nymphs occurred at R3–R6. Of the *Lygus* bugs collected in soybeans in 2009, 3% were nymphs and in 2010, 12% were nymphs. These proportions were lower than those in field beans in the same years (2009: 8%; 2010: 31%) and the differences were significant (2009: $LR\chi^2 = 3.8$, df = 1, $P = 0.049$; 2010: $LR\chi^2 = 42.7$, df = 1, $P < 0.001$). Furthermore, in 2010, when numbers were high enough for the comparison, 52% of nymphs collected from soybeans were of instars 4–5, significantly lower than the 80% of nymphs in instars 4–5 in samples from field beans ($LR\chi^2 = 11.4$, df = 1, $P = 0.01$).

The late season numbers of *L. lineolaris* adults were higher than the early season numbers of nymphs in navy, pinto and soybeans in all years. Of the 22 sampled fields for which adjacent harvests were recorded, the late season peak of adult numbers occurred within 1 week following the harvest of adjacent canola in 17 fields. Adjacent canola fields were harvested between 18 August and 7 September, corresponding to weeks 8–11 in Figs. 1 and 2.

A total of 48 *A. lineolatus* adults were collected in field beans (Table 4) and adults were present from late July to mid-September in 2008 and early August to late September in 2009 and 2010. Only one *A. lineolatus* nymph was collected in field beans. In soybeans, both adults and 4–5 instar nymphs of *A. lineolatus* were found in 2009 and 2010. In 2009, the adults occurred from early August to late September and nymphs were found during early September. In 2010, both nymphs and adults occurred from early August to mid-September.

The numbers of mirids and total seed weight in the 4 m^2 area harvested around each sampling location were never significantly correlated for any of the three crops. Correlation coefficients for navy beans were positive in 2010 (r = +0.02, P = 0.9, n = 18) and negative in other years (2008: r = −0.25, P = 0.1, n = 35: 2009: r = −0.31, P = 0.2, n = 20). Correlation coefficients were negative for pinto beans (2008: r = −0.18, P = 0.7, n = 8; 2009: r = −0.34, P = 0.4, n = 8; 2010: r = −0.34, P = 0.1, n = 20). For soybeans, correlation coefficients were positive in both years (2009: r = +0.13, P = 0.6, n = 20; 2010: r = +0.20, P = 0.4, n = 20). In navy beans, the frequency of insect-damage was generally highest in 2008 when the maximum number of insect-damaged seeds per sub-sample was 16. The highest number of insect-damaged seeds per sub-sample was 9 in 2009 and 4 in 2010. Numbers of insect-damaged seeds in navy beans were not correlated with the numbers of mirids in any year (2008: r = −0.09, P = 0.6, n = 37; 2009: r = +0.01, P = 0.9, n = 20; 2010: r = −0.30, P = 0.2, n = 18). In pinto beans, no insect damage was found in 2008 and 2009 and only one damaged seed was recorded in 2010.

Discussion

The highest number of *Lygus* bugs observed in Manitoba field beans was 0.11 per sweep in stage R1–R3 and 0.5 in R6–R7; lower than the numbers in canola (average of 3 *Lygus* bugs/sweep) (Timlick *et al*. 1993) and buckwheat (average of 6.7 *Lygus* bugs/sweep) (Mostafa 2007) in

Manitoba. In Lima beans in California, an average of 0.7 *Lygus* bugs/sweep has been recorded in stage R1–R3, with 2.9 bugs/sweep later in the season (Bushing *et al*. 1974). In field bean varieties in stage R1–R3, a maximum of 12.6 *Lygus* bugs/sweep was observed in Washington (Hagel 1978). In Manitoba, mirid densities in field beans and soybeans tend to be lower than those observed in other Manitoba annual crops and those observed on beans in the United States of America.

Lygus lineolaris accounted for 78–95% of the mirids collected in the sampled crops in Manitoba. The order of relative abundance of *Lygus* species in beans, *L. lineolaris, L. borealis* and *L. elisus,* is similar to that in canola (Timlick *et al*. 1993), alfalfa (Mostafa 2007) and flax (Wise and Lamb 2000) in Manitoba. This same order of relative abundance is characteristic of canola in the boreal region of Alberta (Cárcamo *et al*. 2003), and *L. lineolaris* and *L. borealis* are the main *Lygus* species in canola adjacent to alfalfa in Alberta, British Columbia and Manitoba (Cárcamo *et al*. 2002).

Lygus spp. nymphs and adults were distributed evenly in the bean fields, so I infer that producers could sample anywhere in the field to obtain reliable information about *Lygus* bug numbers. Tapping of plants and collecting the dislodged insects is an effective method of sampling *Lygus* nymphs in strawberries (Schaeffers 1980; Rancourt *et al.* 2000) and cotton (Snodgrass 1993). However, although relative efficiency of collecting nymphs was higher for tray sampling than for sweep netting in my study, the total catch in tray samples was too small to make them an effective method of sampling in field beans or soybeans.

The arrival of *Lygus* adults in the bean crops in July or early August coincided with the development of plant reproductive tissues. These adults were probably of the first summer

generation, as reproductive first generation adults also colonize flowering canola in Manitoba at the same time of the year (Gerber and Wise 1995). All five stages of nymphs of *Lygus* were found in both bean types sampled. The peak occurrence of nymphs was found shortly before that of adults, a pattern of occurrence that accords with the hypothesis that development was completed in field beans and soybeans. The alternative, that nymphs disperse from adjacent crops, is unlikely: most of my sampled fields were separated from other crops by roads or ditches, which are barriers to *Lygus* bug dispersal (Schaber *et al*. 1990). Furthermore, the proportion of nymphs in samples ≥400 m from adjacent crops in the middle of fields was no lower than in field margin samples. Most *Lygus* nymphs move only short distances (Swezey *et al*. 2013) with maximum recorded dispersal distances of 50–62 m (Khattat and Stewart 1980; Swezey *et al*. 2013). I conclude that nymphs of the second summer generation (Gerber and Wise 1995) complete development on beans in Manitoba and contribute to the second summer generation adult peak in late August or early September.

In late August or early September, there were peaks of *L. lineolaris* adults and late instar *Lygus* nymphs in field beans that were higher than the peaks of early instar nymphs. This phenomenon is attributable, in part, to the higher efficiency of sweep sampling for adults than for nymphs (Byerly *et al.* 1978). Also, the lack of synchronization of developmental stages coupled with the shorter duration of nymphal development compared with adult longevity (Bariola 1969) reduces the probability of nymphs being included in a sample compared with adults (Birley 1977). Nevertheless, adult peaks often coincided with or followed soon after the harvest of adjacent crops, particularly canola. Thus, some of the adults in the study fields late in the season may have dispersed from adjacent crops that had matured to the point where they were no longer suitable for *Lygus* bug feeding or had been harvested.

In soybeans, the seasonal occurrence of *L. lineolaris* was similar to that of field beans and is consistent with adults of the first summer generation colonizing the crop and giving rise to nymphs of the second generation. However, the proportion of nymphs was low in *Lygus* bug collections in soybeans, which is consistent with studies in the southern United States (Freeman and Mueller 1989; Snodgrass *et al*. 2010). The lower proportion of nymphs from soybeans that were of instars 4–5 suggests that the survival of nymphs was poorer on this crop than on field beans. In cage studies, *L. lineolaris* females oviposit less and fewer nymphs survive to complete development on soybeans than on suitable hosts (Snodgrass *et al.* 2010). The patterns I observed are consistent with both these mechanisms operating, and suggest that soybeans are a marginal host for production of the second generation of *L. lineolaris* in Manitoba. As with field beans, late season migration of *Lygus* bugs into soybeans was evident, with peaks of adults observed following the harvest of adjacent crops.

The numbers of alfalfa plant bug, *A. lineolatus*, in field beans and soybeans in my study were too low for conclusions to be drawn about reproduction or development in these crops. *Adelphocoris lineolatus* is primarily a herbivore of alfalfa and red clover, and occurs in low numbers in other crops (Hughes 1943; Craig 1963; Soroka and Murrell 1993; Mostafa 2007). At least some cultivars of *P. vulgaris* are poor hosts for *A. lineolatus*: relative to insects reared on alfalfa, fewer nymphs survive to adulthood and the durations of the oviposition period and adult life are reduced (Qiaosi *et al*. 1994). Adult *A. lineolatus* disperse to soybean plots embedded in alfalfa fields when the alfalfa is cut (Poston and Pedigo 1975). Thus, *A. lineolatus* in bean crops in Manitoba may be mostly transients displaced from harvested alfalfa, as was suggested for buckwheat (Mostafa 2007).

In my study, there was no evidence of economic losses in yield quantity or quality that could be attributed to plant bugs in field beans and no quantity loss that could be attributed to plant bugs in soybeans. The highest numbers of mirids, both adults and nymphs, per 200, 180° sweeps during three years of sampling in navy and pinto beans were 18 and 22 in stage R1–R3, 37 and 16 in R4–R5, and 110 and 97 above R6, respectively. The highest numbers of plant bugs per 200 sweeps in soybeans were 8 in R1–R2, 15 in R3–R4 and 68 in R5–R6. Thus, in Manitoba commercial fields, mirid numbers lower than these cause no economic damage in these crops, and farmers would receive no benefit from control measures applied at these or lower densities. The incidence of seed quality loss through seed pitting depends on the type of edible bean. Seed pitting was negligible in pinto beans and there was a small amount in navy beans. The highest number of insects was in 2010, which had the lowest number of damaged seeds. Hagel (1978) also found little or no seed pitting in pinto beans, in contrast to 5–20% of pitted seeds in other types of beans that did not include navy beans. Soybeans are not preferred hosts for *Lygus* bugs, and peak numbers similar to those I observed cause no loss in soybeans in Mississippi (Snodgrass *et al*. 2010), although high numbers of *Lygus* adults, caged on soybean buds or blooms, cause yield loss (Broersma and Luckmann1970).

Mirid bugs, particularly *L. lineolaris*, occur in field beans and soybeans in Manitoba. *Lygus* species reproduce in these crops and nymphs reach adulthood feeding on the crops. However, during my study the population densities were too low to cause economic losses in these crops. In southern Manitoba, populations of mirids were low in all crops in 2009, but insecticides were applied for *Lygus* bug control in canola, sunflowers, seed alfalfa and strawberries in 2008, and in sunflowers and strawberries in 2010 (Gavloski 2001–2010). The patterns of insecticide application for *Lygus* bug control in 2008 and 2010 appear typical for

southern Manitoba for most years from 2001 to 2010, suggesting that the populations in the region were not unusually low in two of the three years of my study. Hence, the absence of economic loss attributable to plant bugs in field beans and soybeans in my study fields may occur in most years in southern Manitoba. However, my results should not be applied incautiously during the occasional years in which extremely high populations of plant bugs occur in the Prairie Provinces. In 1997 for example, *Lygus* bug numbers reached 20/sweep in canola in Alberta and large areas of canola, legumes and other crops were affected (Western Committee on Crop Pests 1997). It is likely that, if such populations occurred in an area with field beans and soybeans, these crops would suffer economic damage.

Table 3. Crop growth stages of field beans and soybeans.

Field beans*		Soybeans†	
Stage	Description	Stage	Description
V	Emergence to the development of trifoliate leaves	V	Emergence to the development of the all unifoliate and trifoliate leaves
R1–R3	Flowering to early pod set with the pod size up to 2.5 cm long	R1–R2	Open flowers on the stem
R4–R5	50% pods at maximum pod length to one pod with fully developed seeds	R3–R4	Pods are 0.5–2 cm long at one of the four upper most nodes
R6–R7	50% fully developed seeds to the start of colour change in the pod	R5–R6	Seed length varies from 0.3 cm to the pod capacity, at one of the four upper most nodes
R8 and above	80% pods reach maturity	R7–R8	One pod to 95% of the pods have reached mature colour

* Adapted from Colorado State University Integrated Pest Management Program 1998.

† Adapted from Iowa State University Extension 1997.

Table 4. Total numbers of *Adelphocoris lineolatus* and *Lygus* species adults and nymphs, and relative abundance (%) of mirid adults in Manitoba.

Year	Crop	No. of fields (No. of field-weeks)	*Adelphocoris lineolatus*		*Lygus* species		Relative abundance of mirid adults (%)				
			Adults	Nymphs	Adults	Nymphs	*Adelphocoris lineolatus*	*Lygus lineolaris*	*Lygus borealis*	*Lygus elisus*	Other *Lygus* spp.
2008	Navy	9(84)	12	0	140	52	7.9	77.6	2.6	5.3	6.6
	Pinto	2(18)	3	0	156	62	1.9	94.0	1.3	0.6	2.5
	Soybean	—	–	—	—	—	—	—	—	—	—
2009	Navy	4(30)	6	0	80	4	7.0	80.2	12.8	0.0	0.0
	Pinto	4(31)	9	0	86	10	9.5	85.3	5.3	0.0	0.0
	Soybean	5(31)	14	2	159	5	8.1	85.5	5.2	0.6	0.6
2010	Navy	4(23)	10	1	392	220	2.5	95.3	1.0	0.2	1.0
	Pinto	4(22)	8	0	386	137	2.0	95.2	2.3	0.2	0.3
	Soybean	4(26)	10	7	221	31	4.3	92.2	1.2	0.4	0.9

Note: One field-week represents one of the weekly samples of 200 sweep samples and 5 tray samples from a field.

Fig. 1. Seasonal abundance of *Lygus lineolaris* adults and *Lygus* nymphs in navy beans and in pinto beans. Values plotted are weekly averages of total catch from 200, 180° sweep samples and 5 tray samples per field (N is given in Table 4, but was reduced in weeks of inclement weather). Internal tick marks on the horizontal axis are weeks where week 1 is the first week of July; note that the scales of the vertical axes differ among years.

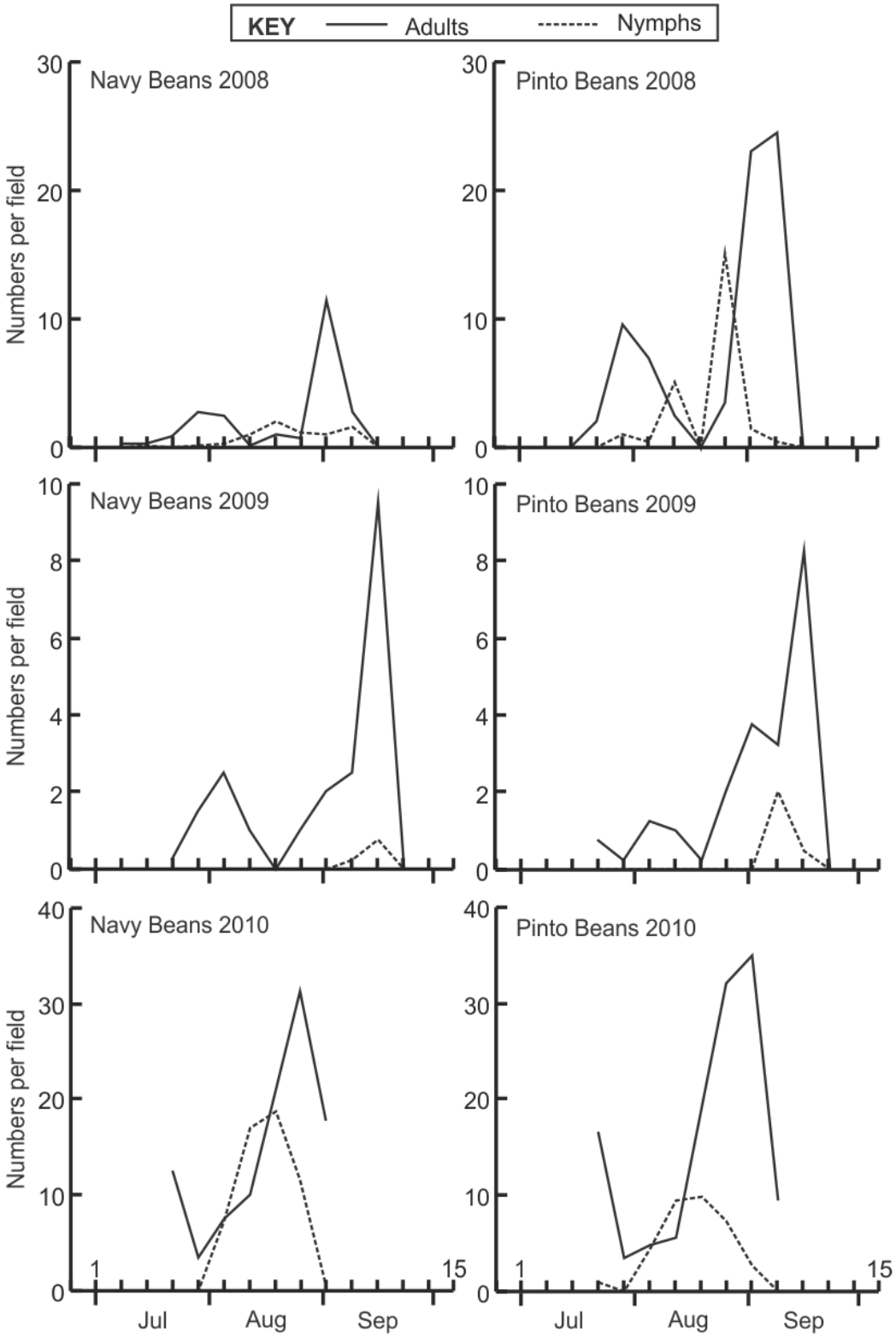
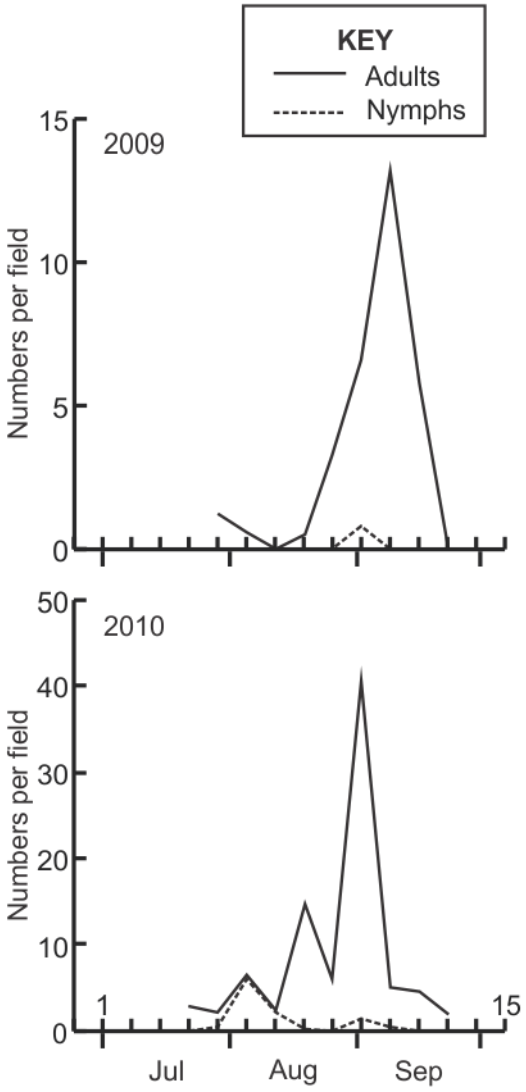

KEY
Adults
Nymphs
Navy Beans 2008
Pinto Beans 2008
Navy Beans 2009
Pinto Beans 2009
Navy Beans 2010
Pinto Beans 2010
Numbers per field
Jul
Aug
Sep
1
15

Fig. 2. Seasonal abundance of *Lygus lineolaris* adults and *Lygus* nymphs in soybeans. Values plotted are weekly averages from 200, 180° sweep samples and 5 tray samples per field (N is given in Table 4, but was reduced in weeks of inclement weather). Internal tick marks on the horizontal axis are weeks where week 1 is the first week of July; note that the scales of the vertical axes differ between years.


KEY
Adults
Nymphs
2009
Numbers per field
15
10
5
0
2010
Numbers per field
50
40
30
20
10
0
1
15
Jul
Aug
Sep

CHAPTER 3 – Manuscript II

Microscopic examination of *Lygus lineolaris* (Hemiptera: Miridae) feeding injury to different growth stages of navy beans

This manuscript focuses on documenting the immediate responses of plants to a short period of feeding by nymphs and adults of *L. lineolaris* in three growth stages of navy beans. Documenting the symptoms of the immediate response to injury aids growers and graders in identifying *Lygus* bug injuries in field conditions. Defining the immediate response may assist in understanding how injury leads to the effects on yield that are reported in manuscript III.

Abstract

Light microscopy was used to study feeding injury by fifth instar nymphs and unmated newly-moulted adults *of Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) to navy beans (*Phaseolus vulgaris* L.: Fabaceae). Insects were caged on individual racemes of potted plants at different growth stages. For growth stages from flowering to early pod set, abortion of pods, flowers or buds was the most common response to feeding injury; this type of injury did not occur at later growth stages. Abortions occurred in both uninfested and infested cages, and were most frequent at the distal end of the raceme; the frequency of abortions was higher in infested treatments and higher in treatments with nymphs than with adults. During the seed development growth stage, feeding resulted in exterior diffuse discoloured patches on seed pods and associated interior necrosis of the vascular tissues supplying seeds. At harvest time, many of the seeds were shrivelled when racemes were fed upon during the seed development growth stage. Frequency of seeds affected by vascular injury was higher in treatments with nymphs than with adults, but was unaffected by position of pods on the raceme or seed position within pods. Direct seed injury, involving penetration of the testa and loss of cotyledon tissue, was occasionally observed during the seed development growth stage, and was the most frequent injury at the developed seed stage. Harvested seeds that were directly injured exhibited crater-like surface pits fringed by brown pigmented areas. There were no observable differences in the type of injury caused by nymphs and the adults at any of the growth stages.

Introduction

Mirid bugs in the genus *Lygus* Hahn cause injury to more than 20 economically important crop plants worldwide (Tingey and Pillemer 1977). In North America, *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae) feeds on plants of more than 385 species (Young 1986), and crop damage has been reported in alfalfa, apples, birdsfoot trefoil, carrots, cotton, snap beans, Lima beans, peaches, pears, soybeans, strawberries, tomatoes, nursery stock (Tingey and Pillemer 1977 and references therein), canola (Butts and Lamb 1990b), buckwheat (Wise *et al*. 2005), flax (Wise and Lamb 2000) and wheat (Wise *et al*. 2000).

Lygus bugs feed on meristematic tissues and developing reproductive organs of plants (Strong 1970). They feed by inserting their piercing-sucking mouth parts into plant tissues and mechanically or enzymatically destroying cell walls and liberating cell contents (Hori 2000; Backus *et al*. 2007). The cell contents are then digested and consumed through cycles of alternating flushing with saliva and ingestion of the resulting fluids (Strong 1970, Miles 1972; Backus *et al*. 2007). Digestive enzymes in *Lygus* bug saliva include polygalacturonases, α-amylases, and proteases (Strong and Kruitwagen 1968; Celorio-Mancera *et al*. 2008; Cooper *et al*. 2013). In addition, saliva contains other proteins that may function in detoxification of plant defensive chemicals (Cooper *et al.* 2013). It was earlier postulated that the saliva of *Lygus* bugs also contains plant hormones (Allen 1947). However, Strong (1970) was unable to detect such hormones and concluded that effects attributed to hormones in saliva are the result of feeding injury disrupting the host plant's hormonal system.

Descriptions of injury by *Lygus* bugs, largely based on macroscopic observations, are numerous. Among them are descriptions of injury to apple leaves, buds and fruits (Michaud *et al*. 1990), stems and reproductive structures of canola (Butts and Lamb 1990b), reproductive

structures of buckwheat (Mostafa 2007), and seeds of sunflower (Charlet 2003) and wheat (Wise *et al*. 2000). Staining and light microscopy have been used to study injury by *Lygus* species to a number of plant organs including cotton stems and petioles (King and Cook 1932), cotton bolls (Williams and Tugwell 2000) various parts of sugar beet and rape (Hori 1971a), pumpkin fruit (Hori *et al*. 1987), and guayule stems (Addicott and Romney 1950). Light microscopy and scanning electron microscopy have been used to study *L. lineolari*s injury to strawberry fruits (Handley and Pollard 1993a, 1993b), and to study injury to different phenological stages of grape vine (Fleury *et al*. 2006).

Macroscopic studies of *Lygus* bug injury to beans of the genus *Phaseolus* (Linnaeus: Leguminosae) have been carried out on Lima beans, *P. lunatus* L., and various cultivars of *P. vulgaris* L. *Lygus* bugs feeding on Lima beans reduce both seed yield and quality (Bushing and Burton 1974). Reduction in seed yield of Lima beans results from shedding of reproductive structures following petiole or pod injury, and from distortion of pods (Elmore 1955); quality is reduced as a result of pitting of the seed surface (Baker *et al*. 1946; Elmore 1955). In green beans, *P. vulgaris*, injury by *L. lineolaris* causes abscission of buds and blossoms, and reduced numbers of seeds per pod and reduced seed weight (Khattat and Stewart 1975). There have been no microscopic studies of *Lygus* bug injury to *Phaseolus*, and no studies of injury to the field bean types of *P. vulgaris*.

The reproductive organs of *P. vulgaris* are compound racemes, arising in the axil of a trifoliate leaf; three flower buds are located in the leaf axil and at each of two to five nodes along the peduncle of the raceme (Adams 1967; Mauk *et al.* 1984; Sage and Webster 1987). Relative to the distal portions of the raceme, flowering and pod development occur earlier at the base of the raceme (Sage and Webster 1987). In healthy plants, abortion of buds, flowers or young pods,

results in far fewer developed pods than the initial number of buds (Tamas *et al*. 1979; Sage and Webster 1987). Pods are modified leaves, and the two halves of the pod are linked by dorsal and ventral sutures, with the dorsal suture corresponding to the midrib (Woodcock 1935). The vascular supply to developing seeds is the placenta, consisting of two vascular bundles running the length of the pod near the ventral suture (Woodcock 1935). Each developing seed is connected to the placenta by the vascular tissue of the funiculus (Woodcock 1935; Sage and Webster 1990). In healthy pods, considerable numbers of seeds fail to develop, and remain flattened and shrivelled (Harris 1915).

Field beans, including navy beans, are an important crop in Manitoba (Pulse and Soybean Growers Association 2015) and there is considerable concern about the effect of *Lygus* bugs on this crop (Gavloski 2001–2010). It has been reported that 5–20% of field bean seed can be damaged by *Lygus* bugs (Agriculture and Agri-Food Canada 2005), and processors in Manitoba diagnose blemishes on seeds as insect damage. In Manitoba, >90% of mirids in field bean crops belong to the genus *Lygus* and most of these are *L. lineolaris* (Nagalingam and Holliday 2015). The lack of information about how *L. lineolaris* affects reproductive growth of field beans and the quality of seed impairs diagnosis of *Lygus* bug injury in the field and accurate assessment of the economic significance of these insects in the crop. Therefore, in this study, I used reflective light microscopy to investigate the feeding injury inflicted by *L. lineolaris* nymphs and adults on reproductive organs of navy beans, *P. vulgaris*, at three different growth stages. I investigated the use of scanning electron microscopy, but these results are not included in this chapter as they did not add information about the nature of feeding injury.

Materials and methods

Determinate bush-type navy beans, cv 'Envoy' were grown from seed in pots (21 cm diameter, 21 cm height) in a controlled environment room at 23 °C, 70% RH and 16:8 h (L:D) photoperiod. Pots were filled with horticultural potting mix (Sunshine LA4, Sungro Horticulture Distribution Inc., Agawam, Massachusetts, United States of America). Two seeds were planted in each pot and, when the first trifoliate leaf opened, plants were thinned to one per pot. Pots were watered every second day, and every two weeks received 0.625 g of NPK 20-20-20 fertilizer in 250 ml of water.

A laboratory colony of *L. lineolaris* was established from over-wintered adults collected each spring from alfalfa fields near Carman, Manitoba. The colony was maintained in a controlled environment chamber at 21 °C, 70% RH with a photoperiod of 16:8 h (L:D). First to third instar nymphs were maintained on broccoli pieces in 60 mm diameter polystyrene Petri dishes. Older nymphs and adults were kept in perforated plastic tubs (12 cm diameter, 15 cm height; Bug Tub® (Royal Oak Point NW, Calgary, Alberta, Canada) and provided with green bean (*P. vulgaris*) pods as food and oviposition substrate. Bean pods that had been confined with adults were held for 5 days, and then the pods were transferred to 145 mm diameter polystyrene Petri dishes. Thereafter, for one week, the Petri dishes containing the pods were inspected every second day and nymphs that hatched were transferred with a paint brush to the small Petri dishes used for rearing early instar nymphs. The colony was re-established each year to ensure colony vigour.

Insect injury treatments were made by caging *L. lineolaris* nymphs or adults for 5 days on reproductive structures of potted navy bean plants growing in the controlled environment room. Cages were sleeve cages (12 cm diameter, 30 cm long) of 40 thread/cm mesh tergal netting

(Fabricland, Winnipeg, Manitoba, Canada) on a wire ring frame. Each cage was supported by attaching it to a wire tomato support frame, the base of which was pushed into the soil of the pot. A single raceme and its subtending trifoliate leaf were inserted into the sleeve cage and the proximal end of the sleeve was secured around the plant stem with a twist tie. The distal end was tightly secured with a rubber band after introduction of insects if any. Control cages had no insects. Insects introduced into infested cages were either 1-day-old fifth instar nymphs or adults that had completed their final moult within the previous 1–2 days. To avoid injury from oviposition or feeding by young nymphs during the five-day exposure, adults were isolated from the colony as fifth instar nymphs and not allowed to mate; sex of adults was not determined. Injury treatments were made at three growth stages (Colorado State University Integrated Pest Management Program 1998) and the number of insects used at each growth stage was chosen to produce injury but not complete destruction of caged plant parts. At growth stages R2–R3 (flowering to early pod set), one nymph or one adult *L. lineolaris* was introduced into each infested cage. At R4–R5 (mid pod set to early seed fill) three nymphs or three adults were introduced, and at R6–R7 (mid seed fill to early pod maturity) five nymphs or five adults were introduced.

After the five-day exposure, the insects and cage were removed and the raceme was detached from the plant and examined under a Leica MS5 stereomicroscope (Magnification range 12.6 to 80). Plant parts that were injured were compared with uninjured parts from control cages, and representative injuries were selected for microphotography. For microphotography, a Nikon D5200 digital camera attached to an Olympus SZX16 light microscope was used. In addition to examination of treated plant portions immediately after exposure to insects, some

plants were grown until the stage of commercial seed harvest, and seeds were examined microscopically as described above.

For growth stages where abortion of reproductive organs occurred, the number of reproductive organs (buds, flowers or pods) at each node of the caged raceme was recorded before caging, and again at the end of the five-day treatment. The pre-treatment numbers of reproductive organs at each node were compared using analysis of variance with Tukey's post hoc test. The assumptions of analysis of variance were tested by Shapiro-Wilk (normality) and Levine test (homogeneity). The relationships between frequency of abortion and treatment and position within racemes or pods were investigated by log-linear modelling of contingency tables (Bishop *et al*. 2007). Analyses were performed in Systat (Systat 2009), with the criterion for significance of $\alpha = 0.05$.

At the R4–R5 stage, two additional experiments were carried out to characterize the injury to racemes. One experiment was with three adults or three nymphs and the other experiment was with five adults or five nymphs. In infested cages, the insects were allowed to feed for 5 days then removed from the cages, then the racemes detached from the plant and examined for the injury. The pods were dissected longitudinally and the injury scars which were present on the funiculus or placental area of the seeds were counted. The position of the pod in the raceme and the position of the seed in a pod were also noted. Data from the three and five insect experiments were analysed together using log-linear modelling of contingency tables to study the frequency of funiculus injury in relation to pod position, seed location and number and stage of insects.

Results

The use of unmated adults to prevent oviposition was successful as there were no eggs or young nymphs seen when sleeve cages were dismantled. Only injury from feeding was evident at the end of the five-day treatment period.

In growth stages R2–R3, buds, flowers and small pods are present (Colorado State University Integrated Pest Management Program 1998). In my study, caged racemes had reproductive structures (buds, flowers or pods) on the axillary node and on the basal three nodes of the peduncle. At the end of the five-day exposure, injuries to pods caused by feeding of either adults and nymphs were characterized by brown spots, about 1 mm in diameter, centred at the feeding puncture (Fig. 3A). In some cases, the exterior surface of injured pods exhibited an irregular appearance because of swelling at feeding sites (Fig. 3B). In other cases, "split lesions" (Painter 1930) in the epidermis occurred at feeding sites on the peduncle of the raceme or on pod surfaces (Fig. 3C–E). Split lesions on the peduncle were found mainly in internode regions but usually close to a node, and often resulted in constriction or breakage (Fig. 3D). On pods, split lesions were elongated areas of torn epidermal tissue (Fig. 3E). The most frequent response to feeding injury during the R2–R3 growth stages was abortion of buds, flowers and pods (Fig. 3F). This abortion injury was readily distinguishable from stem breakage at a feeding site. Abortion of pods was associated with brown necrotic lesions inside the pod (Fig 3G). Injuries caused by feeding by nymphs (Fig. 3A–C, G) showed no observable differences in their nature from those resulting from adult feeding (Fig. 3D–F).

Before caging for the R2–R3 treatments, numbers of reproductive structures at each node in the raceme differed significantly ($F = 22.2$, df = 3, 128, $P < 0.001$), with the number of structures at the distal node lower than at all other positions (Table 5). The frequency of

abortions during the five-day treatment period (Table 5) differed significantly among treatments (Likelihood ratio (LR)χ^2 = 15.4, df = 2, P = 0.001): abortions were more frequent in infested treatments than in controls (LRχ^2 = 9.6, df = 1, P = 0.002) and more frequent in the nymph treatment than in the adult treatment (LRχ^2 = 5.8, df = 1, P = 0.02). Frequency of abortion was also affected by position within the raceme (LRχ^2 = 11.2, df = 3, P = 0.01), mostly attributable to more frequent abortions at the most distal node (LRχ^2 = 8.1, df = 1, P = 0.004). The relationship between abortion frequency and position within the raceme did not differ significantly among treatments (LRχ^2 = 9.7, df = 6, P = 0.2).

In the R4–R5 growth stages, seeds are present in pods, and seed development ranges from just initiated to fully developed; R5 terminates when more than one pod contains fully developed seeds (Colorado State University Integrated Pest Management Program 1998). At these growth stages in my study, there were pods only at the axillary and first peduncular nodes. In my study of injury symptoms, there were two pods at each of these two nodes on 50% of the caged racemes; the remaining racemes had a total of either two (33%) or three (17%) pods, with no more than two pods per node. The maximum number of ovules per pod was seven, and 84% of pods contained either five or six ovules. At the end of the five-day exposure, external pod injury in the R4–R5 growth stage took the form of indistinct brownish areas, and was found mainly near the ventral suture of the pod (Fig. 4A). Stylet entry points near the ventral suture were associated with brown necrotic lesions in the placental region of the seed pod (Fig. 4B); frequently this necrosis also involved the funiculus supplying the developing seed (Fig. 4C).

In the experiments to characterize distribution of funiculus and placental injury, there were a total of 30 caged racemes in which there was some injury of this type, and only data from these racemes were analysed. The total number of pods on the 30 racemes was 100; of these, one

pod had eight seeds, 11% had seven seeds, 79% had either five or six seeds, and the remaining 9% had two–four seeds. Frequency of funiculus or placental injury was not affected by whether there were three or five insects ($LR\chi^2 = 0.5$, df = 1, $P = 0.5$) caged on the raceme. At the end of the five-day treatment, the frequency of feeding injury affecting the vascular supply of more developing seeds was higher for racemes exposed to nymphs than for those exposed to adults (Table 6) ($LR\chi^2 = 4.2$, df = 1, $P = 0.04$); no injuries of this type were seen in the control treatment. The frequency with which seeds were affected by placental or funiculus injury did not differ among pod positions within the raceme ($LR\chi^2 = 2.5$, df = 3, $P = 0.5$), and the distribution of injury among pod positions was unaffected by stage of insect ($LR\chi^2 = 3.0$, df = 3, $P = 0.4$).

Analysis of the effect of seed position within pods on the frequency of placental and funiculus injury was complicated by the variable number of seed positions per pod. In one analysis, only data from the basal six seed positions were analysed because of the few pods with seeds at more distal positions. In this analysis (Table 7), frequency of injury was unaffected by seed position ($LR\chi^2 = 5.7$, df = 5, $P = 0.3$) and the distribution of injuries among seed positions did not differ significantly between treatments with nymphs and adults ($LR\chi^2 = 5.2$, df = 5, $P = 0.4$). In a second analysis, frequency of injury at the two endmost seed positions in pods was compared with that at all other seed positions. Again, the frequency of funiculus injury was unaffected by seed position ($LR\chi^2 = 1.0$, df = 1, $P = 0.3$) and the pattern of injury was unaffected by insect stage ($LR\chi^2 = 0.01$, df = 1, $P = 0.9$). Pooled over insect stages, 36% of endmost seed positions had funiculus injury, compared to 32% for seed positions that were not at the ends of the pod.

Direct injury to developing seeds was also observed in the R4–R5 stages, and affected 3% of developing seeds exposed to feeding by nymphs, and 7% of seeds exposed to adults. In

direct seed injury, the testa was penetrated and a cavity was evident in the underlying cotyledon (Fig 4D). In early stages of seed development, there was little change in pigmentation, but in older seeds, the testa bordering the injury became brown (Fig. 4E). Unlike earlier growth stages, no pod abortion resulted from feeding in the R4–R5 stage. Like earlier stages, the nature of injury in response to feeding by nymphs (Figs. 4A, B, E) and adults (Figs. 4C, D) did not differ.

When seeds from the R4–R5 treatments were examined at the time of seed harvest, many of them were shrivelled (Fig. 4F). The majority of shrivelled seeds showed no signs of direct injury, but some shrivelled seeds did have scars indicating they had been directly injured.

At the R6–R7 stage, bean pods contain fully developed filling seeds (Colorado State University Integrated Pest Management Program 1998). At the end of the five-day exposure, the major injury in these stages was direct injury to the seed and this did not appear to be fundamentally different from the seed injury in the R4–R5 stage. Injury at the R6–R7 stages was more visible because, in more mature seeds, there was greater pigmentation of the testa bordering the injury site (Fig. 5A). The injury was not localized on any particular part of the seed, and more than one injury on a seed was frequently observed. At the site of feeding, the testa was perforated and there was a cavity in the tissues of the cotyledon (Fig. 5B). In most cases, it appears that stylets were inserted through the pod wall and the feeding site on the seed was close to the pod penetration point (Fig. 5C); pod penetration points included the dorsal and ventral sutures and sides of the pod. When seeds injured during the R6–R7 growth stage were examined at the normal time of harvest, lesions were crater-like pits and frequently had surrounding concentric raised ridges of brown tissue (Fig. 5D), No pod abortion was observed at the R6–R7 growth stages, and no differences were observed between the type of injury from nymphs (Figs. 5A) and adults (Fig. 5B, C).

Discussion

I chose the five-day duration for feeding injury treatments to provide the maximum duration of exposure to a specific insect stage, without transition to a following stage. At the end of the injury treatment at the temperatures of my study, the fifth instar nymphs would be almost ready to moult to adults, and female *L. lineolaris* would be expected to begin oviposition at about the end of the period of injury treatment (Bariola 1969; Ugine 2012). It may be unwise to assume my results apply to all ages of nymphs and adult *L. lineolaris* as, in *Lygus hesperus* (Knight), feeding behaviour is dependent on nymphal instar (Cooper and Spurgeon 2013) and reproductive status of adults (Cooper and Spurgeon 2011).

In my study, the type of injury associated with feeding of unmated adults and of fifth instar nymphs was indistinguishable. In both instances where it was analysed, injury from feeding by nymphs was more frequent than from adults. However, because insects could not leave the small portion of the plant to which they were confined, extrapolation of my results to whole plants or crops is unwise. Stage-specific patterns of *Lygus* injury to whole plants can differ from those on isolated plant organs because of stage-specific differences in mobility or distribution on the plant (Cooper and Spurgeon 2013). For similar reasons, the lack of evidence in my study for nymphs or adults having preferred pod or seed positions for inflicting injury is probably applicable to choices within racemes, but cannot be safely extrapolated to the broader range of choices available to insects on crop plants growing in the field.

In this study, brown discoloured lesions were found both externally and internally in navy bean reproductive organs, regardless of growth stage. The exterior 1 mm diameter brown spots I observed appeared to surround individual stylet penetration points, whereas diffuse brown areas on the surface of pods, were probably the result of multiple low-volume injections of saliva, as

described for *L. hesperus* (Backus *et al*. 2007). Brown colouration was also evident at interior feeding locations, including the sites of placental and funiculus injury. Hori (2000) suggested that oxidation of phenols by phenoloxidases is responsible for the production of brown-pigmented compounds following feeding by Heteroptera. In plants, activation of the phenol-phenoloxidase system is a general response to wounding, and so the brown pigmentation could be a response to cellular destruction by the stylets or saliva of *Lygus* (Hori 2000). The pigmentation could also result from the direct effect on plant phenolics of the phenol oxidizing enzymes in *Lygus* bug saliva (Laurema *et al*. 1985; Cooper *et al*. 2013).

Tissue swelling and split lesions resulted from *Lygus* bug feeding in the R2–R3 stage. Split lesions on stems are characteristic injuries from *Lygus* bug feeding on cotton (King and Cook 1932), poplar (Sapio *et al*. 1982) and Douglas-fir (Schowalter *et al.* 1986). Swellings are the result of cell hypertrophy, and interior swelling may strain the epidermis sufficiently to cause it to split, producing a split lesion (Painter 1930; King and Cook 1932). The hypertrophy may result from disruption of the plant's hormonal system by *Lygus* bug feeding (Tingey and Pillemer 1977; Hori 2000).

Abortion of reproductive structures in response to *Lygus* bug feeding has been reported in several leguminous crops including alfalfa (Sorensen 1939), soybeans (Broersma and Luckmann 1970), Lima beans (Baker *et al*. 1946), snap beans (Fisher *et al*. 1946), and green beans (Khattat and Stewart 1975). In my study, abortion of reproductive organs occurred only at the R2–R3 growth stages, and in soybeans, Lima beans and green beans there is a similar restriction to early stages of raceme development (Baker *et al*. 1946; Broersma and Luckmann 1970; Khattat and Stewart 1975). In my study, abortions occurred in all treatments, but were more frequent when racemes were caged with *Lygus* bugs. Each of the four nodes in racemes in my study would

initially have had three buds, but by the time of caging for the R2–R3 studies there were two or less at each node, with fewest at the distal node. During the five-day period of caging, frequency of abortion was always highest for the most distal node. As treatment did not influence the positional pattern of abortions within the raceme, I conclude that *Lygus* bugs did not exhibit strong preferences for feeding on specific pods within the raceme.

The positions of abortions within the caged racemes accord with patterns observed for healthy racemes by Sage and Webster (1987): the third bud at each node aborts before anthesis, and the frequency of subsequent abortions is highest for the later-developing distal nodes. Abortion of pods occurs at specific abscission zones on the pedicel (Ofir *et al.* 1993). Abscission may be a response to reduced flow of auxins from the pod (Osborne 1989; Ofir *et al.* 1993), or a response to reduced photosynthate sink activity of the pod (Binnie and Clifford 1999). I observed that in cages with insects, aborted pods had internal lesions. Such lesions could, as hypothesized by Strong (1970) and Tingey and Pillemer (1977), lower the levels of auxins released by the pods, but could also reduce the pod's sink activity. Either mechanism would represent interference by *Lygus* bug feeding with the normal regulation of the process of abscission in the raceme, a process that occurs only during a critical period within 5 days of anthesis (Sage and Webster 1987). My finding that pod abortion did not occur in later growth stages supports the hypothesis that the elevated levels of abortion were not a direct response to *Lygus* bug feeding, but rather a consequence of that feeding modifying the plant's normal system of regulation of abscission during the critical period.

By the completion of the critical period for pod abortion, more than 95% of reproductive structures will have aborted except for those at two bud positions at each of the two basal nodes

of the raceme (Sage and Webster 1987). The count of four or fewer pods on the racemes caged in the R4–R5 and R6–R7 growth stages are a result of this process.

Necrosis of the vascular tissues in the placental and funiculus regions in pods at the R4–R5 stage occurred only in cages with *Lygus* bugs. Developing seeds within pods receive minerals and photosynthates through these vascular tissues, and shortage of these resources results in abortion and collapse of fertilized ovules (Adams 1967). Hence, injury to the vascular tissues supplying nutrients is the most likely cause of shrivelling of seeds, which were very frequent at pod maturity when *Lygus* bug feeding had occurred at the R4–R5 stages. Seed shrivelling in response to *Lygus* bug feeding also occurs in Lima beans, *P. lunatus* (Baker *et al.* 1946). In *P. vulgaris*, shrivelling of seed is a normal phenomenon in healthy pods: the frequency of undeveloped seeds may exceed 90% for the basal seed position in the pod and be 10–30% in more distal positions in the pod (Harris 1915; Nakamura 1988). In my study, *L. lineolaris* did not show feeding preferences for specific seed positions within the pod. Funiculus injuries were evaluated immediately after the 5 day exposure to the insects, and positional patterns of shrivelled seeds such as those observed in healthy pods by Harris and by Nakamura, could develop later in pod development.

Direct injury to seeds was prevalent in the R6–R7 stages and infrequently occurred at the R4–R5 stages. Direct injury resulted in mature seeds with crater-like seed pits fringed by pigmented areas. The central pit is likely to be the result of physical and enzymatic destruction of the cells of the testa and underlying cotyledon, with the surrounding pigmentation arising from the plant's phenol-phenoloxidase wound response (Hori 2000). Frequent occurrence of such blemishes in a seed sample for grading would reduce the grade (Canadian Grain Commission 2014) and could make beans unsuitable for canning (United States Department of Agriculture

1976). Similar seed pitting occurs in Lima bean when *Lygus* bugs feed on developing seeds (Baker *et al*. 1946; Elmore 1955).

Reduced grade of seeds because of seed pitting is likely to lead directly to economic loss for bean producers. However, it is not clear whether injury at early growth stages will cause economic loss. Plants of *P. vulgaris* respond to removal of early flowers by setting pods from later flowers (Binnie and Clifford 1981) and respond to removal of early pods by reduced frequency of abortion of later developing pods (Tamas *et al*. 1979). Thus, the plant may compensate partially or completely for loss of some reproductive structures due to *Lygus* bug injury. It is also possible that there is compensation for *Lygus* bug-induced seed shrivelling within a pod. Seed filling occurs within an environment regulated by nutrient competition (Adams 1967) and a relatively high proportion of seeds fail to fill (Harris 1915; Nakamura 1988); *Lygus* bug-induced removal of some seeds from the competition could allow filling of unaffected seeds that would otherwise fail to fill.

This study has identified the symptoms of *Lygus* bug injury to navy bean racemes that are observable within a few days of that injury occurring. Knowledge of these symptoms can allow for more accurate diagnosis of *Lygus* bug injury during field scouting in field beans. Early in the growing season, *Lygus* bug adults are detectable in low numbers in field bean crops at growth stages up to R3 (Nagalingam and Holliday 2015). Although *Lygus* bug feeding induces abscission of reproductive structures at these growth stages, detached buds, flowers or small pods are not diagnostic of *Lygus* bug injury because of the prevalence of abscission of reproductive structures in healthy plants. Reliable signs of *Lygus* bug injury up to the R3 growth stage include brown pigmented feeding spots and associated tissue swelling or split lesions. Following the arrival of adults, a generation of nymphs develops in field beans but, probably

because of low sampling efficiency, only low numbers are detected in sweep net samples during the R4–R5 growth stages (Nagalingam and Holliday 2015). At these growth stages, funiculus and placental injury are characteristic, and external examination of developing pods for discolouration near the ventral suture, followed by internal examination for brown lesions near the vascular tissues, would provide a reliable indicator of *Lygus* bug injury. Late in the growing season, there are peaks of sweep net catches of nymphs and adults that occur at R6–R7 (Nagalingam and Holliday 2015). At these growth stages, pods with exterior penetration points and containing seeds with direct injury to the testa are characteristic. Retrospective assessments of *Lygus* bug injury based on harvested seed can clearly show evidence of direct *Lygus* bug injury to seeds, which produces seed pits. Shrivelling of seed is not definitive because many seeds fail to fill in the absence of *Lygus* bugs (Harris 1915). Furthermore, shrivelled seed is likely to be under-represented in harvested samples following threshing and cleaning.

Table 5. Number of reproductive structures (buds, flowers or pods) before treatment, and percentage of these that aborted during treatments in relation to position in the raceme. Treatments were exposure to one *Lygus lineolaris* nymph, one *L. lineolaris* adult or no insects (Control) for five days at the R2–R3 growth stages.

Position in raceme	Mean ± SE number of structures before treatment [a]	Percentage aborted at the end of treatments (*n* of structures)			
		Control	Nymph	Adult	Pooled over treatments
Axil [b]	1.8 ± 0.1 a	10% (20)	38% (21)	10% (20)	20% (61)
Node 1	2.0 ± 0.0 a	0% (22)	27% (22)	13% (23)	13% (67)
Node 2	2.0 ± 0.1 a	5% (20)	39% (23)	29% (24)	25% (67)
Node 3	0.9 ± 0.2 b	67% (6)	46% (13)	30% (10)	45% (29)
Pooled over position	—	10% (68)	37% (79)	19% (77)	

[a] Total for all treatments. Means followed by the same letter do not differ significantly (Tukey's test, $P \leq 0.05$)

[b] Axil is the basal node on the raceme and node 3 is the most distal on the peduncle.

Table 6. Frequency of funiculus or placental injury in relation to pod position within racemes of navy beans exposed to three or five *Lygus lineolaris* adults or nymphs at the R4–R5 growth stage.

	Percentage of seed positions with funiculus or placental injury at each pod position	
Pod position	Adult	Nymph
1	33% (66) [a]	39% (101)
2	21% (67)	37% (95)
3	34% (58)	34% (80)
4	24% (37)	37% (49)
Pooled over all positions	28% (228)	37% (325)

[a] Total numbers of seed positions at each pod position (and pooled) are shown in parentheses.

Table 7. Percentage of funiculus or placental injured positions in relation to the seed position in racemes when they were exposed to three or five *Lygus lineolaris* nymphs, three or five *L. lineolaris* adults at the R4–R5 growth stage of navy beans.

	Percentage of funiculus or placental injured positions (*n* of total positions)	
Seed position	Adult	Nymph
1	25% (40)	28% (60)
2	17% (40)	40% (60)
3	25% (40)	39% (59)
4	37% (40)	32% (57)
5	32% (37)	41% (54)
6	36% (25)	50% (28)

Fig. 3. Injuries caused by *Lygus lineolaris* during feeding on the R2–R3 growth stages of navy beans; scale bars = 1 mm. (A) Necrotic spot (arrow) on pod surface at the site of feeding by a nymph; (B) pod showing swellings (arrow) at sites of nymphal feeding; (C) discoloured area and split lesion (arrow) on peduncle of the raceme resulting from feeding by a nymph; (D) split lesion (arrow) on peduncle following adult feeding; (E) split lesion (arrow) on pod following feeding by an adult; (F) pod aborted following feeding by an adult showing location of feeding sites (arrows); (G) aborted bean pod sectioned longitudinally to show inner discolouration following feeding by a nymph.

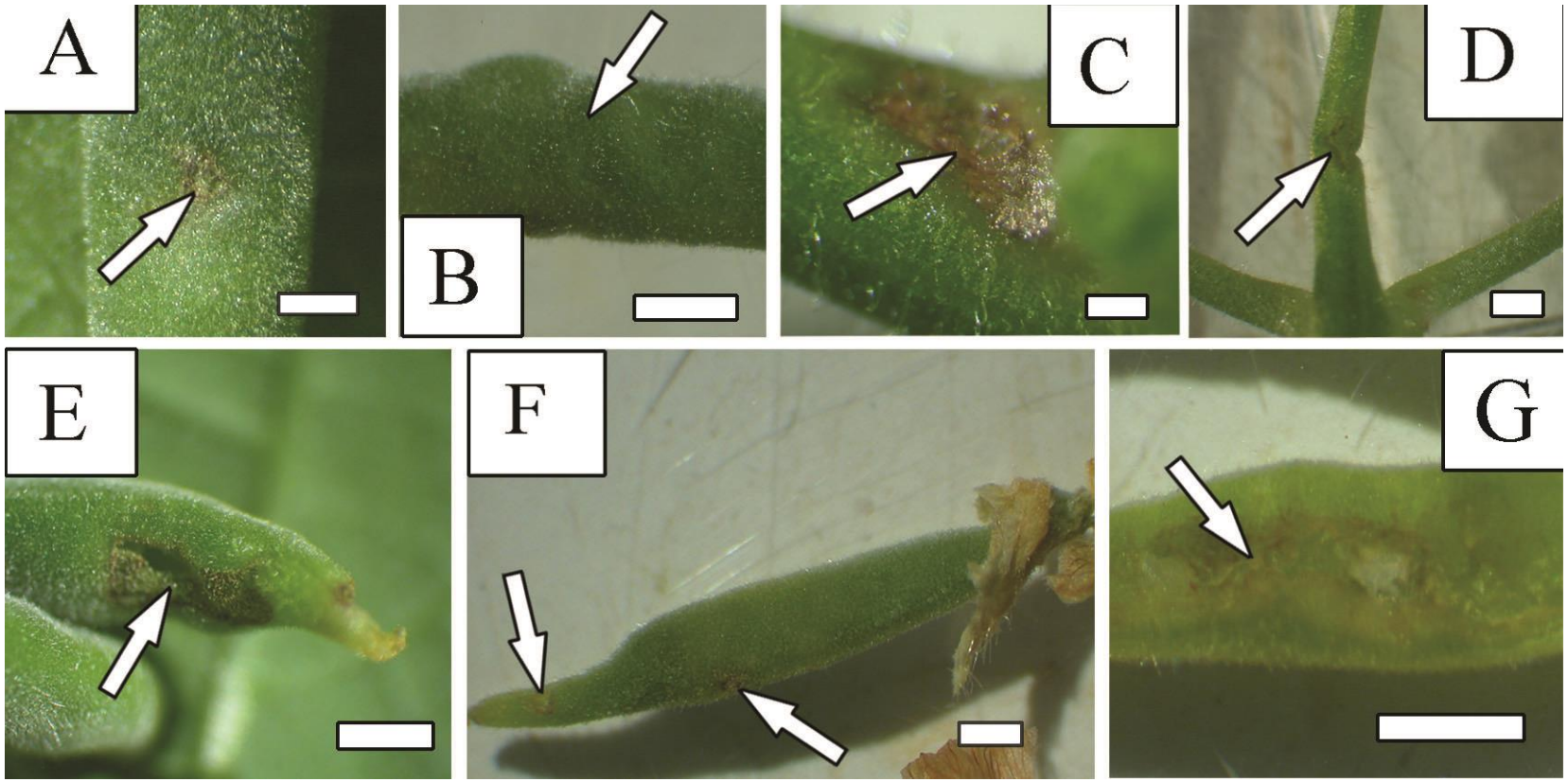

A
B
C
D
E
F
G

Fig. 4. Injuries caused by *Lygus lineolaris* during feeding on the R4–R5 growth stages of navy beans; scale bars = 1 mm. (A) External discolouration (arrow) near the ventral suture (VS) following feeding by nymphs; (B) longitudinal section of a pod showing necrosis of the placental region (brown discolouration) and stylet entry points (se) following feeding by nymphs; (C) necrosis of the placental region (pl) and funiculus (fu) regions following feeding by adults; (D) direct seed injury (arrow) from adult feeding, that penetrates through the testa into the cotyledon of a young developing seed; (E) direct seed injury (arrow) to an older developing seed following feeding by nymphs; (F) shrivelled seeds at harvest maturity, one of which has a scar (arrow) from direct seed injury, following feeding injury by nymphs at the R4–R5 stage.

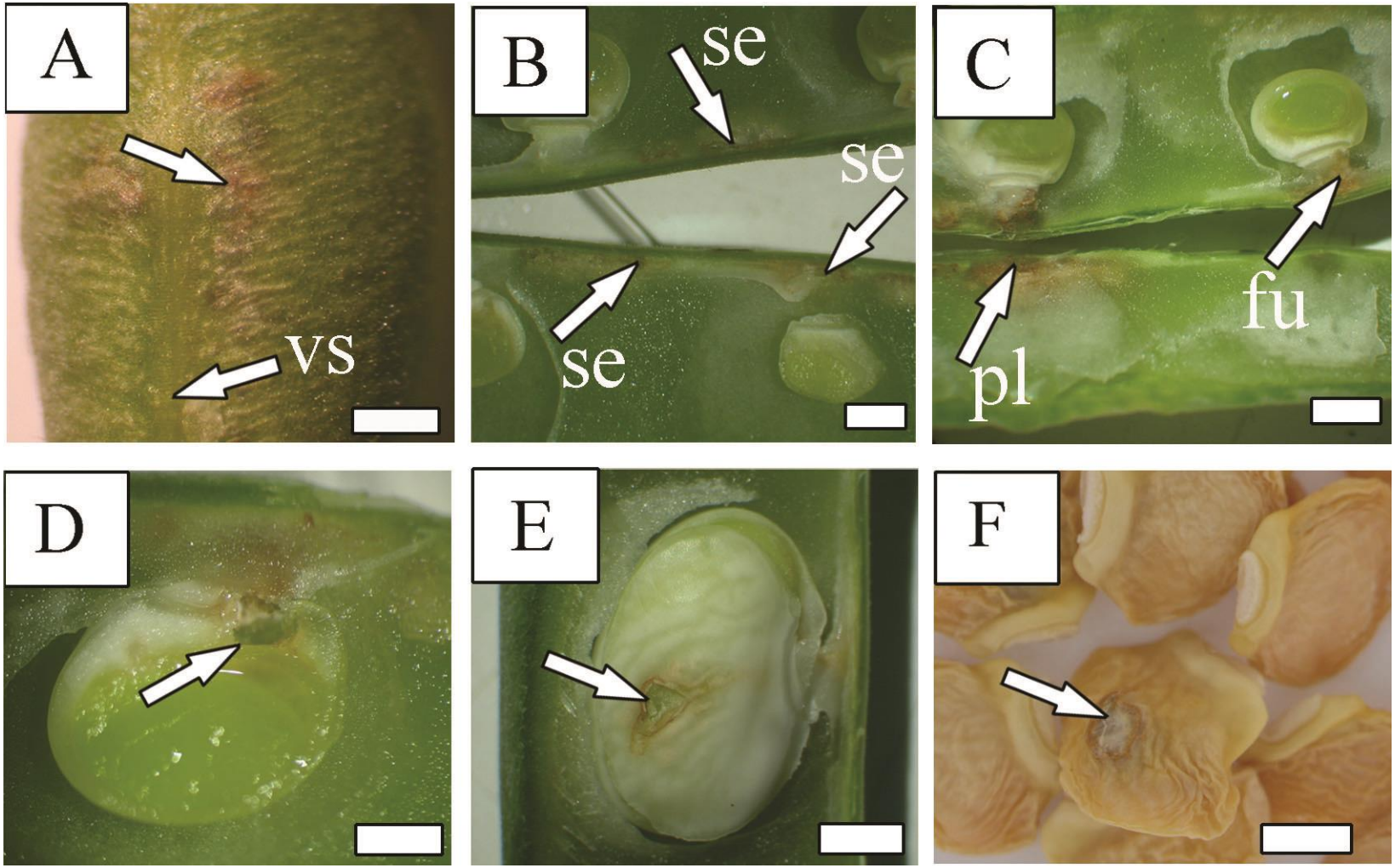
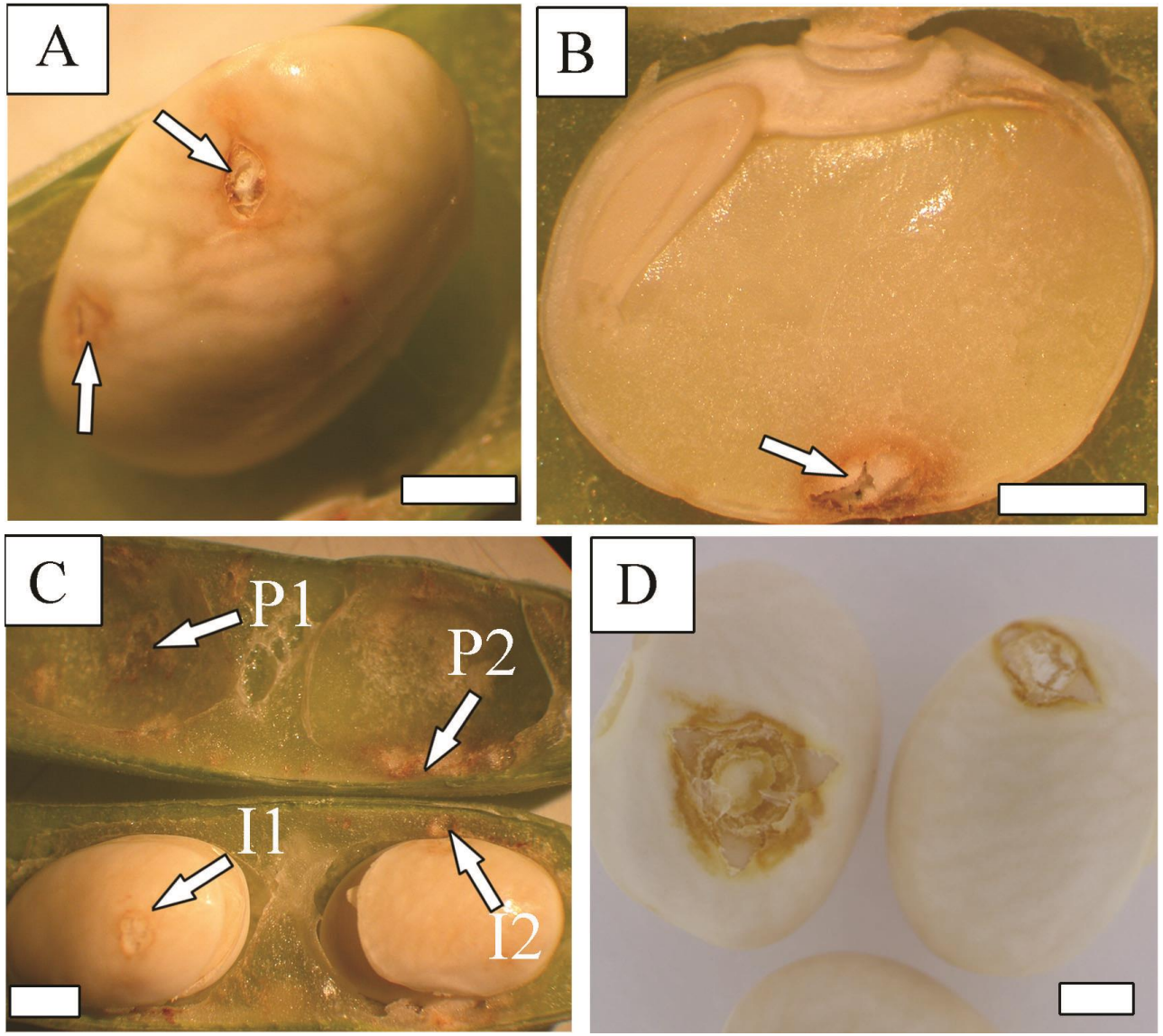

A
vs
B
se
se
se
C
fu
pl
D
E
F

Fig. 5. Injuries caused by *Lygus lineolaris* during feeding on the R6–R7 growth stages of navy beans; scale bars = 1mm. (A) Direct injury to seed (arrows) following feeding by adults; (B) longitudinal section of a seed showing injury to cotyledon (arrow) following feeding by nymphs; (C) corresponding halves of pod joined at the dorsal suture, showing pod penetrations points (P1 and P2) and corresponding seed injury sites (I1 and I2) following feeding by nymphs; (D) seed pits on mature harvested seeds following feeding by nymphs at the R6–R7 growth stages.


A
B
C
P1
P2
I1
I2
D

CHAPTER 3 – Manuscript III

Impact of *Lygus* bug feeding on quantity and quality of navy bean yield

In this manuscript the results from laboratory and field cage experiments are described. In the laboratory study, I attempted to define the consequences for yield of short intervals of feeding, the immediate effects of which were studied in manuscript II. In the field cage experiments, I attempted to define the effect of feeding of *Lygus* bugs on yield in circumstances that were more similar to field conditions. Infestations in the field cages were intended to last from introduction at a specific growth stage until harvest. Hence, comparisons of yield in control and infested treatments were expected to approximate the effects of the alternatives of no control and successful control applied at the time of introduction into the cages.

Abstract

In the laboratory and in the field, experiments were conducted at three spatial scales to study the consequence for harvested yield of feeding injury by *Lygus* bugs at three growth stages of navy beans. Spatial scales in the laboratory were individual racemes and whole plants; in the field, cages enclosing $1m^2$ of crop were used. Growth stages studied were R2–R3 (flowering to early pod set), R4–R5 (mid pod set to early seed fill) and R6–R7 (mid seed fill to maturity). In the laboratory, plants were exposed to fifth instar nymphs or pre-reproductive adults for 5 days at the chosen growth stage. In field cages, insects were introduced at the chosen growth stage and it was intended that their population persist until harvest; however there was evidence that population persistence did not always occur. At the R2–R3 stage, infestation treatments affected the harvested seed weight in raceme level and whole plant experiments, but significant differences from the control did not occur in adult treatments. Total seed weights were lower than in controls when one nymph was confined to a raceme, but when 30 nymphs were caged on a whole plant, seed weight was significantly increased relative to controls. In field cages, there was a yield reduction of 0.5 g/m^2 per *Lygus* bug adult present at the R2–R3 stage in one year, but in a second year plants exhibited no yield loss in infested cages. At the R4–R5 stage, injury resulted in shrivelled seeds and pods: in the raceme level experiment with three insects, 41%, 95% and 72% of the seeds were shrivelled in control, nymph and adult treatments respectively. In the raceme level experiment, total whole seed weights in cages with three nymphs were significantly lower than in uninfested controls, but weights in the cages with three adults did not differ significantly from those in control cages. At the R4–R5 stage, no significant effects on yield occurred in whole plant experiments, which were conducted only with adult insects. In the field cages, ≥60 nymphs reduced yield significantly from that of the control, but yields in adult

treatments did not differ from those of the control. There was no significant linear relationship between the number of *Lygus* adults or nymphs and the whole seed weight. At the R6–R7 stage, *Lygus* bugs fed directly on the seeds, resulting in seed pitting. A maximum of seven pits per seed was found in individual harvested seeds. There were more pitted seeds in most infested treatments than in controls in the raceme and whole plant studies, but significant effects of treatments on numbers of pitted seeds were not evident in field cages. Linear regression analysis showed that there was a significant linear relationship between the number of blemished seeds and the number of adults per plant. Each *Lygus* adult increased the number of blemished seeds by 6.75. Injury at this stage did not reduce total harvested seed weight at any spatial scale, but pitted seed has lower quality and value, and individual seed weight of pitted seeds was lower than that of unblemished whole seeds. There was no significant reduction in total marketable seed weight. In all my experiments, fifth instar nymphs were more injurious than pre-reproductive adults.

Introduction

Field bean (*Phaseolus vulgaris* L.) production is a major part of Manitoba's agricultural economy. In Manitoba, field beans are grown on about 58,000 ha and contribute about $ 43.5 million to the provincial economy (Pulse Growers Association 2015). Navy, pinto, red kidney, great northern, black, small red, pink, and cranberry are important market classes of field beans produced in Canada (Agriculture and Agri-Food Canada 2012).

In Canada, *Lygus* bugs are one of the economically important field crop pests, attacking canola (Butts and Lamb 1991a, 1991b), wheat (Wise *et al*. 2000), alfalfa (Mostafa and Holliday 2008), buckwheat (Wise *et al*. 2005) and flax (Wise and Lamb 2000). Three species of *Lygus* bugs are commonly present in Manitoban field beans: *L. lineolaris* (Palisot de Beauvois), *L. borealis* (Kelton), *L. elisus* (Van Duzee) (Nagalingam and Holliday 2015). Among them *L. lineolaris* is the most abundant species, comprising 78–95% of the adult mirids in the crop (Nagalingam and Holliday 2015). In Manitoba *L. lineolaris* produces two generations annually (Gerber and Wise 1995) and the second of these can be completed in navy beans (Nagalingam and Holliday 2015).

Lygus bugs prefer to feed on meristematic tissues of plants (Tingey and Pillemer 1977). They feed by inserting their piercing and sucking mouth parts into the tissues; by the combined effect of mechanical penetration (Flemion *et al*. 1954) and enzymatic digestion plant tissues are destroyed, and the resulting fluids are ingested (Strong 1970; Cooper *et al*. 2013). *Lygus* bug feeding produces a wide range of symptoms in different types of crops (Strong 1970; Tingey and Pillemer 1977). Strong (1970) broadly categorized *Lygus* bug damage symptoms as "necrosis", "cat facing" or "deformation of young fruits", "production of embryo-less or shrivelled seeds", "abscission of fruiting forms" and "reduction of vegetative growth". In several leguminous

crops, loss of yield quality and quantity from *Lygus* bug feeding has been reported, and the nature of damage is dependent on the plant growth stage injured by the insects. In Lima beans, green beans and alfalfa reduced yield quantity results from flower bud, flower and pod abortions occurring at the flowering and early pod stage; yield losses also result from responses during seed development when *Lygus* bug attack may reduce numbers of seeds in pods and cause seed shrivelling (Sorenson 1936; Hagel 1978; Khattat and Stewart 1975). Quality of bean yield is reduced by seed pitting at the mature stages of pods (Baker *et al*. 1946; Elmore 1955).

In Manitoba, plant bugs, including *Lygus* bugs and alfalfa plant bugs, are a concern for field bean producers; and in 2002 spraying of field beans for plant bugs was widespread in Manitoba (Gavloski 2001–2010), but there were no records of the effect of these plant bugs or their control on the crop during that year. Up to 20% of field bean seed may be damaged by *Lygus* bug infestations in Canada (Agriculture and Agri-Food Canada 2005). However, there have been no studies of the impact of plant bugs in field beans in Manitoba, studies that could enable field bean producers to make informed control decisions. The objective of this study was to quantify the yield loss and the loss in quality caused by *L. lineolaris* feeding on three different growth stages of navy beans.

Materials and methods

Experiments were carried out on three different growth stages of navy bean plants: R2–R3 (flowering to early pod set), R4–R5 (mid pod set to early seed fill) and R6–R7 (mid seed fill to maturity) (Colorado State University Integrated Pest Management Program 1998). Experiments were carried out at three spatial scales. Experiments at the smallest scale were carried out in controlled environmental chambers, with insects enclosed with one raceme of a plant (raceme level experiments). In intermediate scale experiments in controlled environmental chambers,

insects were enclosed in a cage containing a single potted plant (whole plant experiments). In the largest scale experiments, field cages were used to enclose a group of plants growing in the field and known numbers of insects were added (field cage experiments).

Plants: For raceme level and whole plant experiments, navy bean plants, cv 'Envoy' were grown in controlled environment chambers at 23 °C, 70% RH and 16:8 h (L:D) as described previously (Chapter 3 – Manuscript II).

Insects: The methodology for rearing *L. lineolaris* and maintaining the colony is described in the previous chapter (Chapter 3 – Manuscript II**).** In all laboratory experiments, the test insects were from the laboratory colony and were fifth instar nymphs that had moulted within the last 24 h or *L. lineolaris* adults that had moulted within the last 48 h. Adults were of unspecified sex and, to avoid injury to plants from oviposition or feeding by young nymphs during the five day laboratory exposures, adults were isolated from the colony as fifth instar nymphs and not allowed to mate. In field cage experiments, field-collected insects were used. The main sources of field-collected insects were alfalfa and canola fields near Carman, Manitoba. The insects were collected from these fields using a 38 cm-diameter sweep net and they were brought to the laboratory in an insulated cooler in Bug Tub®(Royal Oak Point NW, Calgary, Alberta, Canada) cages containing green bean pods as food. In the laboratory, adult *L. lineolaris* and fifth instar nymphs of *Lygus* spp. were sorted and then held in incubators at 21 °C, 70% RH and photoperiod of 16:8 h (L:D) for 1–2 days until they were used in the experiments. The developmental status of the adults used in field experiments was not determined.

Raceme level experiments: Navy bean racemes were caged with fifth instar nymphs or adults of *L. lineolaris* for 5 days using sleeve cages as described earlier in Chapter 3 – Manuscript II.

Control treatment cages contained no insects. Only one cage was used on each plant (Fig. 6). Treatments were made at three growth stages of navy beans. The infestation levels and numbers of replicates are given in Table 8. Insects were allowed to feed for 5 days and upon removal of the insects, cages were removed and racemes were marked with coloured tapes for later identification. Plants were grown until harvest maturity (R8) and marked racemes were harvested.

The pods were hand threshed in the laboratory and seeds were separated. For harvested racemes, the total numbers of all pods, numbers of sound pods (which were plump and filled with seeds, and not shrivelled), numbers of shrivelled pods (mostly containing only shrivelled seeds but sometimes with one whole seed among them), numbers of whole seeds (fully filled seeds that could have seed blemishes), and numbers of shrivelled seeds were recorded. Pods were dried indoors in perforated polyethylene bags. Seeds were weighed using a Mettler Toledo New Classic MF top loading precision balance (Greifensee, Switzerland). The total weights of all seeds, of whole seeds, and of shrivelled seeds were recorded for each harvested raceme. In the R2–R3 experiment, in addition to the above measures, the reproductive structures were counted prior to the introduction of insects. In R6–R7 experiments, seed quality was assessed by inspecting for seed pits. Numbers and weight of seeds with seed pits were recorded. Seed quality was not assessed in the R2–R3 and R4–R5 experiments, because preliminary studies showed no or little effect of insects on quality at this stage.

Whole plant experiments: In these experiments, potted bean plants were caged for 5 days using tergal (Fabricland, Winnipeg, Manitoba) net cages (30 threads/cm) that were supported by the frame of the tomato cage that was providing support to the plant. Insects were collected from the colony using an aspirator and were carefully introduced through the bottom of the cage which

was then tied and taped with masking tape at the bottom of the pot (Fig. 7). Control plants were caged without insects. The design of the experiment, insect infestations and numbers of replicates are given in Table 8. Plants were grown until harvest maturity (R8), and pods were hand harvested and dried indoors in perforated polyethylene bags. Once pods were dry they were hand threshed. The total number of all pods, numbers of sound and shrivelled pods, and numbers of whole and shrivelled seeds were recorded for the whole plant. For each plant, the weights of whole seeds, shrivelled seeds, and all seeds were determined as described above. In R6–R7 experiments, numbers and weights of pitted seeds were recorded.

Field cage experiments: Field cage experiments were conducted in 2009–2011 in plots at "The Point" research area of the University of Manitoba Fort Garry Campus. Plots were located in different areas of "The Point" each year. In 2009, one experiment was conducted at growth stage R2–R3. Navy bean cv. Envoy was seeded 2.5 cm deep in rows 60 cm apart on 15 June 2009, and fertilizer (46-0-0 NPK) applied at 50 kg/ha. In 2009, because of damp weather, the field was sprayed with Vinclozolin (Ronilan EG 50%) at 1.5 kg (a.i.) /ha on 6 August 2009 to protect from white mould. In 2010, one experiment was conducted at R6–R7. The crop was seeded 1.25 cm deep in rows 60 cm apart on 16 June 2010. Fertilizer (46-0-0 NPK) was applied at 50 kg/ha. The plot was sprayed with the herbicides glyphosate (Vantage Plus, 480 g/L) at 2.75 L (a.i.)/ha and imazethapyr (Pursuit 240 g/L) at 213 mL (a.i.)/ha on 16 June 2010. In 2011, three experiments were conducted, one at R2–R3, one at R4–R5, and one at R6–R7. On 17 June 2011, the field was seeded at about 1.25 cm deep with rows 60 cm apart, and fertilizer (46-0-0 NPK) was applied at 50kg/ha. The crop was sprayed with herbicide imazethapyr (Pursuit 240 g/L) at 213 mL (a.i.)/ha on 24 June 2011.

In each experiment, at the end of the vegetative stage of the crop, plants were caged with 1.21 m high and 1 m square polyethylene monofilament fibre mesh cages (13 meshes/cm). Each cage was supported by an aluminum pole at each corner, and spanned two rows of plants (Fig. 8). Table 8 summarizes the levels of infestation per cage and numbers of replicates for the experiments. Once plants reached the designated stage for the experiment, insects were introduced on to the plants inside the cages, and cages were sealed. Insects remained in the cages from the time of introduction until harvest. At the time of maturity (R8) the cages were opened and the plants were cut at soil level, put in burlap sacks, and allowed to air dry indoors. Once the plants were dry, the pods were removed from the plants and the seeds separated from the pods by hand.

From the time of insect introduction until harvest, field cages were inspected regularly to check for holes in the mesh or to detect unwanted insects in the cages. For the results reported in this manuscript, none of the field cages had damage that would have allowed entry or exit of insects, and there were no cages infested with insects other than *Lygus* bugs.

The data collected from field cage experiments were totals for each cage, and were total number of plants, total number of pods, number of sound pods, number of shrivelled pods, total seed weight, number of whole seeds, number of shrivelled seeds and number of blemished seeds (which included pitted seeds and seeds with other blemishes). Seeds were weighed as described above. In addition, for the R6–R7 experiment, marketable seed weight was measured. Marketable seeds are the whole seeds which are not blemished.

Data analysis: In the raceme level experiment, the initial numbers of reproductive structures (buds, flowers and pods) varied greatly. Therefore, in the R2–R3 experiment, the total number of

pods and the whole seed weight were analysed by analysis of covariance with the initial numbers of reproductive structures as a covariate. In the R4–R5 and R6–R7 raceme level experiments, in 70% of the cases treatment and controls were matched on the basis of the number of reproductive structures in the raceme, and so total number of pods and the whole seed weight were subjected to analysis of variance without a covariate. For the R6–R7 raceme level and whole plant experiments, individual seed weight of pitted and non-pitted seeds were compared using an unpaired t-test. At the R6–R7 stage, a contingency table analysis was used to examine the effect of treatment on the numbers of pitted seeds in both raceme level and whole plant level experiments. The proportion of sound pods, proportion of shrivelled pods, proportion of whole seeds and proportion of shrivelled seeds were analyzed by one-way analysis of variance for the raceme level study and for the whole plant level, these parameters were analyzed by general linear modelling.

Field cage experiments were analyzed using general linear modelling with the effect of blocks included in the model and the total numbers of plants in cages used as a covariate. In addition, for each of the three growth stages of introduction, a weighted linear regression analysis was performed to determine the relationship between the number of insects per plant and the harvested whole seed weight; the model also included the effect of block to control for spatial and between-year effects. At the R6–R7 stage, the relationship between the number of insects per plant and the proportion of blemished whole seeds was determined in a similar way. These regression analyses allowed data from multiple years to be combined in a single analysis.

For all analyses of variance, the assumptions of normality of residuals and homogeneity of residuals were tested by Shapiro-Wilk and Levene tests respectively. Whenever the data failed to meet these requirements, data was transformed. Tukey's tests were performed to compare

means. All analyses were performed using Systat 13 (Systat 2009), with an α level for significance = 0.05.

Results

R2–R3 experiments

Raceme level experiment: At harvest, the total whole seed weight of the raceme differed significantly among treatments (Table 9), with lower seed weight in the nymph treatment than the adult treatment. However, seed weights for infested treatments were not significantly different from the control. The seed weight in the adult treatment was the highest of all treatments. The total numbers of pods present at harvest was significantly different among treatments (Table 9). The numbers of pods did not differ significantly between the control and the two infested treatments but the nymph treatments had lower numbers of pods than the adult treatment. However, despite using the initial number of reproductive structures as a covariate, the apparently higher numbers of pods harvested in the adult treatment than in the control is partly attributable to average numbers of reproductive structures before treatment being lower in the control than in either of the infested treatments. When the number of abortions (difference between the initial number of reproductive structures and the number of pods at harvest) were analyzed, treatment effects were significant ($F = 5.3$, df = 2, 15, $P = 0.018$). The number of abortions was almost the same in the control (3.8 ± 0.5) and adult (3.7 ± 0.5) treatments, and both of these differed from the higher number in the nymph treatment (6.2 ± 0.8) (Tukey's test $P \leq 0.05$). The proportion of shrivelled pods and proportion of shrivelled seeds did not differ among treatments. Shrivelled seeds were found both in shrivelled and sound pods. On some occasions a shrivelled pod contained one whole seed, but in most cases all the seeds in the shrivelled pods were also shrivelled.

Whole plant experiment: At the whole plant level, the total whole seed weights were significantly different among treatments (Table 9). The seed weight in the 30 nymph treatment was higher than the uninfested control and did not differ from that in the 15 adult treatment. The total number of pods, proportion of shrivelled pods, and proportion of shrivelled seeds were not significantly different among treatments (Table 9). Although there were no significant treatment effects for the total numbers of pods, these numbers showed the same trends as those seen for total whole seed weight with higher numbers for the 30 nymph treatment and 15 adult treatments (Table 9). To determine whether compensation occurred by increasing individual weight of whole seeds in response to reductions in the number of seeds being filled, the individual weights of whole seeds among the treatments were analyzed and the average weight per seed in grams did not differ significantly among treatments (control: 0.16 ± 0.02; 15 adults: 0.16 ± 0.02; 30 adults: 0.16 ± 0.01; 30 nymphs: 0.19 ± 0.07) (F = 1.0, df = 3, 12, P = 0.4).

Field cage experiments: Numbers of plants found in the cages varied between 14 and 17 in 2009 and between 9 and 13 in 2011 (Table 10). In 2009, the average numbers of pods per plant were lower than in 2011 (Table 10).

In 2009, total whole seed weight was significantly different among treatments. The 60 adult treatment had significantly lower whole seed weight compared to the control but the mean total seed weight in the 30 adult treatment was not significantly different from that in the other two treatments. A linear model fitted to total whole seed weight indicated that losses in yield were 0.5 g/m^2 per adult present in the 1-m^2 cages. The total numbers of pods were significantly different among treatments, with a generally similar pattern of means to those for total seed weight (Table 10). The proportion of shrivelled pods and proportion of shrivelled seeds did not

differ significantly among treatments in 2009, although the proportion of shrivelled seeds and the proportion of shrivelled pods tended to be higher in infested treatments than in the control.

In 2011, total whole seed weights were significantly different among treatments (Table 10): 90 adult treatments had significantly higher mean total seed weights than the control and the mean for 30 adults was not significantly different from the control but differed significantly from that for the 90 adult treatment. Fitting of a linear model to the means demonstrated an opposite trend in 2011 to that in 2009: in 2011, there was an increase in total seed weight of 0.6 g/m^2 per adult in the 1-m^2 cages. Treatments significantly affected the total numbers of pods with means following a similar pattern to those of total seed weight. The proportion of shrivelled pods and shrivelled seeds were not significantly differed from control treatment.

The proportion of blemished whole seeds in the infested treatments in 2009 ranged between 0.01–0.07, but was much lower in in 2011 when it ranged between 0.001–0.009. The proportion of pitted seeds were significantly affected by treatment in 2009 (Table 10) with all the three treatments having different means. Although the proportion of pitted seeds tended to be higher in treatments with more insects in 2011, the effect of treatment was not significant. The number of pitted whole seeds may be an indicator of the number of insects feeding at the time seeds are maturing, and is shown in Table 11. The pitted seed numbers were higher in treatments with more insects in both years but the numbers of pitted seeds in 2011 was much lower than those in 2009.

When both 2009 and 2011 data were analysed together in relation to numbers of insects per plant, there were significant differences in whole seed weight among levels of insects per plant (Table 12). However, only 31% if this variation was explained by the linear regression,

which was not significant. The positive slope estimate may be attributable to the highest numbers of insects per plant in 2011 being almost twice those in 2009, while total whole seed weight in all treatments in 2011 was more than 2.5 times that in 2009.

R4–R5 experiments

Raceme level experiment: In the experiment with one insect per cage, there were no significant differences in any of the measured variables (Table 13), although the infested treatments tended to have higher proportions of shrivelled pods.

In the experiment with three insects per cage (Table 13), total whole seed weight was significantly affected by treatment, with the mean for nymphs lower than that for controls, and the mean for adults intermediate between them and not significantly different from either. Treatment also significantly affected the proportion of shrivelled pods and the proportion of shrivelled seeds with means higher for nymphs, lowest for controls and intermediate for adults. Number of pods was unaffected by treatment. The numbers of shrivelled pods and shrivelled seeds per nymph was 1.33 ± 0.12 and 6.94 ± 0.95 respectively and for adults it was 1.11 ± 0.16 and 4.06 ± 0.71 respectively.

Whole plant experiment: At the whole plant level, none of the measures of yield differed significantly among the treatments (Table 13). Average weight per whole seed was not significantly different among treatments (control: 0.152 ± 0.018 g; 15 adults: 0.140 ± 0.007 g; 30 adults: 0.136 ± 0.010 g). Mean number of shrivelled pods and shrivelled seeds per adult was 0.38 ± 0.07 and 1.93 ± 0.30 respectively.

Field cage experiment: Total whole seed weight was significantly affected by treatments (Table 14): treatments with nymphs had significantly lower weights than the control, and weights in

adult treatments were intermediate between, and not significantly different from, those for control and nymph treatments. The proportion of shrivelled seeds was not determined as the total numbers of seeds were not known. Numbers of shrivelled seeds were significantly affected by treatment (F = 2.6, df = 6,23, P = 0.047), but Tukey's test revealed no significant differences among treatment means (data not shown) No other measures showed significant responses to treatments.

The average numbers of pitted whole seeds are shown in Table 11. These numbers are highly variable among treatments and treatments were not significantly different (Table 11). The 120 nymph treatment and the 30 adult treatments had high mean numbers of pitted seeds, but the high standard errors for these treatments indicate that there was great variability among cages within the same treatments.

In the analyses of total whole seed weight in relation to numbers of insects per plant (Table 12), there were significant differences in whole seed weight among levels of adults, but the linear regression was not significant and explained only 5% of the variance among insect levels. For nymphs, whole seed weight also varied among-insect levels; the linear regression explained 53% of the among-insect levels variance, but was not significant. Estimated slopes for both nymphs and adults were negative, which one would expect if insects caused reduced yield, but as the regressions were not significant, they may not be reliable estimators of insect effect.

R6–R7 experiments

Raceme level experiment: The total whole seed weight in this experiment was not significantly affected by treatment (Table 15)**.** The only yield quantity parameter that differed significantly among treatments was the proportion of shrivelled seeds where the nymph treatments had significantly higher proportions of shrivelled seeds than the control (Table 15).

The proportion of pitted seeds differed significantly among treatments (Table 15). There were significantly more pitted seeds in the nymph treatment than in the control (Table 15). Among the infested treatments, the average seed weight of whole seeds with pits was 0.108 ± 0.037 g and of whole seeds with no pits was 0.122 ± 0.035 g, differences that were significant (t = 2.0, df = 111, P = 0.046). Marketable seed weights, calculated as the total weight of whole seeds that were not pitted, differed among treatments (F = 7.3, df = 2, 15, P = 0.006). The mean weight of marketable seed per raceme in the nymph treatment (1.0 ± 0.3 g) was lower than that in controls (2.8 ± 0.3 g) and neither differed from that in the adult treatment (1.6 ± 0.5 g) (Tukey's test P = 0.05). The frequency of pitted seeds differed significantly among treatments (LRχ^2 = 91.2, df = 2, P < 0.001). In the adult treatment 26% of whole seeds were pitted whereas 38% of whole seeds in the nymph treatment were pitted. The seeds affected by nymphs had up to four pits per seed and those affected by adults had up to three pits per seed. In the nymph treatment, 81% of the pods had pitted seeds, in the adult treatment 45% of the pods had pitted seeds and the control treatments did not have any pitted seeds in pods. The numbers of pitted seeds per insect in the raceme level experiment with five insects was 1.9 ± 0.4 per nymph and 1.1 ± 0.6 per adult.

Whole plant experiment: In this experiment there were no significant treatment effects on total whole seed weight (Table 15) or on any yield components contributing to the quantity of yield. The proportion of whole seeds with seed pits was significantly different among treatments; the control treatment had no pits, which differed significantly from all infested treatments (Table 15). Although not significant in Tukey's tests, the proportion of pitted seeds tended to be higher in infestations with 30 nymphs than in those with the 30 adults (Table 15). In the treatment with 15 nymphs, seeds had up to five pits per seed; when infested with the same number of adults,

there were up to four pits per seed. For the treatments with 30 insects, infestation with nymphs resulted in up to seven pits per seed and adult infestations produced up to five pits per seed. The number of pitted seeds per insect in the whole plant experiments was 1.5 ± 0.5 per nymph for the 15 nymph treatment, 0.7 ± 0.1 per nymph for the 30 nymph treatment, 0.7 ± 0.2 per adult in the 15 adult treatment and 0.5 ± 0.2 per adult in the 30 adult experiment. Among the infested treatments, the average weight of a pitted whole seed (0.107 ± 0.038 g) differed significantly from that of a whole seed without pits (0.116 ± 0.042 g) (t = 3.6, df = 2000, P < 0.001).The total weight of marketable seed was not significantly affected by treatment (F = 0.2, df = 4, 20, P = 0.9).

On a per capita basis nymphs tended to produce higher numbers of pitted seeds in the raceme level experiment (1.9 ± 0.4 per nymph) than in the whole plant experiment (15 nymphs: 1.5 ± 0.5, 30 nymphs: 0.7 ± 0.1). Similarly, adults tended to produce higher numbers of pitted seeds in the raceme level experiment (1.1 ± 0.6 per adult) than in the whole plant level (15 adults: 0.7 ± 0.2, 30 adults: 0.5 ± 0.2).

Field cage experiments: In 2010, the numbers of plants in cages varied from 15 to 27 while in 2011 the number of plants per cage ranged from 11 to 16 (Table 16). In corresponding treatments, the average number of pods per plant was higher in 2011 than in 2010 (Table 16). In 2010, only the total numbers of pods were counted; pods were not separated into sound and shrivelled pods and total numbers of whole seeds were not counted. Therefore the proportions of shrivelled and blemished seeds were calculated based on the estimate of seed numbers obtained from the total seed weight and the measured 100 seed weight in that experiment.

In neither year was there any significant effect of treatment on measurements of yield quantity or quality (Table 16). In both years, infested cages tended to have higher proportions of blemished seeds than did the corresponding controls. In both years, total marketable seed weight per cage was not significantly affected by treatment (2010: $F = 0.2$, df = 3,6, $P = 0.9$; 2011: $F = 1.6$, df = 4,9, $P = 0.3$).

For each treatment, numbers of insects per pod in 2010 were 60 nymphs: 0.132 ± 0.012, 60 adults: 0.123 ± 0.012, 120 adults: 0.254 ± 0.017 and in 2011 were 60 nymphs: 0.136 ± 0.003, 60 adults: 0.157 ± 0.014, 120 adults: 0.316 ± 0.027, 180 adults: 0.450 ± 0.028. These numbers were lower than those in raceme level experiments (nymphs: 1.167 ± 0.053 per pod, adults: 1.3 ± 0.123), and in the whole plant experiments (15 adults: 0.702 ± 0.138 per pod, 30 adults: 0.820 ± 0.076, 15 nymphs: 0.46 ± 0.07, 30 nymphs: 1.017 ± 0.252). The number of blemished seeds per insect in the field cage experiments varied greatly; the highest numbers were 0.8 ± 0.4 blemished seeds/insect for nymphs and 0.9 ± 0.2 for adults. These numbers were smaller than those for the raceme level studies (nymphs: 1.9 ± 0.4 per insect; adults: 1.1 ± 0.6) but comparable to the highest numbers in the whole plant experiment (nymphs: 1.5 ± 0.5 per insect; adults: 0.7 ± 0.2).

In the grading system of the Canadian Grain Commission, extra number 1 Canada grade of beans must have ≤1% of seeds damaged. Canada number 1 and number 1 select grade must have ≤1.5% of damaged seeds, number 2 grade ≤3% of damaged seeds, number 3 grade ≤5% damaged seeds and number 4 grade ≤8.5% of damaged seeds (Canadian Grain Commission 2014). In my studies, seeds in control treatments exhibited blemishes resulting from factors other than *Lygus* bug damage. These blemishes could be staining from leaves adhering to seeds or physical damage from abrasion of seeds with other seeds, plants and soil. Therefore I subtracted the percentages of blemished seeds in control cages within each replicate block of the field cage

study and constructed corrected percentages of blemished seeds for each infested treatment. In 2010, the corrected percentage of seed with blemishes was 0.8 ± 0.7 in the 60 adult treatment, 0.1 ± 0.4 in the 60 nymph treatment, and 1.1 ± 0.4 in the 120 adult treatment. Based on this, in 2010 the 120 adults reduced the grade of the seeds from extra number 1 grade and other levels of infestation did not have any effect on seed quality. In 2011, the corrected percentage of blemished seed was 4.9 ± 3.2 in the 60 nymph treatment, 3.3 ± 2.0 in the 60 adult treatment, 2.3 ± 1.4 in the 120 adult treatment and 34.5 ± 29.2 in the 180 adult treatment, so there was a loss of quality in all infested treatments.

When analysing total whole seed weight in relation to numbers of insects per plant (Table 12), there were no significant differences in whole seed weight among levels of adults. A linear regression explained 14% of the variance among levels of adults per plant, and was not significant. There was a significant linear regression between the number of blemished seeds and the numbers of adults per plant (Table 12), and this relationship explained 36% of the variance among adult levels. However, there was no significant variation in the number of blemished seeds among adult levels, so the linear relationship may have little practical value. The slope of the regression was positive, which means that when there were more insects per plant there were more blemished seeds. For nymphs, there was no significant linear relationship between the insect levels either for the whole seed weight or for the numbers of blemished seeds (Table 12). For both of these analyses, F tests for among blocks and among insect levels were not performed as the data were sparse.

Discussion

This study was performed at three scales of experimentation and each scale of experimentation can affect plant responses. At the raceme level, insects had little choice of tissues upon which to

feed, and the yield assessment was based on plant organs on which feeding was likely to have occurred. At the whole plant level, insects may have fed on only some of the yield-producing organs and may have fed on other plant tissues as well. Thus, the whole plant level was more similar to a field situation, in that the insect had choices among plant parts and could spend time off the plants (Cooper and Spurgeon 2013), although, unlike the field situation, there was no choice among plants. More realism was also conferred at the whole plant scale by the harvest being of all yield-producing organs, regardless of whether they had been fed upon. Both raceme- and whole plant-level experiments were laboratory studies under controlled conditions with infestations for a short defined period of time, so that the details of stage-specific plant responses could be elucidated.

At the R2–R3 stage, the bud, flowers and small pods develop, and abortion of these structures occurs naturally in healthy bean plants (Adams 1967). In my study, pod abortions occurred only at this stage. Pod abortion results from a hormonally-regulated system of abscission at a critical period of growth, rather than from direct injury (Chapter 3 – Manuscript II of this thesis). In healthy bean plants, the critical period for abscission is the 5 days following anthesis (Sage and Webster 1987). At later growth stages the critical period for pod abortion has past and racemes no longer respond to insect feeding by pod abortion.

At the individual raceme level, a plant can compensate for insect-induced abortions earlier in the development of that raceme by not aborting the reproductive structures that would otherwise abort. At the whole plant level, compensation for early pod abortions may also happen within injured racemes, but additionally there can be compensation in uninjured racemes that were not fed upon or which develop later in the plant's development. Navy beans follow the determinate type of growth (Manitoba Pulse Growers Association 2015) and produce all their

flowers and pods in a short period of time (Ontario Ministry of Agriculture, Food and Rural Affairs 2009b). Because of the short flowering time they are more prone to stresses (Ontario Ministry of Agriculture, Food and Rural Affairs 2009b), possibly because there is diminished time in which compensation by later-developing racemes can occur.

My raceme-level study demonstrated the great importance of the numbers of pods and numbers of seeds to the yield of the raceme at harvest. In this study, whole seed yield was reduced in the treatment with nymphs, and this appears to be a consequence of pod abortion, resulting in reductions in the numbers of pods and hence of seeds. In adult treatments there tended to be higher total whole seed weights and numbers of pods than in controls, although this was not significant. This is partly attributable to the higher number of initial reproductive structures before treatment in the adult experiments, when compared to the control. Therefore, it is not clear whether or not plants compensated for the injury from adults in my raceme level study.

In the whole plant study, the total numbers of pods at harvest were not significantly different in different treatments, suggesting that compensation through regulation of pod abortions may have occurred. However, in this experiment the pre-existing numbers of pods were not known and therefore firm conclusions on the occurrence of compensation through suppressed pod abortion cannot be reached based on the available data. Compensation for feeding at the R2–R3 stage could also occur by increasing the weight per seed of surviving seeds; however this did not occur as average weight per seed did not differ significantly among treatments.

In the R2–R3 stage, feeding activity of *Lygus* bugs caused bud, flower and pod abortions. This is similar to the other crops such as canola (Butts and Lamb 1990b), tomatoes (Davis *et al*. 1963), apples (Prokopy and Hubbell 1981), cabbage (Getzin 1983), alfalfa (Sorenson 1939), green beans (Khattat and Stewart 1975.) and Lima beans (Baker *et al*. 1946). In several other crops, *Lygus* bug injury at the bud, flower and early pod stage may increase yield due to compensation or reduce yield because of abortions. Exposure for 24 h to *L. hesperus* and *L. elisus* during the bloom period of alfalfa resulted in increased dropping of buds and flowers at increased infestation levels; compensation was not evident as the infestation resulted in fewer pods and seeds at harvest (Sorenson 1939). The effects of *Lygus* spp. on canola, another annual crop, have been well studied (Butts and Lamb 1990a, 1990b, 1991a, 1991b; Turnock *et al*. 1995; Wise and Lamb 1998). Compensation for bud and flower loss caused by *L. elisus* feeding on caged canola racemes occurs in some situations, resulting in no net reduction in the number of pods (Butts and Lamb 1990b). Khattat and Stewart (1975) studied damage to green beans by *L. lineolaris* feeding at the flower bud stage, the flower blossom stage and the pod initiation stage. Feeding for 3 or 5 days at the bud stage by two fifth instar nymphs or two adults reduced numbers of blossoms and pods and the total weight of pods. Feeding for 3 days by two fifth instar nymphs or two adults at blossom or pod set reduced average weight of seeds per pod and numbers and total weight of the pods. Like my results, those of Khattat and Stewart (1975) provide no evidence that *P. vulgaris* plants compensate for injuries to reproductive structures that occur at or before pod set.

At the R4–R5 stage, increased proportions of shrivelled pods and of shrivelled seeds were the major injuries in raceme level experiments. In the raceme level experiment with three insects there was an associated significant reduction in total whole seed weight in the nymph treatment.

Nymphs in three insect treatments produced more seed shrivelling than did adults and as a consequence seed weights were reduced. In navy beans, seed shrivelling results from injury to the vascular tissues supplying nutrients to the filling seed and this is caused by the necrosis of the vascular tissues in the placental and funiculus regions (Chapter 3 – Manuscript II of this thesis).

In legumes, seed shrivelling is caused not only by *Lygus* bug infestations but also by other environmental factors. For example, Sorenson (1936) reported seed shrivelling in caged young alfalfa pods where there were no insects. This was also true from my studies: seed shrivelling was evident in control treatments in experiments at all spatial scales. *Lygus* bug injury at the young pod stage of alfalfa results in increased seed shrivelling, and even 24 hours of exposure to *Lygus* bug infestation is sufficient to increase frequency of shrivelled seeds (Sorenson 1936). Percent of shrivelled seeds increases with increasing numbers of *Lygus* bugs (Sorenson 1936, 1939). Seed shrivelling in response to *Lygus* bug feeding also occurs in Lima beans, *P. lunatus*, early in seed development (Baker *et al.* 1946).

At the whole plant level, I found no significant amount of seed shrivelling or reduction in total whole seed weight in infested treatments at the R4–R5 stage. In my whole plant experiment, I used only adults in infested treatments and the outcome of the experiments were similar to that of raceme level experiment in which adults did not produce significant amount of seed shrivelling or reduction in seed yield. The infestation levels in the whole plant study were similar to, or higher than, those of the raceme level study (raceme level study: 0.7 insects per pod, whole plant study: 0.6 per pod for the 15 *Lygus* adult experiment and 1.0 per pod for the 30 *Lygus* adult experiment), so I would expect a similar intensity of injury leading to a similar levels of damage at the two scales. There was no evidence for the escape of *Lygus* bugs from the cages and no mortality of *Lygus* bugs in cages was observed. Possible reasons for the non-significant result at

the whole plant level are either the adults did not cause enough injury to produce the level of damage seen in the raceme level study or there might have been compensation in seed weight in uninjured racemes, through reallocation of photosynthates among fruiting structures (Mostafa 2007). However, in my whole plant experiment, there was a tendency for the higher average weight per whole seed in the controls than in the other treatments suggesting that there was no compensation from increased weight of surviving seeds.

My results in R4–R5 experiments provide evidence that adult *Lygus* bugs in whole plant cages behaved differently than on caged racemes. The numbers of shrivelled pods per insect in the whole plant experiment were less than in the raceme level study; similarly, the numbers of shrivelled seeds per insect were lower in whole plant cages. In cotton, the distribution of *L. hesperus* on reproductive and vegetative tissues and off the plant differs among young and old nymphs and adults, and corresponds to the pattern of plant injury (Cooper and Spurgeon 2013). Adults spend time off the plant (Cooper and Spurgeon 2013) and, as only adults were used in the whole plant experiment, the insects may have fed less on the reproductive structures that produce yield than when caged on racemes.

In the R6–R7 stage of navy beans, feeding by *L. lineolaris* did not cause reductions in seed weight, but quality loss due to seed blemishes, particularly seed pits, was evident in raceme level and whole plant experiments. The number of pitted seeds per insect was higher in the raceme level experiment than the whole plant experiments for both nymphs and adults. As with shrivelling of seeds at the R4–R5 stage, it seems that in the smaller scale experiments the insects inflict more per capita feeding injury on seeds than in the larger scale experiments. In the larger cages, insects have more choice of reproductive as well as vegetative tissues, and can also leave the plant.

Direct damage by seed pitting is reported in several types of beans. In Lima beans in California, 0–5.2% of seeds were pitted when 200 *Lygus* bugs were confined on clusters of older pods in cloth cages for 7 days (Baker *et al*. 1946) and a maximum of 52 punctures was seen on a single Lima bean seed (Baker *et al*. 1946). Bush beans and wax beans are also susceptible to seed pitting damage by *Lygus* bugs (Hagel 1978), as are great northern (Shull 1933) and red bean classes (Hagel 1978) of field beans. Seed pitting damage varies with the type of bean crop (Hagel 1978; Shull 1933), with thin walled bean varieties more prone to seed pitting damage (Shull 1933). Nevertheless, in my studies navy beans exhibited pitting, even though they are a hard walled bean type (P. Balasubramanian, Agriculture and Agri-Food Canada, personal communication). In Hagel's (1978) studies in field plots, field beans of the pinto bean market class showed relatively little pitting damage, and in my commercial field surveys, there was more pitting in navy beans than in pinto beans (Nagalingam and Holliday 2015).

Feeding behaviour of *Lygus* bugs is dependent on the instar of the nymphs (Cooper and Spurgeon 2011) and reproductive status of adults (Cooper and Spurgeon 2011, 2012). In my laboratory experiments, fifth instar nymphs generally caused more damage than did adults. It is known that the late instar nymphs are more injurious than other nymphal instars (Bariola 1969; Gutierrez *et al*. 1977; Zink and Rosenheim, 2005). In cotton, fifth instar nymphs of *L. hesperus* cause more squares to be shed than do third or fourth instars (Gutierrez *et al*. 1977). The injuriousness of first and second instars is negligible (Gutierrez *et al*. 1977; Zink and Rosenheim 2005). The stylets of the earlier instars are shorter than those of later nymph instars and adults so young nymphs are unable to penetrate and reach the internal tissues (Zink and Rosenheim, 2005). The quantity of food consumed per day by *L. disponsi* nymph instars in the laboratory increased with nymphal instar, with fifth instars consuming the most (8 mg/day) (Hori 1971b).

The food intake by adult *L. disponsi* just after emergence is similar to that of fifth instar nymphs and increases within the first 5 days of adult life to 13 mg/day, then tends to decrease until the 12th day when it levels off (Hori 1971b). Hori did not report the reproductive status of the adults. Adult *L. lineolaris* are pre-reproductive until 8 days after moulting from the nymph (Stewart and Gaylor 1994). Zink and Rosenheim (2005) found that fourth and fifth instar nymphs cause less damage than do adults, but in their study they did not differentiate the reproductive state of the adults. The adults used in my experiments were 1–2 days old and therefore were in the pre-reproductive stage. In cotton, pre-reproductive adults of *L. hesperus* cause more squares (flower buds) to be aborted than do reproductive adults, and fifth instar nymphs cause more square abortions than do pre-reproductive adults (Cooper and Spurgeon 2013). Pre-reproductive adults are more active than nymphs and are more likely to occur on vegetative plant parts or leave the plant, rather than remain on the squares (Cooper and Spurgeon 2013). Based on my laboratory studies, the *L. lineolaris* fifth instar nymphs are more injurious than pre-reproductive adults as was the case for *L. hesperus* (Cooper and Spurgeon 2013). The same trend was seen in which two flowering buckwheat plants were caged with either five late instar *Lygus* nymphs or five *L. lineolaris* adults; plants exposed to nymphs had lower seed yields and more injuries than uninfected controls, but plants exposed to adults did not differ from controls (Mostafa 2007).

Field cage experiments were intended to mimic the commercial production system in which control decisions would normally occur. Ideally, populations of *Lygus* bugs in field plots would have been manipulated using insecticides as was done by Mostafa (2007) in alfalfa and buckwheat. Unfortunately, in the years of study, there were insufficient insects for such manipulations. In my study, insects were introduced into the field cage at a specific plant growth stage, and the plants and insects then remained undisturbed until harvest. Because of the cryptic

and mobile nature of *Lygus* bugs it was not possible to manipulate numbers in cages once infestations were initiated, an approach successfully used for Colorado potato beetles on potato (Senanayake and Holliday 1990). Ideally, the introduced *Lygus* bugs would undergo population processes in the cage in parallel to those in the field; however, this could not be verified. If the ideal did occur, then the system would be similar to that of a field in which a control decision was made at the time of *Lygus* bug introduction, and affected the population for the rest of the season. In all my field cage experiments the *Lygus* bug numbers used per pod were less than that of the laboratory studies, but if the population persisted until harvest, the insect days per pod would be higher in field cages than in the laboratory cages.

In field cage experiments, treatment effects seldom occurred, or did not follow the patterns expected from those of the laboratory experiments or differed between experiments conducted in different years. Part of this outcome may be attributable to the greater intrinsic variability of the field cage system than the laboratory cage studies. Plant condition can vary among years and spatially within field plots. Also, the insects used in field experiments were collected from the field and their age and reproductive state were not known. However, the field cage methodology appeared to work well in the first experiment, which was in 2009. In that year, there was a linear yield loss response to infestation level at the R2–R3 stage. A large scale experiment with several growth stages was planned for 2010, but only one portion of the experiment could be carried out because deer destroyed parts of the plots and some cages, and ultra-violet curing of cage fabric rendered other cages unusable. Thus, all the remaining field cage studies had to be performed in 2011, and did not generate the same quality of data as that from the 2009 experiment. As the problems in 2011 affected experiments with all growth stages, the possible reasons for their occurrence are discussed first, before detailed consideration of the

individual experiments. Many of these problems appear to result from a lack of persistence of the population from time of introduction to harvest in 2011 and possibly 2010. In these two years in R2–R3 and R4–R5 experiments, the numbers of pitted seeds per cage (Table 11), which provide evidence of persistence of the population at least until R6, were low relative to the numbers in 2009.

The lack of population persistence in 2010 and 2011 was not likely to have been due to insects escaping from the cages. Each year, field cages were carefully examined from time to time and there was no evidence that insects could escape from the cages. The same type of cages was used in each of the three years, so if escape was a problem, this would also have occurred in 2009.

The lack of persistence is most likely the result of mortality attributable to high temperatures. There is evidence that *L. lineolaris* suffers stress at temperatures higher than 27–30 °C: compared with lower temperatures, adult longevity is reduced at 30–32 °C (Ugine 2012). Lifetime fecundity and net reproductive rate at 27 °C is more than double that at 32 °C (Ugine 2012). Therefore it is likely that insects suffered mortality or reduced population growth if temperatures exceeded 30 °C. In the R2–R3 experiments, insects were in the cages from 14 July to 15 September in both 2009 and 2011. In this period in 2009, there were no days in which the maximum temperature was ≥30 °C in Winnipeg (Environment Canada 2015) whereas there were 15 days in 2011 with maxima ≥30 °C. The highest maximum temperature in the period was 27.9 °C in 2009 and 37.2 °C in 2011. For the R4–R5 experiment in 2011, the insects were in the cages from 11 August to 14 September and there were 12 days in this period in which the maximum temperature was ≥30 °C, with the extreme of 37.2 °C (Environment Canada 2015). In the R6–R7 stage the insects were in cages from the third week of August to the second week of September

in 2010 and 2011. In 2011, there were 6 days in which the temperature was ≥30 °C with the maximum of 37.2 °C. In 2010, there were 2 days of ≥30 °C and the maximum temperature was 31.3 °C. Microclimate in the cages would differ from air temperatures measured in a standard weather station. Maximum temperatures in cages with soybeans are slightly higher than exterior air temperatures and exceed exterior temperatures by >0.5 °C for most of the daylight hours (Perillo *et al.* 2015), so I expect that daytime temperatures in my cages would have been slightly higher than those reported by Environment Canada (2015).

It is noticeable that the total number of pods produced by the caged plants was much higher in 2011 than in 2009 in the R2–R3 experiments (Table 10) and were higher in 2010 than 2011 in the R6–R7 experiments (Table 16). The average numbers of pods per plant in control cages in 2009 was lower than in 2010 and 2011. This suggests that the growing conditions for the plants were poorer in 2009 than in the other two years. It may be that the weather conditions of 2009 were particularly favourable to the insects and unfavourable to the plants.

In the R2–R3 field cage study in 2009, *Lygus* adults caused a reduction in total whole seed yield and an associated reduction in the numbers of pods and of seeds. This pattern is similar to the laboratory studies where reduction in yield occurred in the nymph treatment. In contrast to the results of year 2009, in 2011 a yield increase was observed in the 90 adult treatment. It was intended that in the field cage experiment, there would be injury from the time of introduction until harvest as a result of a persistent *Lygus* bug population in the cages. If the population did persist, I expect that seed pitting would be detectable at harvest, resulting from *Lygus* bug feeding on seeds at R6 or later. Numbers of pitted seeds per cage in infested treatments were much higher in 2009 than 2011; there was a treatment with 30 adults per cage in both years, and the number of pits per cage in this treatment in 2009 was 5.4 times that in 2011

(Table 11). In both 2009 and 2011, the number of pitted seeds did not vary greatly among cages in the same treatment of the R2–R3 study. It seems that 2011 was not as favourable a year for the persistence of the population as 2009. Thus in 2011, the absence of insects late in the experiment may have allowed plants to compensate for injuries inflicted early in the study, a phenomenon that I conclude could have occurred in the whole plant level laboratory studies at this growth stage. In 2009, the persistent insect population would have inflicted ongoing injury from R2–R3 to harvest, allowing little opportunity for compensation.

The field cage experiment with introduction of insects at the R4–R5 stage was conducted only in 2011. In these experiments, the total weight of whole seeds was significantly lower than that of the control for both levels of the nymph treatments. These results are comparable to those of the raceme level study in which nymphs also reduced seed weight. The number of pitted seeds per cage in this experiment varied greatly among cages within the same treatment (Table 11). In most treatments with adults and nymphs, numbers of pitted seeds per cage were very low relative to the number of insects introduced. In the few treatments with means of >10 pitted seeds per cage, standard errors were large as a consequence of most cages having few pitted seeds with a few cages having very many. Thus, in most cages, population persistence to the R6 stage was poor. In only four cages were there a reasonably high number of pitted seeds (≥32); when data from these four cages were checked to see whether there were correlations between yield and pitted seed numbers or yield and shrivelled seed numbers, those correlations were not significant. In the adult treatments, the number of pitted seeds was highest with 30 insects, and lower with 60, 90 or 120 insects. Also, the lack of a significant difference between the total whole seed weights in the 60 and 120 nymph treatments in field cages is unexpected. These patterns may have arisen because of the extreme variability among cages, but it is possible that at higher

densities *Lygus* nymphs and adults may feed less because of interference between individuals or cannibalism. Cannibalism of *L. lineolaris* nymphs has been seen at high densities of nymphs (Khattat and Stewart 1977; Snodgrass and McWilliams 1992). Despite evidence that the populations in field cages seldom persisted to the R6 stage, plants did not show evidence of compensation for injury by nymphs feeding at the R4–R5 stage.

Compared to experiments at the other growth stages, the R6–R7 field cage experiment and laboratory experiments were more similar, as the period of exposure to insects was relatively short in both. In the laboratory, insects were on the plants for 5 days and there was a short insect-free period before harvest; in the field cages, insects were introduced two weeks before harvest in both experimental years, and remained in the cages until harvest time. Despite this greater similarity of laboratory and field studies, numbers of blemished seeds were significantly affected by treatments in both raceme and whole cage laboratory experiments but were not affected by treatments in the field cages. In the field cages, it is noticeable that in some of the infested treatments there were many more blemished seeds than in the controls, but standard errors were very high, indicating much variability within treatments, and a low analytical sensitivity. Potential reasons for these different results include differences in the insect population used in the field and laboratory studies. The pre-reproductive adults used in the laboratory studies are likely to be more injurious than reproductive adults (Cooper and Spurgeon 2011); in the R6–R7 field cage experiments insects were field collected and were a mixture of adults and nymphs of unspecified age. Adult *Lygus* bugs in August and September in southern Manitoba are ready to find over-wintering sites and undergo diapause (Otani 2000) and therefore they may not be producing as much damage as non-diapausing pre-reproductive adults. Also, the lower numbers of insects per pod in field cages than in the laboratory studies suggests that the numbers of

insects introduced in the field cage experiments may not have been sufficient to cause statistically significant effects on yield. Nevertheless, the numbers of blemished seeds/insect in the field cage study was comparable to the highest numbers in the whole plant study, although both were lower than in the raceme level study. Surveys in commercial fields (Nagalingam and Holliday 2015) revealed a low incidence of seed pitting, so the field cage results may be representative of field conditions

From my field cage experiments at R2–R3 stage, I found that when growing conditions are poor and *Lygus* adults at the densities of 2009 are not controlled, yield loss did occur. But if growing conditions are good, and *Lygus* bugs at the densities of 2011 do not persist naturally or if they are controlled there may be no yield loss and yield compensation may occur. At the R4–R5 stage, except for the raceme level experiment with one *Lygus* bug, nymphal infestations resulted in yield loss in raceme level and field cage experiments. At the R4–R5 stage, nymphs are the most prevalent stage in field bean fields (Nagalingam and Holliday 2015) and yield loss did occur in field cages at nymph densities of $\geq 60/m^2$.

Unlike in earlier growth stages where injury is indirect, at the R6–R7 stage *Lygus* bug directly and permanently injure seeds and therefore no compensation can happen. In my studies, 120 adult treatments in 2010 and all the infested treatments in 2011 caused quality reductions based on Canadian Grain Commission classifications. Although it was the original aim of the field cage study, no definite economic injury level could be determined from the results. More experiments are needed before definitive economic injury level determination. Ideally these would be to be done in the field by manipulating insect numbers with insecticides, but my data suggest that insect numbers in Manitoba are seldom adequate for this approach to work. Multiple

repetitions of the field cage studies with enhanced numbers of replicates could provide the necessary data for an economic injury level, but the logistics and costs could prove prohibitive.

Laboratory studies were intended to investigate the process of yield loss resulting from a narrowly defined window of feeding injury. Even though some studies have used laboratory studies to determine economic injury levels of *Lygus* bugs (e.g. Charlet 2003), these may not be reliably applied in the field if *Lygus* bug behaviour and plant conditions in the field differ from those in the laboratory environment. In general, laboratory studies are used to learn plant response to injury and at what growth stages should *Lygus* bugs be of greatest concern to producers. My laboratory studies were designed to study stage-specific responses, and the five day duration of exposure makes it impossible to use them to derive an economic injury level.

Table 8. Summary of experimental designs for examining the effect of *Lygus* bugs on navy beans in Manitoba

Experimental scale	Growth stage	Experimental design	Treatment (Number of replicates)
Raceme level	R2–R3	CRD*	Control (6), 1 adult(6), 1 nymph(6)
	R4–R5	CRD	Control (6), 1 adult (6), 1 nymph (6)
	R4–R5	CRD	Control (6), 3 adults (6), 3 nymphs (6)
	R6–R7	CRD	Control (6), 5 adults (6), 5 nymphs (6)
Whole plant level	R2–R3	CRD	Control (5), 15 adults (5), 30 adults (5), 30 nymphs (5)
	R4–R5	CRD	Control (3), 15 adults (4), 30 adults (5)
	R6–R7	CRD	Control (5), 15 nymphs (5), 15 adults (5), 30 nymphs (5), 30 adults (5)
Field cages	R2–R3(2009)	RCBD*	Control (6), 30 adults (6), 60 adults (6)
	R2–R3(2011)	RCBD	Control (6), 30 adults (6), 90 adults (6)
	R4–R5(2011)	IBD*	Control (9), 30 adults (8), 90 adults (6), 120 adults (4), 60 nymphs (3), 120 nymphs (3)
	R6–R7(2010)	RCBD	Control (4), 60 adults (4), 120 adults (4), 60 nymphs (4)
	R6–R7(2011)	RCBD	Control (4), 60 nymphs (4), 60 adults (4), 120 adults (4), 180 adults (4)

*CRD: Complete randomized design; RCBD: Randomized complete block design; IBD: Incomplete Block Design

Table 9. Total number of pods, proportion of shrivelled pods, proportion of shrivelled seeds and total weight of seeds at harvest from raceme level and whole plant experiments, when *Lygus* bug treatments occurred at the R2–R3 stage of navy beans. The duration of treatment was 5 days.

Growth stage	Treatments	Total number of pods at harvest (Mean ± SE)	Proportion of shrivelled pods (Mean ± SE)	Proportion of shrivelled seeds (Mean ± SE)	Total whole seed weight (g) (Mean ± SE)
Raceme level	Control	2.7 ± 0.5ab	0.00 ± 0.00	0.21 ± 0.03	1.7 ± 0.3ab
	1 Nymph	1.3 ± 0.6b	0.08 ± 0.08	0.22 ± 0.08	0.6 ± 0.3b
	1 Adult	3.3 ± 0.5a	0.07 ± 0.05	0.23 ± 0.04	2.0 ± 0.3a
	Statistics	$F = 3.8$	$F = 1.0$	$F = 0.05$	$F = 5.7$
		df = 2,14	df = 2,13	df = 2,13	df = 2,15
		$P = 0.048$[1]	$P = 0.40$	$P = 0.95$	$P = 0.015$[1]
Whole plant level	Control	23.4 ± 1.0	0.09 ± 0.02	0.10 ± 0.03	16.1 ± 1.1a
	15 Adults	25.2 ± 2.0	0.07 ± 0.03	0.07 ± 0.04	18.3 ± 1.5ab
	30 Adults	22.2 ± 0.9	0.05 ± 0.03	0.06 ± 0.02	15.4 ± 1.3a
	30 Nymphs	27.8 ± 2.1	0.09 ± 0.04	0.10 ± 0.02	22.9 ± 2.8b
	Statistics	$F = 2.3$	$F = 0.6$	$F = 0.7$	$F = 6.0$
		df = 3,16	df = 3,16	df = 3,16	df = 3,12
		$P = 0.1$	$P = 0.61$[2]	$P = 0.57$[2]	$P = 0.010$

Within each analysis with a significant *F* statistic, treatment means with the same letters were not significantly different $P \leqslant 0.05$ (Tukey's test).

[1]Initial numbers of reproductive structures were considered as covariate in the analysis and the means and standard errors reported here are adjusted for covariate effect; [2] Analysis was performed on arcsine transformed values and the means and standard errors are untransformed unadjusted values.

Table 10. Number of plant per cage, number of pods per plant, numbers of total pods, proportion of shrivelled pods, proportions of shrivelled seeds, and total weight of seeds from field cages experiments at harvest, when *Lygus* bug treatments began at the R2–R3 stage of navy beans and continued until harvest.

Experiment type	Treatments	Number of plants per cage (Mean ± SE)	Number of pods per plant	Total number of pods at harvest (Mean ± SE)	Proportion of shrivelled pods (Mean ± SE)	Proportion of shrivelled seeds (Mean ± SE)	Proportion of pitted whole seeds (Mean ± SE)	Total whole seed weight (g) (Mean ± SE)
Field cage study 2009	Control	15.3 ± 0.2	9.90 ± 0.60	152.8 ± 5.3a	0.05 ± 0.01a	0.03 ± 0.01	0.00 ± 0.00a	95.9 ± 4.6a
	30 Adults	15.2 ± 0.4	9.63 ± 0.62	151.0 ± 5.4 ab	0.06 ± 0.01ab	0.05 ± 0.01	0.04 ± 0.01b	89.8 ± 4.7ab
	60 Adults	15.6 ± 0.4	8.74 ± 0.70	130.6 ± 5.5b	0.08 ± 0.01b	0.05 ± 0.01	0.06 ± 0.01c	65.0 ± 4.8b
	Statistics	$F = 0.7$	$F = 2.5$	$F = 4.8$	$F = 3.6$	$F = 1.5$	$F = 32.6$	$F = 11.7$
		df = 2,10	df = 2,10	df = 2,9	df = 2,9	df = 2,9	df = 2,9	df = 2,9
		$P = 0.5$	$P = 0.132$	$P = 0.038$[1]	$P = 0.072$[1]	$P = 0.27$[1]	$P = 0.000$[1]	$P = 0.003$[1]
Field cage study 2011	Control	10.3 ± 0.3	38.8 ± 4.0	401.5 ± 18.6a	0.13± 0.01	0.14 ± 0.02	0.000 ± 0.000	257.2 ± 8.3a
	30 Adults	11.5 ± 0.3	35.7 ± 2.3	406.9 ± 17.8a	0.10 ± 0.01	0.13 ± 0.00	0.002 ± 0.001	274.3 ± 7.9a
	90 Adults	11.2 ± 0.4	42.8 ± 2.0	474.5 ± 18.6b	0.13 ± 0.01	0.16 ± 0.01	0.003 ± 0.001	312.0 ± 7.5 b
	Statistics	$F = 2.1$	$F = 2.9$	$F = 5.8$	$F = 3.0$	$F = 1.0$	$F = 3.7$	$F = 13.1$
		df = 2,10	df = 2,10	df = 2,9	df = 2,9	df = 2,9	df = 2,9	df = 2,9
		$P = 0.2$	$P = 0.1$	$P = 0.024$[1]	$P = 0.10$[1]	$P = 0.40$[1]	$P = 0.07$[12]	$P = 0.002$[1]

Within each analysis with a significant *F* statistic, treatment means with the same letters were not significantly different $P \leqslant 0.05$ (Tukey's test). [1]Total numbers of plants were considered as covariate in the analysis; [2] Analysis was performed on arcsine transformed values and the means and standard errors are untransformed unadjusted values.

Table 11. Numbers of pitted seeds in field cage experiments starting at the R2–R3 and R4–R5 stages of navy beans

Crop stage	Stage of the plant	Year of the experiment	Number of pitted seeds
R2–R3	2009	Control	0.0 ± 0.0a
		30 Adults	18.8 ± 2.8b
		60 Adults	25.0 ± 3.2b
		Statistics	$F = 25.6$
			df = 2,9
			$P < 0.001$
R2–R3	2011	Control	0.0 ± 0.0
		30 Adults	3.5 ± 1.1
		90 Adults	6.8 ± 2.3
		Statistics	$F = 3.7$
			df = 2,9
			$P = 0.07$
R4–R5	2011	Control	0.0 ± 0.0
		30 Adults	22.6 ± 13.0
		60 Adults	1.3 ± 0.7
		90 Adults	3.0 ± 1.3
		120 Adults	11.0 ± 7.0
		60 Nymphs	15.0 ± 5.0
		120 Nymphs	24.3 ± 21.8
		Statistics	$F = 1.3$
			df = 6,23
			$P = 0.30$[1]

Within each analysis with a significant *F* statistic, treatment means with the same letters were not significantly different $P \leqslant 0.05$ (Tukey's test). [1]Total numbers of plants were considered as covariate in the analysis

Table 12. Weighted regression analyses showing overall relationships between the whole seed weight of navy beans (g) and the number of insects per plant in R2–R3, R4–R5 and R6–R7 field cage experiments and the relationship between the number of blemished whole seeds and the number of insect per plant in the field cages in the R6–R7 experiment.

Experiment type (Insect stage)	Whole seed weight (g)			Number of blemished whole seeds	
	Statistics from weighted regression model		Parameter estimates	Statistics from weighted regression model	Parameter estimates
R2–R3 (Adults)	Among blocks	F = 40.1, df = 11,13, *P* < 0.001	Intercept = 171.37		
	Among insect levels	F = 4.6, df = 11, 13, *P* = 0.006	Slope = 3.99/insect/plant		
	Regression	F = 4.5, df = 1,10, *P* = 0.06			
R4–R5 (Adults)	Among blocks	F = 3.6, df = 4,14, *P* = 0.03	Intercept = 239.11		
	Among insect levels	F = 2.7, df = 10,14, *P* = 0.04	Slope = -1.58/insect/plant		
	Regression	F = 0.4, df = 1,9, *P* = 0.52			
R4–R5 (Nymphs)	Among blocks	F = 4.1, df = 4,5 *P* = 0.08	Intercept = 252.17		
	Among insect levels	F = 8.9, df = 5,5, *P* = 0.02	Slope =-7.00 /insect/plant		
	Regression	F = 4.6, df = 1,4, *P* = 0.10			
R6–R7 (Adults)	Among blocks	F = 2.0, df = 8,3 , *P* = 0.30	Intercept = 260.65	F = 0.3, df = 9,3 , P = 0.94	Intercept = 24.6
	Among insect levels	F = 1.7 , df = 15,3, *P* = 0.39	Slope = 3.95 /insect/plant	F = 0.6 , df = 14,3, P = 0.77	Slope = 6.75 /insect/plant
	Regression	F = 2.4, df = 1, 14 , *P* = 0.15		F = 7.2 , df = 1,13 , P = 0.019	
R6–R7 (Nymphs)	Among blocks	-[1]	Intercept = 240.48	-[1]	Intercept = 29.5
	Among insect levels	-[1]	Slope = 9.67 /insect/plant	-[1]	Slope = 4.48/insect/plant
	Regression	F = 1.7 , df = 1,6, P = 0.25		F = 3.2, df = 1,6, P = 0.12	

[1] F statistic was not calculated as data were sparse.

Table 13. Numbers of total pods, proportion of shrivelled pods, proportion of shrivelled seeds, and total weight of seeds from raceme level and whole plant experiments at harvest, when *Lygus* bug treatments occurred at the R4–R5 stage of navy beans. The duration of treatment was 5 days.

Experiment type	Treatment	Total number of pods (Mean ± SE)	Proportion of shrivelled pods (Mean ± SE)	Proportion of shrivelled seeds (Mean ± SE)	Total whole seed weight (g) (Mean ± SE)
Raceme level	Control	5.3 ± 0.2	0.28 ± 0.07	N/A	3.0 ± 0.6
	1 Nymph	5.8 ± 0.5	0.47 ± 0.17	N/A	2.2 ± 0.7
	1 Adult	5.7 ± 0.2	0.54 ± 0.12	N/A	2.0 ± 0.6
	Statistics	$F = 0.6$	$F = 1.1$		$F = 0.7$
		df = 2,15	df =2,15		df = 2,15
		$P = 0.6$	$P = 0.4$		$P = 0.5$
Raceme level	Control	4.5 ± 0.4	0.39 ± 0.40a	0.41 ± 0.11a	2.0 ± 0.6a
	3 Nymphs	4.5 ± 0.4	0.90 ± 0.05b	0.95 ± 0.03b	0.2 ± 0.1b
	3 Adults	4.5 ± 0.4	0.75 ± 0.09b	0.72 ± 0.10ab	0.9 ± 0.4ab
	Statistics	$F = 0.0$	$F = 9.5$	$F = 9.2$	$F = 4.8$
		df = 2,15	df = 2,15	df = 2,15	df = 2,15
		$P = 1.0$	$P = 0.002$	$P = 0.003$	$P = 0.025$
Whole plant level	Control	38.0 ± 3.2	0.26 ± 0.05	0.29 ± 0.07	17.0 ± 2.5
	15 Adults	38.0 ± 3.2	0.20 ± 0.06	0.24 ± 0.05	14.3 ± 2.5
	30 Adults	44.8 ± 7.5	0.21 ± 0.04	0.31 ± 0.05	14.1 ± 0.6
	Statistics	$F = 0.9$	$F = 0.3$	$F = 0.5$	$F = 0.7$
		df = 2,9	df = 2,9	df = 2,9	df = 2,9
		$P = 0.4$	$P = 0.30$	$P = 0.64$	$P = 0.5$

Within each analysis with a significant *F* statistic, treatment means with the same letters were not significantly different $P \leqslant 0.05$ (Tukey's test).

Table 14. Number of navy bean plants per cage, number of pods per plant, total number of pods per cage, proportion of shrivelled pods, and total weight of whole seeds at harvest from the 2011 field cage experiment, when *Lygus* bug treatments began at the R4–R5 stage and continued until harvest. The proportions of shrivelled and blemished seeds were not determined because total number of seeds was not known in this experiment

Treatment	Number of plants per cage (Mean ± SE)	Number of pods per plant (Mean ± SE)	Total number of pods (Mean ± SE)	Proportion of shrivelled pods (Mean ± SE)	Total whole seed weight (g) (Mean ± SE)
Control	11.6 ± 0.7	34.6 ± 3.6	394.2 ± 19.2	0.12 ± 0.01	261.6 ± 13.0a
30 Adults	11.7 ± 1.0	30.0 ± 2.8	329.5 ± 20.0	0.12 ± 0.01	201.6 ± 13.5ab
60 Adults	9.0 ± 1.0	42.5 ± 3.9	355.0 ± 45.0	0.10 ± 0.02	223.8 ± 30.5ab
90 Adults	12.2 ± 1.0	27.7 ± 1.0	342.4 ± 23.2	0.10 ± 0.02	233.6 ± 15.7ab
120 Adults	10.7 ± 0.2	33.6 ± 1.4	354.3 ± 28.0	0.14 ± 0.01	232.8 ± 19.0ab
60 Nymphs	11.7 ± 0.3	25.8 ± 0.8	306.5 ± 33.0	0.16 ± 0.01	177.7 ± 22.3b
120 Nymphs	9.7 ± 1.2	31.6 ± 5.7	303.2 ± 34.0	0.17 ± 0.01	172.0 ± 23.0b
Statistics	$F = 4.7$	$F = 1.6$	$F = 1.8$	$F = 2.4$	$F = 3.7$
	df = 6,24	df = 6,23	df = 6,23	df = 6,23	df = 6,23
	$P = 0.4$	$P = 0.2$	$P = 0.1$[1]	$P = 0.06$[1]	$P = 0.01$[1]

Within each analysis with a significant *F* statistic, treatment means with the same letters were not significantly different $P \leqslant 0.05$ (Tukey's test).

[1]Plants per cage was considered as covariate in the analysis

Table 15. Total numbers of pods, proportion of shrivelled pods, proportion of shrivelled seeds, proportion of whole seeds with pits, and total weight of whole seeds at harvest from raceme level and whole plant experiments, when *Lygus* bug treatments occurred at the R6–R7 stage of navy beans. The duration of treatment was 5 days.

Experiment type	Treatments	Total number of pods (Mean ± SE)	Proportion of shrivelled pods (Mean ± SE)	Proportion of shrivelled seeds (Mean ± SE)	Proportion of pitted seeds (Mean ± SE)	Total whole seed weight (g) (Mean ± SE)
Raceme level	Control	4.2 ± 0.3	0.00 ± 0.00	0.08 ± 0.03a	0.00 ± 0.00a	2.8 ± 0.3
	5 Nymphs	4.3 ± 0.2	0.20 ± 0.12	0.42 ± 0.11b	0.40 ± 0.10b	1.7 ± 0.3
	5 Adults	4.0 ± 0.4	0.17 ± 0.17	0.26 ± 0.08 ab	0.25 ± 0.12ab	2.0 ± 0.3
	Statistics	$F = 0.3$	$F = 0.8$	$F = 4.7$	$F = 4.6$	$F = 3.2$
		df = 2,15	df = 2,15	df = 2,15	df = 2,15	df = 2,15
		$P = 0.7$	$P = 0.46$	$P = 0.03$	$P = 0.027$[1]	$P = 0.069$
Whole plant level	Control	27.0 ± 5.8	0.33 ± 0.04	0.42 ± 0.03	0.00 ± 0.00a	9.7 ± 1.0
	15 Nymphs	35.4 ± 4.8	0.26 ± 0.03	0.40 ± 0.04	0.15 ± 0.05ab	11.3 ± 1.6
	15 Adults	24.8 ± 4.4	0.26 ± 0.04	0.39 ± 0.03	0.16 ± 0.05ab	10.1 ± 1.7
	30 Nymphs	38.0 ± 8.9	0.31 ± 0.05	0.44 ± 0.04	0.28 ± 0.06b	12.1 ± 2.5
	30 Adults	37.8 ± 3.3	0.24 ± 0.05	0.36 ± 0.04	0.14 ± 0.04ab	12.3 ± 1.1
	Statistics	$F = 1.2$	$F = 0.7$	$F = 0.7$	$F = 4.5$	$F = 0.5$
		df = 4,20	df = 4,20	df = 4,20	df = 4,20	df = 4,20
		$P = 0.4$	$P = 0.59$	$P = 0.59$	$P < 0.01$	$P = 0.7$

Within each analysis with a significant *F* statistic, treatment means with the same letters were not significantly different $P \leqslant 0.05$ (Tukey's test).

[1] Analysis was performed on arcsine transformed values and the means and standard errors are untransformed unadjusted values.

Table 16. Numbers of total pods, proportion of shrivelled pods, proportion of shrivelled seeds, proportion of whole seeds with blemishes, and total weight of seeds in field cage experiments with navy beans in which treatments began at the R6–R7 stage and continued until harvest.

Experiment type	Treatments	Number of plants per cage (Mean ± SE)	Number of pods per plant (Mean ± SE)	Total number of pods (Mean ± SE)	Proportion of shrivelled pods (Mean ± SE)	Proportion of shrivelled seeds (Mean ± SE)	Proportion of whole seeds with blemishes (Mean ± SE)	Total whole seed weight (g) (Mean ± SE)
Field cage study 2010	Control	21.6 ± 0.7	21.7 ± 1.3	465.4 ± 33.2	N/A	0.25 ± 0.01	0.02 ± 0.00	283.1 ± 32.4
	60 Adults	24.3 ± 1.0	20.2 ± 0.8	490.3 ± 31.1	N/A	0.28 ± 0.01	0.03 ± 0.01	310.3 ± 30.4
	60 Nymphs	24.1 ± 2.4	19.7 ± 1.9	482.6 ± 34.7	N/A	0.28 ± 0.01	0.03 ± 0.01	277.6 ± 33.9
	120 Adults	22.5 ± 1.3	21.5 ± 1.9	472.5 ± 34.5	N/A	0.27 ± 0.01	0.03 ± 0.00	284.7 ± 33.6
	Statistics	$F = 1.8$ df = 3,7 $P = 0.2$	$F = 0.8$ df = 3,7 $P = 0.5$	$F = 0.1$ df = 3,6 $P = 1.0$[1]	N/A	$F = 3.2$ df = 3,6 $P = 0.1$[1]	$F = 1.7$ df = 3,6 $P = 0.3$[1]	$F = 0.2$ df = 3,6 $P = 0.9$[1]
Field cage study 2011	Control	12.0 ± 0.9	28.5 ± 1.5	339.8 ± 30.4	0.11 ± 0.01	0.25 ± 0.01	0.02 ± 0.00	189.0 ± 28.6
	60 Adults	13.0 ± 0.9	30.1 ± 0.9	392.4 ± 28.8	0.10 ± 0.01	0.22 ± 0.01	0.06 ± 0.02	266.8 ± 27.1
	60 Nymphs	13.0 ± 1.5	34.8 ± 3.0	429.5 ± 34.8	0.11 ± 0.01	0.32 ± 0.03	0.07 ± 0.04	274.6 ± 32.7
	120 Adults	13.5 ± 1.0	30.0 ± 5.5	388.5 ± 29.4	0.08 ± 0.02	0.24 ± 0.01	0.05 ± 0.02	261.3 ± 27.6
	180 Adults	13.3 ± 0.7	30.2 ± 0.3	388.6 ± 34.6	0.13 ± 0.02	0.30 ± 0.06	0.40 ± 0.29	265.1 ± 32.6
	Statistics	$F = 0.4$ df = 4,10 $P = 0.8$	$F = 0.4$ df = 4,10 $P = 0.8$	$F = 1.0$ df = 4,9 $P = 0.5$[1]	$F = 1.2$ df = 4,9 $P = 0.39$	$F = 2.4$ df = 4, 9 $P = 0.13$[1]	$F = 1.7$ df = 4,9 $P = 0.24$[12]	$F = 1.4$ df = 4,9 $P = 0.3$[1]

Within each analysis with a significant F statistic, treatment means with the same letters were not significantly different $P \leqslant 0.05$ (Tukey's test). [1] Number of plants were considered as covariate in the analysis; [2] Analysis was performed on arcsine transformed values and the means and standard errors are untransformed unadjusted values.

Fig. 6. Net cage used to enclose navy bean raceme and a trifoliate leaf for raceme level experiments for studying the effect of *Lygus* bug feeding on yield quantity and quality

Fig. 7. Net cages used to enclose navy bean plants for whole plant experiments for studying the effect of *Lygus* bug feeding on yield quantity and quality

Fig. 8. Cages in a plot of navy bean plants for field cage experiments to assess the effect of *Lygus* bug feeding on yield quantity and quality

CHAPTER 4: General Discussion

General Discussion

The major objectives of this dissertation were to establish the seasonal patterns and species composition of mirid bugs in field beans and soybeans in southern Manitoba, to document the types of injury caused by nymphs and adults of *Lygus lineolaris* (Palisot de Beauvois) on different reproductive growth stages of navy beans and to examine in controlled conditions and in field conditions the effect of feeding of *Lygus* bugs on navy bean yield.

From field surveys reported in Chapter 3 – Manuscript I, I determined that the R2–R3 stage of field beans occurs during mid-July to early-August in navy beans and the R6–R7 stage occurs during late August to early September with R4–R5 in between. In Manitoba, numbers of adult *Lygus* bugs begin to rise in field beans in July, which corresponds to the beginning of the R1–R3 stage of the navy beans. Nymphs were collected from the end of the July until September. Peak numbers of adults and late instar nymphs occurred from late August to early September, which corresponds to the R6–R7 stage. Based on the seasonal pattern of occurrence the potential stages of *Lygus* bug to cause injury to the navy beans are the adults in the R2–R3 stage; nymphs at the R4–R5 stage and adults and late instar nymphs at R6–R7.

Chapter 3 – Manuscript II of this thesis describes *L. lineolaris* injury to the three growth stages of navy beans and these could be useful for producers and processors to identify symptoms of *Lygus* bug feeding in field conditions. I also characterized the injury at the R4–R5 and R6–R7 stages with scanning electron microscopy but unfortunately the results did not add new information to that from light microscopic studies.

Chapter 3 – Manuscript III of the thesis describes the implications for yield quantity and quality of the injury by *L. lineolaris* feeding at the three growth stages in experiments at three

spatial scales. At the R2–R3 stage, seed weight in raceme level experiments was lowered in treatments with *Lygus* nymphs. At the whole plant level, the 30 nymph treatment increased seed yield. In field cages, *Lygus* bugs introduced at the R2–R3 stage sometimes reduced yield quantity, but sometimes plants compensated for the injury. In 2009, a yield loss of 0.5 g/m^2 per *Lygus* bug adult occurred in the field cage study but no loss of yield occurred in 2011. At the R4–R5 stage, shrivelled seeds and pods occurred as a result of the injury. Three nymphs caused significant loss of total whole seed weight in the raceme level experiment. In field cage experiments, treatments with ≥60 nymphs/cage significantly reduced yield relative to controls. No loss of yield was evident in adult treatments at any spatial scale. At the R6–R7 stage, direct feeding by *Lygus* bugs resulted in seed pitting. In the raceme and whole plant studies, significantly higher numbers of pitted seeds were recorded in cages with *Lygus* bugs but this was not so in field cages. Seed pitting lowered the individual seed weight but total weight at harvest was not reduced at any spatial scale. Reduced seed quality is the major effect at this stage. In all these cage studies, fifth instar nymphs tended to be more injurious than pre-reproductive adults.

My study reveals that in the R2–R3 stage field cage experiment in 2011, plant compensation may have occurred and resulted in increased total seed weight at harvest. A similar phenomenon occurred in the whole plant experiment in the laboratory. In both these treatments feeding did not persist from R2–R3 until harvest (Chapter 3 – Manuscript III). Relative to plants that do not compensate for insect injury, compensation may enhance fitness of the plants or increase yield of the plant (Trumble *et al*. 1993). In the agricultural context, increases in yield are considered to be more important than increased fitness (Southwood and Norton 1973).

The physiological mechanisms of compensation in plants are complex and vary greatly. Compensation ability also varies with type of crops, time of the attack, pest pressure, nutrient

availability and the length of time remaining in the growing season (Maschinski and Whithan 1989). Plants can either compensate fully or partially for injuries or stresses. Some other plants compensate by breaking apical dominance of shoots, thereby allowing induction of branching. This is a common phenomenon in plant species with strong apical dominance (Maschinski and Whithan 1989; Doak 1991; Huhta *et al*. 2000). Other plants compensate for injury by adjusting their resource allocation. For example, in seedlings of the tropical tree, *Gustavia superba* (H.B.K.) Berg (Lecythidaceae), the resources stored in cotyledon tissues are allocated immediately in injured plants while in uninjured plants it happens over a longer period of time (Dalling and Harms 1999). Partial compensation of lost yield by water stress was observed in spring wheat (*Triticum aestivum* L.) by remobilization of dry matter from the vegetative nodes (Ma *et al*. 2013). In wild sunflowers (*Helianthus annuus* L. (Asteraceae)), simulated damage of the head-clipping weevil *Haplorhynchites aeneus* Boheman (Curculionidae) resulted in production of additional inflorescences in the higher level branches, more seed being filled in the remaining heads than in heads on uninjured plants, and increased seed size in later-developing seed heads (Pilson and Decker 2002). Among legumes, compensation for injury to reproductive structures by resource reallocation has been observed in a number of species. In cowpea, *Vicia unguiculata* (L.) Walp., more flower buds open in order to compensate for flower buds removed within 9 days of anthesis, and seed yield does not decline (Ojehomon 1970). In pigeonpea, *Cajanus cajan* (L.) Millsp., removal of flowers and young pods up to 5 weeks after anthesis does not affect yield as injured plants produce pods from later-forming flowers (Sheldrake *et al.* 1979). In French beans, which like field beans are *P. vulgaris*, removal of opened flowers results in compensation by retention (rather than abortion) of pods from flowers that open later either in the same raceme or in later-developing racemes (Binnie and Clifford 1981).

Reproductive organs in beans are compound racemes, borne in the axil of a trifoliate leaf. Each raceme consists of five nodes in a peduncle (Sage and Webster 1987). At the axil of the trifoliate leaf and at each nodal position there are initially three buds, so that in a raceme with five racemic nodes there is a potential for 18 buds to be developed (Sage and Webster 1987). There is no evidence that the racemes will develop new buds beyond these potential numbers. Flowering in a bean plant occurs acropetally with the buds in the basal position flowering first (Sage and Webster 1987). Not all the formed buds in the raceme develop into pods; instead many naturally abort even in the absence of stress (Sage and Webster 1987). The reasons for abortions may be limitation of assimilates and nutrients (Binnie and Clifford 1980).

In navy beans, numbers of branches are limited and therefore the plant has little ability to compensate by producing more shoots (Ontario Ministry of Agriculture, Food and Rural Affairs 2009b). As described by Sage and Webster (1987), there is little chance for a navy bean raceme to produce more buds than the potential number of buds described above. For compensation to occur in navy beans, the plants can produce flowers in the buds which would otherwise abort in an injured raceme, suppress abortions in uninjured racemes and so increase the number of pods, or else increase the weight per seed in a pod. Binnie and Clifford (1981) report the first two of these mechanisms in French beans. In my studies, I demonstrated that compensation through increasing weight per seed did not happen in the whole plant study or in the field cage experiments. So the possible mechanisms for a navy bean plant to compensate are either to retain flowers in the injured raceme that would otherwise abort, or retain them in uninjured racemes that form later in the season.

Further studies are needed to elucidate the mechanism of compensation in navy beans. The mechanism could be evaluated in a laboratory study by caging different numbers of *Lygus* bugs on early-developing single racemes of whole navy bean plants for <5 days. Data would be collected at harvest from these racemes, the later-developing racemes on the same plant, and from all racemes on untreated control plants. Compensatory mechanisms can be determined by comparing the number and weight of seeds in each pod, and the number and position of pods, for injured and uninjured racemes on the treated plants, as well as comparing these data with those for untreated control plants. Overall compensatory ability can be assessed by comparing total seed yield of treated and untreated plants.

When *Lygus* bugs feed on the R4–R5 stage of navy beans, seed shrivelling occurs due to injury in the funiculus and placental area. Chapter 3 – Manuscript II of this thesis quantifies the injury occurring at the funiculus and placental area of the pods by counting the feeding scars and punctures on the pods and relating the injury to the position in the pod. As part of the studies reported in Chapter 3 – Manuscript II, I looked for evidence that weight of individual seeds was affected by funiculus injury at other seed locations within the same pod, and tried to link this to the architecture of vascular supply in the pod. These data are not presented in the thesis, as I concluded that they were inadequate in several ways. Among the difficulties were that, in the absence of insect feeding, weight of seeds varied according to seed position within pods, and the within-pod pattern differed according to pod position on the raceme. In navy beans, mean seed weights of the middle seeds are higher than those of end seeds in pods (Harris 1915). Seed weight patterns vary based on the number of seeds in the pod (Harris 1915) and the size and cultivar of the plant (Nakamura 1988). Consequently, seed weight patterns need to be defined in plants with no insect injury in order to understand how insect feeding on the funiculus affects the

distribution of nutrients to seeds at different positions in the pod. In my studies, I weighed seed at the end of the five day period of injury, which made the detection of effects more difficult, as seed filling was at an early stage and the effects of injury had had little time to accumulate. It would be informative to repeat this study with more replicates, better matching of control and treated plants, and control of pod position. The data collection should be extended by weighing seeds immediately after insect exposure and in a separate set of plants, weighing seeds at harvest time. It would be necessary to record the position of funiculus injuries within pods shortly after the exposure period to insects as funiculus feeding scars were visible after the 5 days of exposure of *Lygus* bugs (Chapter 3 – Manuscript II of this thesis) but are not visible at harvest time.

At the R6–R7 stage, seed pitting is the major injury, and leads to quality loss of field beans (Chapter 3 – Manuscript II and Chapter 3 – III of the thesis). The susceptibility of the bean market classes for seed pitting varies. My results demonstrate that navy beans are susceptible to seed pitting damage, and my surveys confirmed that pitting of navy bean seeds occurs in the field in Manitoba. My surveys and Hagel (1978) suggest that pinto beans are less susceptible to seed pitting than other bean classes. Further studies are needed to compare seed pitting damage in the different market classes of field beans as well as in the different varieties in each market class that are relevant to Manitoba. The level of resistance or tolerance and the mechanisms involved could be studied in field beans grown in commercial fields in Manitoba. Weekly surveys of *Lygus* bug numbers and pitting injury in commercial fields should be started at the R4–R5 stage as some seed pitting injury occurs at this stage (Chapter 3 – Manuscript III); surveys should be continued up to harvest time. Sweep net sampling for *Lygus* bugs and bean pod samples can be taken at any place in fields as Chapter 3 – Manuscript I showed that distribution of *Lygus* bugs is relatively even throughout fields. Weekly samples of bean plants

would be harvested from the same location as the insect samples. Plant samples would be brought to the laboratory and the number of pitted seeds and pits per seed in these pods would be recorded; in addition the thickness of the pods would be measured at the centre of the pod as described by Shull (1933), and the seed coat thickness and toughness would be measured. The toughness of the seed coat can be measured using a penetrometer as described by Martin *et al*. (2004). Relationships between the number of *Lygus* bugs present in the field, the numbers of pitted seeds, and the pod and seed coat thickness and toughness investigated to establish the determinants of seed pitting damage.

The best method to derive an economic injury level is to manipulate pest numbers by insecticides as was done by Mostafa (2007) in alfalfa and buckwheat or by adding insects to the fields by collecting them from other fields. Initially, my study aimed to determine the economic threshold of *Lygus* bugs in navy beans in Manitoba, using field plots in which these techniques of manipulating numbers would be used. I hoped to use either insecticides or augmentation to maintain low, medium and high levels of *Lygus* bugs in plots and assess how these numbers affected quality and quantity of harvested beans. Unfortunately, in the years of study, there were insufficient insects in field bean plots for manipulation using insecticides, and insufficient numbers in other crops to collect for augmentation. Therefore plans for manipulations in plots were abandoned and instead I used field cage experiments to fulfill the objectives.

As discussed in Chapter 3 – Manuscript III, one promising field cage study in 2009 suggested that this was a suitable technique for developing economic injury levels, but results in the following two years were difficult to interpret, probably because conditions in the cages were unfavourable for *Lygus* bug survival. So I was unable to derive economic injury levels from my experimental studies. Instead, I used field survey data (Chapter 3 – Manuscript I) as a substitute

to establish, in Manitoban field conditions, numbers of mirids in each stage of the crop that do not produce economic loss (Chapter 3 – Manuscript I). These numbers are no doubt lower than the true economic injury levels but do provide producers with some information on which to base control decisions.

Even though I could not derive a formal economic threshold based on my experiments, I suggest a nominal threshold of 10 *Lygus* adults/m^2 at the R2–R3 stage of the crop. When the levels of insects reach above this level it is recommended to spray the crop. This threshold is based on my finding in the R2–R3 field cage experiment in 2009, when 1 adult/m^2 reduced yield by 0.6 g/m^2 = 6 kg/ha. Given a cost of an insecticide application (material and application cost) of $35/ha and a crop value of $0.59/kg, a yield loss of 58.92 kg/ha is equal in value to the cost of the control measure, and would occur when there were 58.92/6 = 9.8 adults/m^2. This threshold applies only when the conditions are not favourable for the plants and are favourable for the *Lygus* bugs as described in Chapter 3 – Manuscript III; in other circumstances, the threshold would be higher.

The numbers of *Lygus* bugs present in field beans were low during 2008–2010 and the commercial field surveys revealed that the mirid bugs did not affect the yield quantity and quality of field beans. When considering other crops during these years, in 2008 *Lygus* bugs were of concern in confection sunflowers and in canola, and in 2010 *Lygus* bugs were problematic in sunflowers and strawberries; spraying for *Lygus* bugs occurred in these crops in these years (Gavloski 2001–2010). In 2009, the numbers of *Lygus* bugs in other crops were low and no spraying for *Lygus* bug occurred (Gavloski 2001–2010). Based on this information, it may be that the population of *Lygus* bugs in field beans is low—and lower than in other crops—in most years in Manitoba. Low numbers in field beans may be attributable to low population

growth rates of *Lygus* bugs in field beans compared to those in other host plants such as green beans, canola and alfalfa (personal observations). A quantitative study to address the reproduction and survival of *Lygus* bugs in field beans and soybeans is necessary to understand their population growth on beans. There are several studies in the literature on the reproduction of different North American *Lygus* species but these are laboratory rearing studies of some or all of the period of development, and the insects were reared using detached plant parts or diets as food (Ridgway and Gyrisco 1960; Bariola 1969; Slaymaker and Tugwell 1982; Leferink 1992; Gerber 1995; Gerber 1997; Otani 2000; Ugine 2012). To determine the host suitability of a plant for *Lygus* bugs, it is important to conduct a study on living plants and preferably in controlled environmental chambers. The population growth rate of navy beans can be determined by caging *L. lineolaris* adults on a whole plant at the R2–R3 stage as this is a potential stage for egg-laying in beans (Baker *et al*. 1946). Laboratory-reared reproductive adults of known age can be introduced to the cages for a period of time and then removed from the plants. Counting the numbers of stages of *Lygus* bugs on a whole bean plant is difficult as eggs are laid inside plant tissues, nymphs are cryptic and the adults are mobile. In order to handle this situation, separate sets of experiments would be carried out, and at a selected time each set would be terminated and the numbers of insects assessed. Appropriate times for the terminations and assessments would be immediately after the removal of adults from cages to assess egg numbers, 7–10 days after removal of adults to assess numbers of early instar nymphs, 16–20 days after adult removal to assess late stage nymphs, and at about 24 days after adult removal when adults of the next generation are available. These times would be appropriate at 27 °C, which is an optimal temperature for the development of *L. lineolaris* (Ugine 2012); however, it may be necessary to adjust conditions to have the insects' stages developing on the same plant growth stages that they

would use in the field. Population growth on field beans should be compared with that on host plants thought to be favourable hosts for *Lygus* bugs, such as canola.

I conclude that economic loss due to plant bugs in field beans does not occur in most years in southern Manitoba. However, when numbers of *Lygus* bugs are extremely high in an area, field beans would probably also be damaged by them. Although all growth stages could be vulnerable in such a season, the late season (R6–R7) stage of field beans would be most at risk because most other crops have been harvested and the displaced *Lygus* bug population would move to beans. In that case beans could suffer quality loss. An example where most crops were attacked by *Lygus* bugs because of extreme numbers of *Lygus* bugs occurred in 1997 in Alberta and in Saskatchewan. In that year, more than 200,000 ha of crops including canola, mustard, alfalfa and peas were sprayed for *Lygus* bugs (Western Committee on Crop Pests 1997).

REFERENCES

Adams, M.W. 1967. Basis of yield component compensation in crop plants with special reference to the field bean, *Phaseolus vulgaris*. Crop Science **7**: 505–510.

Addicott, F.T. and Romney, V.E. 1950. Anatomical effects of *Lygus* injury to guayule. Botanical Gazette **112**: 133–134.

Agblor, A., Henderson, H.M., and Madrid, F.J. 1994. Characterization of alpha-amylase and polygalacturonase from *Lygus* spp. (Heteroptera: Miridae). Food Research International **27**: 321–326.

Agriculture and Agri-Food Canada. 2005. Crop profile for dry bean in Canada [online]. Available from http://publications.gc.ca/collections/collection_2009/agr/A118-10-4-2005E.pdf [accessed 15 October 2014].

Agriculture and Agri-Food Canada. 2012. Pulses and special crops- Canadian Industry. [online] Available from http://www.agr.gc.ca/eng/industry-markets-and-trade/statistics-and-market-information/by-product-sector/crops/pulses-and-special-crops-canadian-industry/?id=1174420265572 [accessed 30 July 2013].

Agusti, N. and Cohen, A.C. 2000. *Lygus hesperus* and *L. lineolaris* (Hemiptera: Miridae), phytophages, zoophages, or omnivores: evidence of feeding adaptations suggested by the salivary and midgut digestive enzymes. Journal of Entomological Science **35**: 176–185.

Allen, T.C. 1947. Suppression of insect damage by means of plant hormones. Journal of Economic Entomology **40:** 814–817.

Allen, W.W. and Gaede, S.E. 1963. The relationship of lygus bugs and thrips to fruit deformity in strawberries. Journal of Economic Entomology **56**: 823–825.

Arnott, D.A. and Bergis, I. 1967. Causal agents of silver top and other types of damage to grass seed crops. Canadian Entomologist **99**: 660–670.

Backus, E.A., Cline, A.R., Ellerseick, M.R., and Serrano, M.S. 2007. *Lygus hesperus* (Hemiptera: Miridae) feeding on cotton: new methods and parameters for analysis of

nonsequential electrical penetration graph data. Annals of the Entomological Society of America **100**: 296–310.

Baker, F.K., Snyder, W.C., and Holland A.H. 1946. *Lygus* bug injury of Lima bean in California. Phytopathology **36**: 493–503.

Bariola, L.A. 1969. The biology of the tarnished plant bug, *Lygus lineolaris* (Beauvois), and its nature of damage and control on cotton. Ph.D. thesis, Texas A&M University, College Station, TX, United States of America.

Binne, R.C. and Clifford, R.E. 1980. Effect of some defoliation and decapitation treatments on French beans. Annals of Botany **46**: 811–813.

Binnie, R.C. and Clifford, P.E. 1981. Flower and pod production in *Phaseolus vulgaris*. Journal of Agricultural Science (Cambridge) **97:** 397–402.

Binnie, R.C. and Clifford, P.E. 1999. Sink characteristics of reproductive organs of dwarf bean in relation to likelihood of abscission. Crop Science **39:** 1077–1082.

Birley, M. 1977. The estimation of insect density and instar survivorship from census data. Journal of Animal Ecology **46**: 497–510.

Bishop, Y.M.M., Feinberg, S.E., and Holland, P.W. 2007. Discrete multivariate analysis: Theory and practice. MIT Press, Cambridge, MA, United States of America.

Boivin, G. and Stewart, R.K. 1982. Identification and evaluation of damage to Mcintosh apples by phytophagous mirids (Hemiptera, Miridae) in Southwestern Quebec. Canadian Entomologist **114**: 1037–1045.

Bolton, J.L. and Peck, O. 1946. Alfalfa seed production in northern Saskatchewan as affected by *Lygus* bugs, with a report on their control by burning. Scientific Agriculture **26**: 130–137.

Bostanian, N.J., Mailloux, G., Binns, M.R., and Thibodeau, P.O. 1990. Seasonal fluctuations of *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera, Miridae) nymphal populations in strawberry fields. Agriculture Ecosystems and Environment **30**: 327–336.

Braun, L., Erlandson, M., and Baldwin, D. 2001. Seasonal occurrence, species composition, and parasitism of *Lygus* spp. in alfalfa, canola, and mustard. Canadian Entomologist **133**: 565–577.

Broersma, D.B. and Luckmann, W.H. 1970. Effects of tarnished plant bug feeding on soybean. Journal of Economic Entomology **63**: 253–256.

Bushing, R.W. and Burton, V.E. 1974. Partial pest management programs on dry large Lima beans in California: regulation of *L. hesperus*. Journal of Economic Entomology **67:** 259–261.

Bushing, R.W., Burton, V.E., and Tucker, C.L. 1974. Dry large Lima beans benefit from *Lygus* bug control. California Agriculture **28**: 14–15.

Butts, R.A. 1989. Biology and damage assessments of *Lygus* spp. infesting oilseed Brassicas grown in Alberta. Ph.D. Thesis, University of Manitoba, Winnipeg, MB.

Butts, R.A. and Lamb, R.J. 1990a. Comparison of oilseed *Brassica* crops with high or low levels of glucosinolates and alfalfa as hosts for three species of *Lygus* (Hemiptera, Heteroptera, Miridae). Journal of Economic Entomology **83**: 2258–2262.

Butts, R.A. and Lamb, R.J. 1990b. Injury to oilseed rape caused by mirid bugs (*Lygus*) (Heteroptera: Miridae) and its effect on seed production. Annals of Applied Biology **117**: 253–266.

Butts, R.A. and Lamb, R.J. 1991a. Seasonal abundance of three lygus species (Heteroptera: Miridae) in oilseed rape and alfalfa in Alberta. Journal of Economic Entomology **84**: 450–456.

Butts, R.A. and Lamb, R.J. 1991b. Pest status of *Lygus* bugs (Hemiptera: Miridae) in oilseed *Brassica* crops. Journal of Economic Entomology **84**: 1591–1596.

Byerly, K.F., Gutierrez, A.P., Jones, R., and Luck, R.F. 1978. Comparison of sampling methods for some arthropod populations in cotton. Hilgardia **46**: 257–282.

Calnaido, D. 1959. Notes on the distribution and biology of the lygus bug, *Lygus virandanus* Motch. (Heteroptera: Miridae), a pest of tea in Ceylon. Tea Quarterly **30**: 108–112.

Canadian Grain Commission. 2014. Official grain grading guide [online]. Available from http://www.grainscanada.gc.ca/oggg-gocg/ggg-gcg-eng.htm [accessed 13 March 2015].

Cárcamo, H., Otani, J., Herle, C., Dolinski, M., Dosdall, L., Mason, P., Butts, R., Kaminski, L., and Olfert, O. 2002. Variation of lygus species assemblages in canola agroecosystems in relation to ecoregion and crop stage. Canadian Entomologist **134**: 97–111.

Cárcamo, H.A., Otani, J., Gavloski, J., Dolinski, M., and Soroka, J. 2003. Abundance of *Lygus* spp. (Heteroptera: Miridae) in canola adjacent to forage and seed alfalfa. Proceedings of the Entomological Society of British Columbia **100**: 55–63.

Celorio-Mancera, M.P. Allen, M.L., Powell, A.L., Ahmadi, H., Salemi, M.R., Phinney, B.S., Shackel, K.A., Greve, L.C., Teuber, L.R., and Labavitch J.M. 2008. Polygalacturonase causes lygus-like damage on plants: cloning and identification of western tarnished plant bug (*Lygus hesperus*) polygalacturonases secreted during feeding. Arthropod-Plant Interactions **2:** 215–225

Celorio-Mancera, M.D., Greve, L.C., Teuber, L.R., and Labovitch, J.M. 2009. Identification of endo- and exo-polygalacturonase activity in *Lygus hesperus* (Knight) salivary glands. Archives of Insect Biochemistry and Physiology **70**: 122–135.

Champlain, R.A. and Butler, G.D. 1967. Temperature effects on the development of egg and nymphal stages of *Lygus hesperus* (Hemiptera: Miridae). Annals of the Entomological Society of America **60**: 519–521.

Charlet, L.D. 2003. Plant stage susceptibility and economic injury level for tarnished plant bug *Lygus lineolaris* (Heteroptera: Miridae), on confection sunflower. Helia **26**: 83–92.

Cherry, R.H., Wood, K.A., and Ruesink, W.G. 1977. Emergence trap and sweep net sampling for adults of the potato leafhopper from alfalfa. Journal of Economic Entomology **70**: 279–282.

Cohen, A.C. and Wheeler, A.G. 1998. Role of saliva in the highly destructive fourlined plant bug (Hemiptera: Miridae: Mirinae). Annals of the Entomological Society of America **91**: 94–100.

Colorado State University Integrated Pest Management Program 1998. Dry bean growth stages [online]. Available from http://www.colostate.edu/Orgs/Vegnet/vegnet/dbgs.html [accessed 10 May 2013].

Cook, B.J., Clay, R.P., Bergmann, C.W., Albersheim, P., and Darvill, A.G. 1999. Fungal polygalacturonases exhibit different substrate degradation patterns and differ in their susceptibilities to polygalacturonase- inhibiting proteins. Molecular Plant Microbe Interactions **12**: 703–711.

Cooper, W.R., Nicholson, S.J., and Puterka, G.J. 2013. Salivary proteins of *Lygus hesperus* (Hemiptera: Miridae). Annals of the Entomological Society of America **106:** 86–92.

Cooper, W.R. and Spurgeon, D.W. 2011. Laboratory studies of variations in feeding behaviors among *Lygus hesperus* (Hemiptera: Miridae) of different gender and reproductive states. Environmental Entomology **40:** 367–373.

Cooper, W.R. and Spurgeon, D.W. 2012. Injury to cotton by adult *Lygus hesperus* (Hemiptera: Miridae) of different gender and reproductive states. Environmental Entomology **41:** 342–348.

Cooper, W.R. and Spurgeon, D.W. 2013. Feeding injury to cotton caused by *Lygus hesperus* (Hemiptera: Miridae) nymphs and pre-reproductive adults. Environmental Entomology **42:** 967–972.

Craig, C.H. 1963. The alfalfa plant bug, *Adelphocoris lineolatus* (Goeze) in northern Saskatchewan. Canadian Entomologist **95**: 6–13.

Craig, C.H. 1983. Seasonal occurrence of *Lygus* spp. (Heteroptera: Miridae) on alfalfa in Saskatchewan. Canadian Entomologist **115**: 329–331.

Craig C.H. and Loan C.C. 1984. *Adelphocoris lineolatus* (Goeze), alfalfa plant bug (Heteroptera: Miridae). *In* Biological control programmes against insects and weeds in Canada 1969–1980. *Edited by* J.S. Kelleher and M.A. Hulme. Commonwealth Agricultural Bureau, London, United Kingdom. Pp 9–10.

Dale, J.E. and Coaker, T.H. 1958. Some effects of feeding by *Lygus vosseleri* Popp. (Heteroptera: Miridae) on the stem apex of the cotton plant. Annals of Applied Biology **46**: 423–429.

Dalling, J.W. and Harms, K.E. 1999. Damage tolerance and cotyledonary resource use in the tropical tree *Gustavia superba*. Oikos **85**: 257–264.

Davis, A.C., McEwen, F.L. and Robinson, R.W. 1963. Preliminary studies on the effect of lygus bugs on the set and yield of tomatoes. Journal of Economic Entomology **56**: 532–533.

Doak, D. 1991. The consequences of herbivory for dwarf fireweed: different time scales, different morphological scales. Ecology **72**: 1397–1407.

Easterbrook, M.A. 2000. Relationship between the occurrence of misshapen fruit on late-season strawberry in the United Kingdom and infestation by insects, particularly the European tarnished plant bug, *Lygus rugulipennis*. Entomologia Experimentalis et Applicata **96**: 59–67.

Elmore, J.C. 1955. The nature of lygus bugs injury to Lima beans. Journal of Economic Entomology **48**: 148–151.

Environment Canada. 2015. Daily data report for Winnipeg Richardson AWOS for 2009–2011. [online] Available from http://climate.weather.gc.ca/index_e. [accessed 19 September 2015].

Fisher, E.H., Riker, A.J., and Allen, T.C. 1946. Bud, blossom, and pod drop of canning string beans reduced by plant hormones. Phytopathology **36**: 504–523.

Fleischer, S.J., Gaylor, M.J., and Edelson, J.V.1985. Estimating absolute density from relative sampling of *Lygus lineolaris* (Heteroptera: Miridae) and selected predators in early to mid-season cotton. Environmental Entomology **4**: 709–717.

Flemion, F., Ledbetter, M., and Schafer, M. 1954. Penetration and damage of plant tissues during feeding by tarnished plant bug (*Lygus lineolaris*). Contributions from Boyce Thompson Institute **17**: 347–357.

Flemion, F. and Olson, J. 1950. Lygus bugs in relation to seed production and occurrence of embryoless seeds in various umbelliferous species. Contributions from Boyce Thompson Institute **16**: 39–46.

Fleury, D., Pare, J., Vincent, C., and Mauffette, Y. 2006. Feeding impact of *Lygus lineolaris* (Heteroptera : Miridae) on *Vitis vinifera*: a behavioural and histological study. Canadian Journal of Botany **84:** 493–500.

Freeman, R.J. and Mueller, A.J. 1989. Seasonal occurrence of the tarnished plant bug, *Lygus lineolaris* (Heteroptera: Miridae) on soybean. Journal of Entomological Science **24**: 218–223.

Garcia, A., Gonzalez, D., and Leigh, T. F. 1982. Three methods for sampling arthropod numbers on California cotton. Environmental Entomology **11**: 565–572.

Gavloski, J. 2001–2010. Summaries of Insect Pests in Crops in Manitoba. [online] Available from http://www.gov.mb.ca/agriculture/crops/insects [accessed 05 March, 2013].

Gerber, G.H. 1995. Fecundity of *Lygus lineolaris* (Heteroptera, Miridae). Canadian Entomologist **127**: 263–264.

Gerber, G.H. 1997. Oviposition preferences of *Lygus lineolaris* (Palisot de Beauvois) (Heteroptera: Miridae) on four *Brassica* and two *Sinapis* species (Brassicaceae) in field cages. Canadian Entomologist **129**: 855 – 858..

Gerber, G.H. and Wise, I.L. 1995. Seasonal occurrence and number of generations of *Lygus lineolaris* and *Lygus borealis* (Heteroptera, Miridae) in southern Manitoba. Canadian Entomologist **127**: 543–559.

Getzin, L.W. 1983. Damage to inflorescence of cabbage seed plants by the pale legume bug (Heteroptera: Miridae). Journal of Economic Entomology **76**: 1083–1085.

Gleason, M.L. and Lewis, D.R. 1992. Integrated pest management for Iowa commercial fruit and vegetable growers. Iowa State University Extension Publication, Ames, IA, United States of America.

Grafius, E. and Morrow, E.A. 1982. Damage by the tarnished plant bug and alfalfa plant bug (Heteroptera: Miridae) to asparagus. Journal of Economic Entomology **75**: 882–884.

Gutierrez, A.P., Leigh, T.F., Wang, Y., and Cave. R.D. 1977. Analysis of cotton production in California - *Lygus hesperus* (Heteroptera-Miridae) injury evaluation. Canadian Entomologist **109**: 1375–1386.

Hagel, G.T. 1978. *Lygus* spp.: damage to beans by reducing yields, seed pitting, and control by varietal resistance and chemical sprays. Journal of Economic Entomology **71**: 613–615.

Handley, D.T. and Pollard, J.E. 1993a. Microscopic examination of tarnished plant bug (Heteroptera: Miridae) feeding damage to strawberry. Journal of Economic Entomology **86:** 505–510.

Handley, D.T. and Pollard, J.E. 1993b. Tarnished plant bug (*Lygus lineolaris*) behaviour on cultivated strawberries. Acta Horticulturae **348:** 463–468.

Harris, J.A. 1915. The influence of position in the pod upon the weight of the bean seed. The American Naturalist **49:** 44–47.

Haws, B.A. 1978. Economic impacts of *Labops hesperius* on the production of high quality range grasses. Utah Agricultural Experimental Station Report. Logan, UT, United States of America. pp. 18–51.

Hill, D.S. 1983. Agricultural insect pests of the tropics and their control. Cambridge University Press, Cambridge, United Kingdom.

Hori, K. 1967. Studies on the salivary gland, feeding habits, and injury of *Lygus disponsi* Linnavuori (Hemiptera: Miridae) I. Morphology of the salivary gland and the symptoms of host plants. Research Bulletin of Obihiro University **5**: 55–74.

Hori, K. 1971a. Studies on the feeding habits of *Lygus disponsi* Linnavuori (Hemiptera: Miridae) and the injury to its host plants I. Histological observations of the injury. Applied Entomology and Zoology **6:** 84–90.

Hori, K. 1971b. Studies on the feeding habits of *Lygus disponsi* Linnavuori (Hemiptera: Miridae) and the injury to its host plants II. Frequency, duration and the quantity of the feeding. Applied Entomology and Zoology **6:** 119–125.

Hori, K. 2000. Possible causes of disease symptoms resulting from the feeding of phytophagous Heteroptera. *In* Heteroptera of economic importance. *Edited by* C.W. Schaefer and A.R. Panizzi. CRC Press, Boca Raton, FL, United States of America, Pp. 11–35.

Hori, K., Torikura, H., and Kumagai, I. 1987. Histological and biochemical changes in the tissue of pumpkin fruit injured by *Lygus disponsi* Linnavuori (Hemiptera:Miridae). Applied Entomology and Zoology **22:** 259–265.

Hughes, J.H. 1943. The alfalfa plant bug, *Adelphocoris lineolatus* (Goeze) and other Miridae (Hemiptera) in relation to alfalfa seed production in Minnesota. University of Minnesota Agricultural Experiment Station Technical Bulletin **161**: 1–101, St. Paul, MN, United States of America.

Huhta, A.P., Lennartsson, T., Tuomi, J., Rautio, P., and Laine, K. 2000. Tolerance of *Gentianella campestris* in relation to damage intensity: an interplay between apical dominance and herbivory. Evolutionary Ecology **14**: 373– 392.

Iowa State University Extension. 1997. Soybean growth stages [online]. Available from http://extension.agron.iastate.edu/soybean/production_growthstages.html [accessed 10 May 2013].

Jay, C.N., Cross, J.V., and Burgess, C. 2004. The relationship between populations of European tarnished plant bug (*Lygus rugulipennis*) and crop losses due to fruit malformation in everbearer strawberries. Crop Protection **23**: 825–834

Jeppson, L.R. and Macleod, G.F. 1946. *Lygus* bug injury and its effect on the growth of alfalfa. Hilgardia **17**: 165–188.

Kelton, K.A. 1975. The lygus bugs (Genus *Lygus* Hahn) of North America (Heteroptera: Miridae). Memoirs of the Entomological Society of Canada **95**: 1–101.

Kelton, L.A. 1980. The plant bugs of the Prairie Provinces of Canada (Heteroptera : Miridae). Agriculture Canada Publication 1703, Ottawa, ON.

Khattat, A.R. and Stewart, R.K. 1975. Damage by tarnished plant bug to flowers and setting pods of green beans. Journal of Economic Entomology **68**: 633–635.

Khattat, A.R. and Stewart, R.K. 1977. Development and survival of *Lygus lineolaris* exposed to different laboratory rearing conditions. Annals of Entomological Society of America **70**: 274–278.

Khattat, A.R. and Stewart, R.K. 1980. Population fluctuations and interplant movements of *Lygus lineolaris*. Annals of the Entomological Society of America **73**: 282–287.

King, W.V. and Cook, W.S. 1932. Feeding punctures of mirids and other plant feeding insects and their effect on cotton. USDA Technical Bulletin **296**: 1–11.

Knight, H.H. 1921. Nearctic records for species of Miridae known heretofore only from the Palearctic region (Heteroptera). Canadian Entomologist **53**: 287–288.

Knight, H.H. 1930. "Alfalfa plant-bug", a common name for an introduced European bug (*Adelphocoris lineolatus* Goeze) found in Iowa (Hemiptera, Miridae). Journal of Economic Entomology **23**: 331–334.

Kogan, M. and Herzog, D.C. 1980. General sampling methods for above ground populations of soybean arthropods. *In* Sampling methods in soybean entomology. *Edited by* M. Kogan and H.N. Pitre. Springer, New York, NY, United States of America. Pp. 30–60.

Kovach, J., Wilcox, W., Agnello, A., and Pritts, M. 1993. "Strawberry IPM Scouting Procedures". New York State Integrated Pest Management Program Publication number 203b. Cooperative Extension Service, Cornell University, Ithaca, NY, United States of America.

Laurema, S. and Varis, A.L. 1991. Salivary amino-acids in *Lygus* species (Heteroptera, Miridae). Insect Biochemistry **21**: 759–765.

Laurema, S., Varis, A.L., and Miettinen, H. 1985. Studies on enzymes in the salivary glands of *Lygus rugulipennis* (Hemiptera: Miridae). Insect Biochemistry **15**: 211–224.

Leferink, J.H.M. and Gerber, G.H. 1997. Development of adult and nymphal populations of *Lygus lineolaris* (Palisot de Beauvois), *L. elisus* Van Duzee, and *L. borealis* (Kelton) (Heteroptera: Miridae) in relation to seeding rate and stage of plant development on canola (Brassicae) in Southern Manitoba. Canadian Entomologist **129**: 777–787.

Leferink, J.M. 1992. The biology of *Lygus* spp. (Heteroptera: Miridae) on oilseed rape in Manitoba. M.Sc. Thesis, University of Manitoba, Winnipeg, MB.

Ma, J., Huang, B., Yang, D., Chai, Q. 2013. Dry matter remobilization and compensatory effects in various internodes of spring wheat under water stress. Crop Science **54**: 331–339.

Mailloux, G. and Bostanian, N.J. 1988. Economic injury level model for tarnished plant bug, *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) in strawberry fields. Environmental Entomology **17**: 581–586.

Manitoba Agriculture Food and Rural Development. 2011a. Field beans - production [online]. Available from http://www.gov.mb.ca/agriculture/crops/production/field-beans.html [accessed 23 April 2014].

Manitoba Agriculture Food and Rural Development. 2011b. Soybean - production and management [online]. Available from http://www.gov.mb.ca/agriculture/crops/production/soybeans.html [accessed 23 April 2014].

Manitoba Agriculture Food and Rural Development. 2012. Soybean Sector. [online]. Available from: http://www.gov.mb.ca/agriculture/market-prices-and-statistics/crop-statistics/pubs/crop_soybean_sector.pdf [accessed 20 October 2014].

Manitoba Agriculture Food and Rural Development. 2014a. Agriculture statistics. [online]. http://www.gov.mb.ca/agriculture/market-prices-and-statistics/crop-statistics/pubs/2014-june-intentions.pdf [accessed 20 May 2015].

Manitoba Agriculture Food and Rural Development. 2014b. Guide to field crop protection 2014. [online]. Available from http://www.gov.mb.ca/agriculture/crops/guides-and-publications/pubs/crop-protection-guide-insect.pdf [accessed 20 October 2014].

Manitoba Pulse Growers Association. 2015. 2013 Pulse variety evaluation. [online] Available from http://www.manitobapulse.ca/wp-content/uploads/Pulse-Variety-Evaluation-2013_FINAL_WR.pdf [accessed 20 September 2015].

Martin, S.A., Darrah, L.L., and Hibbard, B.E. 2004. Divergent selection for rind penetrometer resistance and its effects on European corn borer damage and stalk traits in corn. Crop Science **44**: 711–717.

Maschinski, J. and Whitham. T. G. 1989. The continuum of plant responses to herbivory: the influence of plant association, nutrient availability, and timing. American Naturalist **134**: 1–19.

Mauk, C.S., Breen, P.J., and Mack, H.J. 1984. Flowering-pattern and yield components at inflorescence nodes of snap bean as affected by irrigation and plant density. Scientia Horticulturae **23:** 9–19.

Mauk, C.S., Breen, P.J., and Mack, H.J. 1987. Flower and pod abscission in snap bean as influenced by inflorescence position, raceme node, irrigation and plant density. Canadian Journal of Plant Science **67**: 1193-1202.

McKinlay, K.S. and Geering, R.S. 1957. Studies on the crop loss following insect attack on cotton in East Africa. I. Experiments in Uganda and Tanganyika. Bulletin of Entomological Research **48**: 833–849.

McMahan, H. and Arrand, J.C. 1955. Control of plant bugs in northern alfalfa seed fields. Canadian Department of Agriculture Publication 949. Ottawa. ON.

Michaud, O.D., Stewart, R.K., and Boivin, G. 1990. Susceptibility of apples to damage by *Lygocoris communis* and *Lygus lineolaris* (Hemiptera, Miridae). Phytoprotection **71**: 25–30.

Miles, P.W. 1972. The saliva of Hemiptera. Advances in Insect Physiology **9**: 183–255.

Miles, P.W. and Lloyd, J. 1967. Synthesis of a plant hormone by the salivary apparatus of plant sucking Hemiptera. Nature **213**: 801–802.

Morris, R.F. 1960. Sampling insect populations. Annual Review of Entomology **5**: 7–20.

Mostafa, A.M. 2007. Plant bugs (Hemiptera: Miridae) on buckwheat and seed alfalfa crops in Manitoba: dynamics, yield implications and management. Ph.D. Thesis, University of Manitoba, Winnipeg, MB.

Mostafa, A.M. and Holliday, N.J. 2008. Insecticidal control of late-season plant bug (Hemiptera: Miridae) infestations in Manitoba has no effect on alfalfa seed quantity and quality. Canadian Journal of Plant Science **88**: 763–770.

Mueller, S.C., Summers, C.G., and Goodell, P.B. 2003. A field key to the most common *Lygus* species found in agronomic crops of the central San Joaquin valley of California. University of California, Agriculture and Natural Resources, Oakland, CA, United States of America.

Murrell, D.C. 1987. Alfalfa seed field insect survey 1984–86. Final report, New Crop Development Fund. Agriculture Canada, Ottawa, ON.

Musser. F.R., Catchot, A.L., Stewart, S.D., Bagwell, R.D., Lorenz, G.M., Tindall, K.V., Studebaker, G.E., Leonard, B.R., Scott, A.D., Cook, D., and Daves A.V. 2009. Tarnished plant bug (Hemiptera: Miridae) thresholds and sampling comparisons for flowering cotton in the mid Southern United States. Journal of Economic Entomology **102**: 1827–1836.

Musser. F.R., Stewart, S.D., Bagwell, R., Lorenz, G., Catchot, A., Burris, D.,Cook, D., Robbins, J., Greene, Studebaker, G.E., and Gore, J. 2007. Comparison of direct and indirect sampling methods for tarnished plant bug (Hemiptera: Miridae) in flowering cotton. Journal of Economic Entomology **100:** 1916–1923.

Nagalingam, T. and Holliday, N.J. 2015. Plant bugs (Hemiptera: Miridae) on field beans and soybeans in Manitoba: species composition and seasonal occurrence. Canadian Entomologist **147**: 766–775.

Nakamura, R.R. 1988. Seed abortion and seed size variation within fruits of *Phaseolus vulgaris*: pollen donor and resource limitation effects. American Journal of Botany **75:** 1003–1010.

Nuorteva, P. 1956. Studies on the effect of the salivary secretions of some Heteroptera and Homoptera on plant growth. Annales Entomologicae Fennicae **22**: 108–117.

Ofir, M., Gross, Y., Bangerth, F., and Kigel, J. 1993. High temperature effects on pod and seed production as related to hormone levels and abscission of reproductive structures in common bean (*Phaseolus vulgaris* L.). Scientia Horticulturae **55:** 201–211.

Ojehomon, O.O. 1970. Effect of continuous removal of open flowers on the seed yield of two varieties of cowpea, *Vigna unguiculata* (L.) Walp. Journal of Agricultural Science **74**: 375–381.

Ontario Ministry of Agriculture Food and Rural Affairs. 2009a. Dry Edible Beans: Harvest and Storage. [online] http://www.omafra.gov.on.ca/english/crops/pub811/5harvest.htm [Accessed **20** October 2014]

Ontario Ministry of Agriculture Food and Rural Affairs. 2009b. Dry edible beans: planting and crop development. [online] Available from http://www.omafra.gov.on.ca/english/crops/pub811/5planting.htm [accessed 20 September 2015].

Osborne, D.J. 1989. Abscission. Critical Reviews in Plant Sciences **8:** 103–129.

Otani, J.K. 2000. *Lygus elisus* (Van Duzee) and *Lygus borealis* (Kelton) reproduction in canola and forage crops in southern Alberta. M.Sc. Thesis, University of Manitoba, Winnipeg, MB.

Pack, T.M. and Tugwell, N.P. 1976. Clouded and tarnished plant bugs on cotton: a comparison of injury symptoms and damage on fruit parts. Arkansas Agriculture Experimental Station Service Report 226, Fayetteville, AR, United States of America. pp. 17.

Painter, R.H. 1929. The tarnished plant bug, *Lygus pratensis* L.: A progress report. *In* Annual report of the Entomological Society of Ontario **60**: 102–107.

Painter, R.H. 1930. A study of the cotton flea hopper, *Psallus seriatus* Reut., with especial reference to its effect on cotton plant tissues. Journal of Agriculture Research **40:** 485–516.

Park, S.J. 1985. Growing field beans in Canada. Agriculture and Agri-Food Canada Bulletin 1787E, Ottawa, ON. pp.30.

Pearson, E.D. and Maxwell-Darling, R.C. 1958. The insect pests of cotton in tropical Africa. Empire Cotton Growers Corporation, London, United Kingdom.

Peksen, E. 2007. Dynamics of flower appearance, flowering, pod and seed setting performance and their relations to seed yield in common bean (*Phaseolus vulgaris* L.). Pakistan Journal of Botany. **39**: 485–496.

Perillo, A.C., Kucharik, C.K., Meehan, T.D., Serbin, S.P., Singh, A., Townsend. P.A., Whitney, K.S., and Gratton, C.2015. Use of insect exclusion cages in soybean creates an altered microclimate and differential crop response. Agricultural and Forest Meteorology **208**: 50–61.

Phillips, J.H. and Deronde, J.H. 1965. Relationship between seasonal development of tarnished plant bug *Lygus lineolaris* (Beauv) (Hemiptera-Miridae) and its injury to peach fruit. Proceedings of the Entomological Society of Ontario **96**: 103–107.

Pilson, D. and Decker, K.L. 2002. Compensation for herbivory in wild sunflower: response to simulated damage by the head-clipping weevil. Ecology **83**: 3097–3107.

Poston, F.L. and Pedigo, L.P. 1975. Migration of plant bugs and the potato leafhopper in a soybean-alfalfa complex. Environmental Entomology **4**: 8–10.

Prokopy, R.J. and Hubbell, G.L. 1981. Susceptibility of apple to injury by tarnished plant bug adults. Environmental Entomology **10**: 977–979.

Pulse Canada. 2003. Crop profile for dry beans. [online]. Available from http://www.pulsecanada.com/uploads/a2/09/a2097ea4c4b74e2f8ca52c406c144233/Bean-Profile.PDF [accessed 5 July 2013].

Pulse Canada. 2007. Global pulse industry. [online] Available from http://www.pulsecanada.com/uploads/wv/nr/wvnr5qGWIi8Rqbl22UyY8g/Canadas-Pulse-Industry-in-the-Global-Market-Sep-07.pdf. [accessed 10 November 2014].

Pulse Canada. 2013. Pulse Industry. [online] Available from. http://www.pulsecanada.com/pulse-industry [accessed 5 July 2013].

Pulse Canada. 2014. Pulse Industry [online]. Available from http://www.pulsecanada.com/statistics [accessed 30 January 2014].

Pulse and Soybean Growers Association. 2015. Pulses in Manitoba [online]. Available from http://www.manitobapulse.ca/for-producers/pulses-in-manitoba/ [accessed 11 December, 2015].

Qiaosi, L., Qinxuan, L., and Wangxi, D. 1994. The effect of different host plants on the population dynamics of the alfalfa plant bug. Acta Phytophylacica Sinica **21**: 351–355.

Race, S.R. 1960. A comparison of two sampling techniques for lygus bugs and stink bugs on cotton. Journal of Economic Entomology **53**: 689–690.

Rancourt, B., Vincent, C., and De Oliveira, D. 2000. Circadian activity of *Lygus lineolaris* (Hemiptera: Miridae) and effectiveness of sampling techniques in strawberry fields. Journal of Economic Entomology **93**: 1160–1166.

Rice, P.L. 1938. Cat-facing of peaches by the tarnished plant bug *Lygus pratensis* L. Transactions of the Peninsula Horticultural Society **1938**: 131–136.

Ridgway, R. and Gyrisco, G.G. 1960. Studies of the biology of the tarnished plant bug *Lygus lineolaris*. Journal of Economic Entomology **53**: 1063–1065.

Rings, R.W. 1958. Types and seasonal incidence of plant bug injury to peaches. Journal of Economic Entomology **51**: 27–32.

Rosenheim, J. A., Steinmann, K., Langellotto, G.A., and Zink, A.G. 2004. Estimating the impact of *Lygus hesperus* on cotton: the insect, plant, and human observer as sources of variability. Environmental Entomology **35**: 1141–1153.

Sage, T.L. and Webster, B.D. 1987. Flowering and fruiting patterns of *Phaseolus vulgaris*. Botanical Gazette **148:** 35–41.

Sage, T.L. and Webster, B.D. 1990. Seed abortion in *Phaseolus vulgaris*. Botanical Gazette **151:** 167–175.

Salt, R.W. 1945. Number of generations of *Lygus hesperus* Knt. and *L. elisus* Van D. in Alberta. Scientific Agriculture **25**: 573–576.

Sapio, F.J., Wilson, L.F., and Ostry, M.E. 1982. A split stem lesion on young hybrid *Populus* trees caused by the tarnished plant bug, *Lygus lineolaris* (Hemiptera) (Heteroptera: Miridae). Great Lakes Entomologist **15**: 237–246.

Schaber, B.D. 1992. Insects infesting seed alfalfa in the prairie provinces: a field guide. Agriculture Canada Publication 1881/E. Agriculture Canada, Ottawa, ON.

Schaber, B.D., Harper, A.M., and Entz, T. 1990. Effect of swathing alfalfa for hay on insect dispersal. Journal of Economic Entomology **83**: 2427–2433.

Schaeffers, G.A. 1980. Yield effects of tarnished plant bug feeding on June-bearing strawberry varieties in New York State. Journal of Economic Entomology **73**: 721–725.

Schowalter, T.D., Overhulser, D.L., Kanaskie, A., Steins, J.D., and Sexton, J. 1986. *Lygus hesperus* as an agent of apical bud abortion in Douglas-fir nurseries in western Oregon. New Forests **1**: 5–15

Schwartz, M.D. and Foottit, R.G. 1992a. *Lygus* species on oilseed rape mustard, and weeds: a survey across the Prairie Provinces of Canada. Canadian Entomologist **124**: 151–158.

Schwartz, M.D. and Foottit, R.G. 1992b. Lygus bugs on the prairies. Biology, systematics and distribution. Agriculture Canada Technical Bulletin 1992-4E, Ottawa, ON.

Scott, D.R. 1969. Lygus bug feeding on developing carrot seed: effect on plants grown from that seed. Journal of Economic Entomology **62**: 504–506.

Sedivy, J. and Kocourek, F. 1988. Comparative studies on two methods for sampling insects in an alfalfa seed stand in consideration of a chemical control treatment. Journal of Applied Entomology **106**: 312–318.

Sevacherian, V. and Stern, M.V. 1972. Sequential sampling plans for lygus bugs in California. Environmental Entomology **1**: 704–710.

Senanayake, D.G. and Holliday, N.J. 1990. Economic injury levels for Colorado potato beetle (Coleoptera: Chrysomelidae) on ‘Norland’ potatoes in Manitoba. Journal of Economic Entomology **83**: 2058–2064.

Shackel, K.A., Celorio-Mancera, M., Ahmadi, H., Greve, L.C., Teuber, L.R., Backus, E.A., and Labavitch, J.M. 2005. Micro-injection of lygus salivary gland proteins to simulate feeding damage in alfalfa and cotton flowers. Archives of Insect Biochemistry and Physiology **58**: 69–83.

Sharp, J.W. and Bagwell, R.D. 2006. Evaluation of multiple bug sampling techniques for effectiveness and efficiency. *In* Proceedings Beltwide Cotton Conference, 3–6 January San Antonio,TX, United States of America. pp 954–956.

Sheldrake, A.R., Narayanan, A., and Nenkataratnam, N. 1979. The effect of flower removal on the seed yield of pigeon peas (*Cajanus cajan*). Annals of Applied Biology **91**: 383–390.

Shull, W.E. 1933. An investigation of the *Lygus* species which are pests of beans (Hemiptera, Miridae). Idaho Agriculture Experiment Station Research Bulletin 11. Moscow, ID, United States of America.

Shull, W.E., Rice, P.L., and Cline, H.F. 1934. *Lygus hesperus* Knight (Hemiptera: Miridae) in relation to plant growth, blossom drop, and seed set in alfalfa. Journal of Economic Entomology **27**: 265–269.

Slaymaker, P.H. and Tugwell, N.P. 1982. Low-labor method for rearing the tarnished plant bug (Hemiptera: Miridae). Journal of Economic Entomology **75**: 487–488.

Snodgrass, G.L. and McWilliams, J.M. 1992. Rearing the tarnished plant bug (Heteroptera: Miridae) using a tissue paper oviposition site. Journal of Economic Entomology 85: 1162–1166.

Snodgrass, G.L. 1993. Estimating absolute density of nymphs of *Lygus lineolaris* (Heteroptera: Miridae) in cotton using drop cloth and sweep net sampling methods. Journal of Economic Entomology **86**: 1116–1123.

Snodgrass, G.L. 1998. Distribution of tarnished plant bug (Heteroptera: Miridae) within cotton plants. Environmental Entomology **27**: 1089–1093.

Snodgrass, G.L. 2003. Role of reproductive diapause in the adaption of tarnished plant bug (Heteroptera: Miridae) to its winter habitat in the Mississippi River Delta. Environmental Entomology **32**: 945–952.

Snodgrass, G.L., Jackson, R.E., Abel, C.A., and Perera, O.P. 2010. Utilization of early soybeans for food and reproduction by the tarnished plant bug (Hemiptera: Miridae) in the Delta of Mississippi. Environmental Entomology **39**: 1111–1121.

Snodgrass, G.L. and Scott, W.P. 1997. A conversion factor for correcting numbers of tarnished plant bugs (Heteroptera: Miridae) captured with a sweep net in cotton. Southwestern Entomologist **22**: 189–193.

Sorensen, C.J. 1936. *Lygus* bugs in relation to occurrence of shriveled alfalfa seed. Journal of Economic Entomology **29**: 454–457.

Sorensen, C.J. 1939. *Lygus hesperus* Knight and *Lygus elisus* Van Duzee in relation to alfalfa seed production. Utah Agricultural Experiment Station Bulletin, **284:** 1–61,Utah State Agricultural College, Logan, UT, United States of America.

Soroka, J.J. and Murrell, D.C. 1993. The effects of alfalfa plant bug (Hemiptera: Miridae) feeding late in the season on alfalfa seed yield in northern Saskatchewan. Canadian Entomologist **125**: 815–824.

Southwood, T.R.E. 1978. Ecological methods with particular reference to the study of insect populations. 2nd edition. Chapman and Hall, London, United Kingdom.

Southwood, T.R.E. and Norton, G.A. 1973. Economic aspects of pest management strategies and decisions. *In* Insects: Studies in Population Management, ed. P. Geier, L. Clark, D. Anderson, H. pp168–184. Ecological Society of Australia, Canberra, Australia.

Spurgeon, D.W. and Cooper, W.R. 2011. Among sampler variation in sweep net samples of adult *Lygus hesperus* (Hemiptera: Miridae) in cotton. Journal of Economic Entomology **104**: 685–692.

Spurgeon, D.W. and Cooper, W.R. 2013. Sweepnet captures of *Lygus hesperus* (Hemiptera: Miridae) adult genders and age-classes in cotton. Journal of Entomological Science **48**: 195–205.

Statistics Canada. 2014. Principal field crop areas, June 2014. [online]. Available from: http://www.statcan.gc.ca/daily-quotidien/140627/dq140627b-eng.htm [Accessed 20 October 2014].

Stewart, R.K. and Khattat, A.R. 1980. Economic injury levels of the tarnished plant bug, *Lygus lineolaris* (Hemiptera (Heteroptera): Miridae), on green beans in Quebec. Canadian Entomologist **112**: 306–310.

Stewart, R.K. and Khoury, H. 1976. The biology of *Lygus lineolaris* (Palisot de Beauvois) (Hemiptera: Miridae) in Quebec. Annals of the Entomological Society of Quebec **21**: 52–63.

Stewart, S.D. and Gaylor, M.J. 1994. Effects of age, sex, and reproductive status on flight by the tarnished plant bug (Heteroptera: Miridae). Environmental Entomology **23:** 80–84.

Strong, F.E. 1968. The selective advantage accruing to lygus bugs that cause blasting of floral parts. Journal of Economic Entomology **61**: 315–316.

Strong, F.E. 1970. Physiology of injury caused by *Lygus hesperus*. Journal of Economic Entomology **63**: 808–814.

Strong, F.E. and Kruitwagen, E.C. 1968. Polygalacturonase in the salivary apparatus of *Lygus hesperus* (Hemiptera). Journal of Insect Physiology **14**: 1113–1119.

Summerfield, R.J., Muehlbauer, F.J., and Short, R.W. 1982. Lygus bugs and seed quality in lentils (*Lens Culinaris* Medik). Science and Education Administration Publications **29**: 1–43.

Swezey, S.L., Nieto, D.J., Hagler, J.R., Pickett, C.H., Bryer. J.A., and Machtley, S.A. 2013. Dispersion, distribution, and movement of *Lygus* spp. (Hemiptera: Miridae) in trap-cropped organic strawberries. Environmental Entomology **42**: 770–778.

Systat 2009. Systat 13, Systat Software Inc., Chicago, IL, United States of America.

Tamas, I.A., Wallace, D.H., Ludford, P.M., and Ozbun, J.L. 1979. Effect of older fruits on abortion and abscisic acid concentration of younger fruits in *Phaseolus vulgaris* L. Plant Physiology **64**: 620–622.

Thimann, K.V. 1972. The natural plant hormones. *In* Plant physiology: a treatise *Edited by* F. C. Stewart. Academic Press, New York.

Timlick, B.H., Turnock, W.J., and Wise, I. 1993. Distribution and abundance of *Lygus* spp. (Heteroptera: Miridae) on alfalfa and canola in Manitoba. Canadian Entomologist **125**: 1033–1041.

Tingey, W.M., Leigh, T.F., and Hyer, A.H. 1975. *Lygus hesperus* growth, survival, and egg-laying resistance of cotton genotypes. Journal of Economic Entomology **68**: 28–30.

Tingey, W.M. and Pillemer, E.A. 1977. *Lygus* bugs: crop resistance and physiological nature of feeding injury. Entomological Society of America Bulletin **23:** 277–287.

Triplehorn, C.A. and Johnson, N.F. 2005. Borror and DeLong's Introduction to the Study of Insects. Thomson Brooks/Cole, Belmont, CA, United States of America.

Trumble, J.T., Kolodny-Hirsch, D.M., and Ting, I.P. 1993. Plant compensation for arthropod herbivory. Annual Review of Entomology **38**: 93–119.

Tugwell, N.P., Young, S.C., Dumas, B.A., and Phillips, J.R. 1976. Plant bugs in cotton: Importance of infestation time, types of cotton injury, and significance of wild hosts near cotton. Arkansas Experimental Station Report. pp. 24. Fayetteville, AR, United States of America.

Turnock, W.J., Gerber, G.H., Timlick. B.H., and Lamb, R.J. 1995. Losses of canola seeds from feeding by Lygus species (Heteroptera: Miridae) in Manitoba. Canadian Journal of Plant Science **75**: 731–736.

Uddin, M.J. 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. Ph.D. Thesis, University of Manitoba, Winnipeg, MB.

Ugine, T.A. 2012. Developmental times and age-specific life tables for *Lygus lineolaris* (Heteroptera: Miridae), reared at multiple constant temperatures. Environmental Entomology **41:** 1–10.

United States Department of Agriculture. 1976. United States standards for grades of canned dried beans, canned pork and beans, canned baked beans [online]. Available from

www.ams.usda.gov/AMSv1.0/getfile?dDocName=STELDEV3010019 [accessed 13 March 2015].

Varis, A.L. 1972. The biology of *Lygus rugulipennis* Popp. (Het., Miridae) and the damage caused by this species to sugar beet. Annales Agriculturae Fenniae **11**: 1–56.

Western Committee on Crop Pests. 1997. Annual meeting minutes for 1997 [online]. Available from http://www.westernforum.org/WCCP%20Minutes.html [accessed 17 October 2013].

Williams, L., Phillips, J.R., and Tugwell, N.P. 1987. Field technique for identifying causes of pinhead square shed in cotton. Journal of Economic Entomology **80**: 527–531.

Williams, L. and Tugwell, N.P. 2000. Histological description of tarnished plant bug (Heteroptera : Miridae) feeding on small cotton floral buds. Journal of Entomological Science **35:** 187–195.

Wise, I.L., Elliott, B.G., and Mostafa, A.M. 2005. Seasonal occurrence and within-field distribution of *Lygus lineolaris* on buckwheat in Manitoba. Canadian Entomologist **137**: 598–601.

Wise, I.L. and Lamb, R.J. 1998. Sampling plant bugs, *Lygus* spp. (Heteroptera: Miridae), in canola to make control decisions. Canadian Entomologist **130**: 837–851.

Wise, I.L. and Lamb, R.J. 2000. Seasonal occurrence of plant bugs (Hemiptera: Miridae) on oilseed flax (Linaceae) and their effect on yield. Canadian Entomologist **132**: 369–371.

Wise, I.L., Tucker, J.R. and Lamb R.J. 2000. Damage to wheat seeds caused by a plant bug, *Lygus lineolaris.* Canadian Journal of Plant Science **80**: 459–461.

Woodcock, E.G. 1935. Carpel anatomy of the bean (*Phaseolus vulgaris* L.). Papers of the Michigan Academy of Science Arts and Letters **20:** 267–274.

Word, S.J. and Hutchison, W.D. 2003. Phenology of *Lygus lineolaris* (Hemiptera: Miridae) in Minnesota June-bearing strawberries: comparison of sampling methods and habitats. Journal of Economic Entomology **96**: 1814–1820.

Young , O.P. 1986. Host plants of the tarnished plant bug, *Lygus lineolaris* (Heteroptera : Miridae). Annals of the Entomological Society of America **79:** 747–762.

Zeiss, M.R. and Klubertanz, T.H. 1993. Sampling program for soybean arthropods. *In* Handbook of sampling methods for arthropods in Agriculture Edited by Pedigo, L.P. and Buntin, G.D. CRC Press, Boca Raton, FL, United States of America. pp. 539–601.

Zink, A.G. and Rosenheim, J.A. 2005. Stage dependent feeding behavior by western tarnished plant bugs influences flower bud abscission in cotton. Entomologia Experimentalis et Applicata **117**: 235-242.

Zink, A.G. and Rosenheim, J.A. 2004. State-dependent sampling bias in insects: implications for monitoring western tarnished plant bugs. Entomologia Experimentalis et Applicata **113**: 117–123.