University of Alberta

Sustainable management of the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae), a new invasive insect pest of cereal crops in western Canada

by

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Abstract

The cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae) is a new invasive insect pest of cereals in western Canada, and has expanded its geographic range significantly throughout the region. Its establishment has economic implications for grain production, trade and export. Biological control with its principal larval parasitoid, *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), introduced from Europe has been the most successful management strategy in North America. In southern Alberta, the parasitoid has established naturally along with the beetle and provides an opportunity for integration of biological control with other management tactics. My investigation focused on tritrophic interactions between the cereal host plants, *O. melanopus* and *T. julis*. I investigated life histories and host preferences of *O. melanopus* and *T. julis*, their spatio-temporal distribution dynamics, and explored host-plant resistance mechanisms in exotic wheat genotypes to discern interrelations between these species.

My studies on developmental patterns of *O. melanopus* on potential cereal hosts in western Canada (oat, wheat, barley, corn, rye and triticale) indicated that the preferences for these hosts and their utilization differed within the fundamental host range of *O. melanopus*. Prolonged developmental times and low survivorship on a local cultivar of oat, Waldern, indicated a potential avenue for designing strategies such as trap cropping. My studies on the biology of *T. julis* indicated that *T. julis* females prefer advanced larval instars for parasitization; such a selection lead to higher clutch size, and improved fitness. Under field

conditions, the relationship of *O. melanopus* and *T. julis* indicated a tightly coupled host-natural enemy system. *Tetrastichus julis* exhibited strong density dependence. Host plant characteristics influenced field dynamics of *O. melanopus* which in turn influenced *T. julis* distribution.

Three of the six central Asian wheat genotypes tested (NN-100, NN-78 and NN-27) were less attractive for *O. melanopus* oviposition and feeding and further trials on biology and fitness of the beetle suggested prolonged development and low fitness on these genotypes. This indicated presence of both antixenosis and antibiosis mechanisms. The resistant lines identified can act as effective genotypes for breeding explorations in North America.

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m	metre
cm	centimetre
km	kilometre
ha	hectare
kg	kilogram
µg	microgram
mg	milligram
d	day(s)
h	hour(s)
L:D	light to dark ratio
cv.	cultivar
rh	relative humidity
SADIE	Spatial Analysis by Distance Indices
δ/D	SADIE index of distance to regularity
X	SADIE association index
$\bar{V}_I$	SADIE Patch Index
$\bar{V}_J$	SADIE Gap Index
°C	degrees Celsius
ICARDA	International Center for Agricultural Research in Dry Areas
PRS	Plant Root Simulator
GEE	Generalized Estimating Equations
BIC	Bayesian Information Criterion
dpi	dots per inch

Chapter 1: Introduction

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1.1. General introduction

The cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), is a relatively recent alien invasive pest in western Canada that infests a range of important cereal crops including wheat, oat and barley (Leibee and Horn 1979; USDA Fact Sheet 1995; Dosdall et al. 2011). Native to Eurasia, *O. melanopus* is a common pest of cereals throughout Europe and particularly in the Balkan region (Kostov 2001). The beetle was discovered in North America in 1962 in Michigan, U.S.A. (Dysart et al. 1973; Evans et al. 2006; LeSage et al. 2007). Its exact portal and mode of entry into North America are unknown but it may have arrived in straw material from Europe (Haynes and Gage 1981; LeSage et al. 2007). The beetle has expanded its range in recent years to encompass most regions of cereal production in the U.S.A. (Ihrig et al. 2001; Buntin et al. 2004), and portions of Alberta, Saskatchewan and Manitoba (CFIA 2008; Dosdall et al. 2011).

Western Canada is the major cereal grain production region of the country (McCallum et al. 2007), and provides a favourable climate for population expansion of *O. melanopus*. Western Canada lacks geographic or ecological barriers to prevent further dispersal, and provides a diverse host plant range that can enhance the ability of the species to spread and establish in new sites (Olfert

et al. 2004; Dosdall et al. 2011). Climate change may also affect the future distribution of the beetle and predictive models based on current conditions and different incremental temperature scenarios suggest that the insect could expand its geographic range across the entire cereal-growing region of Canada (Olfert et al. 2004; Olfert and Weiss 2006). Canada contributes as much as seven percent of the world`s wheat and barley production. In terms of exports, Canada contributes 15 and 20% of the world's total exports of wheat and barley, respectively. Assuming that *O. melanopus* causes only 10% yield losses, even this amounts to substantial economic losses. Establishment of this pest thus has several economic implications for grain production, trade and export and presents a potential threat to cereal production in western Canada; thus, research is required to understand its local biology, ecology and to develop integrated management strategies.

In view of the economic importance of *O. melanopus* in its new eco-region, here I present an overview of its taxonomic status, distribution and range expansion, life history and field dynamics, and management strategies. In the concluding section, I present the major research themes and objectives of my research.

1.2. Taxonomic status of *O. melanopus*

Oulema melanopus belongs to the subfamily Criocerinae of the family Chrysomelidae of the order Coleoptera. The subfamily Criocerinae consists of about 1500 described species with a distribution across temperate, tropical and subtropical zones throughout the world (Schmitt 1988). The Chrysomelidae (leaf

beetles) is the second largest family of Coleoptera with many described species (about 50,000) spread over terrestrial, aquatic and sub-aquatic habitats; adults and larvae feed on roots, leaves, stems and flowers of their host plants (Schmitt 1988; Staines 2008).

Oulema melanopus belongs to the tribe Lemini (Haynes and Gage 1981). The chromosome number of the species is $2n=15+Xy_p$ (Xy_p represents a heteromorphic chromosomal pair consisting of a large X chromosome and a small Y chromosome forming a typical parachute shaped association (represented by p) during metaphase), and it belongs to the group of primitive chrysomelids (Ninan et al. 1968). Many researchers have used the genus name *Lema* as being synonymous to *Oulema*. Earlier studies conducted in Europe referred to the cereal leaf beetle as *Lema melanopa* (Hodson 1929; Venturi 1942). Although the differences in the two genera are too small to distinguish morphologically, Ninan et al. (1968) suggested that they are distinct based on chromosome number and genetic makeup; the other difference being that of the host range. While *O. melanopus* feeds on small grains, *Lema* spp. feed mainly on broad-leaved plants (Ninan et al.1968).

In Europe, several congeneric *Oulema* species form a group of leaf beetles feeding mainly on small grain crops, and are referred to collectively as "cereal leaf beetles" (Schmitt 1988; Poszgai and Saringer 2006). *Oulema melanopus* is a dominant and widely distributed member of this group (Schmitt and Rönn 2011), and has successfully invaded new eco-regions. Trans-continental invasion of North America is the classic example of its invasive potential (Dysart et al. 1973).

Other important congeneric cereal leaf beetle species in Europe include *Oulema duftschmidi* (Redtenbacher), *Oulema gallaeciana* Hayden, and *Oulema lichenis* Voet (Stilmant 1995; Ulrich et al. 2004). Another species, *Oulema rufocynaea* (Suffrian), is referred to as a distinct species in the literature (Berti 1989); however, Schmitt and Rönn (2011) have argued that both *O. rufocynaea* and *O. duftschimidi* are synonymous. In a European context, *O. melanopus* is mostly established in northern Europe while the closely related *O. duftschmidi* is mostly found in Mediterranean regions (LeSage et al. 2007). Several differences in species distribution patterns have been observed. For example, in Hungary *O. duftschmidi* (comprising about 25-35% of the total cereal leaf beetle population) has an interspersed distribution with *O. melanopus* (Pozsgai and Saringer 2006), while in central Europe, *O. melanopus* and *O. gallaeciana* are more abundant and economically important (Ulrich et al. 2004). Only *O. melanopus* has been recorded in North America and no other congeneric species are known to have invaded this region (LeSage et al. 2007).

1.3. Diagnostic characters

Oulema melanopus adults are about 5 mm long with bright green-bluish elytra with red legs. The larva has its head wider than the body and covers its body with its own fecal material (Piesik and Piesik 1998). Wellso (1978) developed a key to identify pre- and post-aestival beetles emerging in spring. The pre- and post-aestival beetles can be differentiated based on the coloration of tergites under their elytra in that the former have a light yellow-colored elytra

while the latter have dark-colored elytra that develop after continued feeding on their hosts for about four days. Both newly emerged and post-diapausing adults are capable of sound production by rubbing their elytral apices on striated areas of the last abdominal tergite. When disturbed, the adults of *O. melanopus* usually fly away, tend to hide by dropping to the ground, or feign death (Ninan et al. 1968). There are no obvious morphological characters reported to differentiate between the sexes of *O. melanopus* (Myser and Schultz 1967). Differences in the head capsule widths of larval instars after each consecutive molt have been observed (Hoxie and Wellso 1974). Males and females cannot be distinguished based on the head capsule morphology of the larvae. However, Myser and Schultz (1967) proposed that morphological differences between the intercoxal processes can be used to distinguish between the sexes of *O. melanopus.* While the intercoxal processes are rounded and convex in females, the males possess pointed intercoxal processes that are either flat or concave. These characters enable identification of sexes without dissecting their genitalia.

1.4. *Oulema melanopus* distribution and range expansion

Oulema melanopus has a long association with cereal cultivation in Europe (Hahn 1968). In Europe, crop monoculture practices and intensification of food production following industrialization of the food sector have contributed significantly to the increasing abundance of the beetle (Piesik and Piesik 1998; Ulrich et al. 2004). It is a significant pest in Hungary (Papp and Masterhazy 1996; Pozsgai and Saringer 2006), Poland (Ulrich et al. 2004), Moldova (Livia 2006),

Russia (Sphanev and Golubev 2008), Bulgaria (Kostov 2001), Serbia (Dimitrijević et al. 1999, 2001), The Netherlands (Daamen and Stol 1993), Belgium and France (Stilmant 1995), Germany (Schmitt 1988), and Italy (Morlacchi et al. 2007), and was also reported in India (Hussain and Ahmad 2006), Pakistan (Khan et al. 2008) and Iran (Nikbakhtzedh and Tirgari 2002).

Following its arrival in the north-central U.S.A. in 1962, *O. melanopus* quickly spread throughout the region (Gutierrez et al. 1974; Haynes and Gage 1981; McPherson 1983a; Wellso and Hoxie 1981a). Its eastwardly spread was attributed to prevailing winds toward the Atlantic Ocean (Battenfield et al.1982) that further continued to southeastern Canada (Webster et al. 1972). Eastward dispersal in North America was also facilitated by favourable environmental conditions (Grant and Patrick 1993). Expansion in wheat-growing areas, the availability of overwintering sites, and agronomic practices such as no tillage were considered to contribute to its westward spread in the U.S.A. (Bailey et al. 1991). In Canada, it was reported in southern Ontario in 1965 (Battenfield et al. 1982; LeSage et al. 2007), in the Maritime Provinces in 1994 (LeSage et al. 2007), in the Creston Valley of British Columbia in 1998 (CFIA 1999), and more recently in Alberta (2005), Saskatchewan (2008) and Manitoba (2009) (Dosdall et al. 2011) (Fig.1.1). New disjunct populations were reported in various sites of the three Prairie Provinces in 2013: east of Red Deer in central Alberta, Moosomin in southeastern Saskatchewan and Treherne in southwestern Manitoba (H. Carcamo, personal communication). Annual surveys in Alberta indicate an increase in its range and abundance since 2006 (Dosdall et al. 2011) (Fig. 1.1).

1.5. Life history and field dynamics of *O. melanopus*

The biology and field dynamics of *O. melanopus* have been well studied and documented across different regions in the world including southern Alberta. The phenology and host adaptability of the beetle differ from region to region. In general, the beetle is active from mid-March to July. Peak oviposition occurs in late March to early April when succulent hosts become available (Anderson and Paschke 1970; Wellso et al. 1973). Semiochemicals released by adult beetles may play a role in mate location (Cossé et al. 2002). Oat and barley are favoured for oviposition whereas pubescent varieties of wheat are least preferred (Gallun et al. 1966). Female fecundity and oviposition behaviour are determined by a variety of factors including plant nutrition, host morphology and other micro-climatic factors. Adult host feeding before and during oviposition greatly influences the rate of oviposition (Wellso et al. 1973).

Mating takes place on plants and eggs are laid along the leaf margins or close to the midrib (Piesik and Piesik 1998). Eggs are laid preferentially on central upper leaf surfaces on barley and wheat and on the central to basal region on oat (Wilson and Shade 1964). Leaf width influences the rate of oviposition. Sunlight and light intensities orient female beetles for oviposition (Wilson and Shade 1964). Eggs are laid singly or in multiple clusters of two or three eggs touching end to end (McPherson 1983a). The eggs hatch in about four to six days and the most favourable developmental temperature is about 21^{o}C (Barton and Stehr 1970). The ideal temperature range for egg development, however, is 12-

32°C (Guppy and Harcourt 1978). Each female lays about 50 to 275 eggs (Schmitt 1988). There are four larval instars and larvae tend to feed mainly on upper leaf surfaces between veins (Smith et al. 1971). Larvae in the field are smeared with a fecal coat during feeding which is lost eventually with the formation of the prepupa (Wellso 1973). Temperatures ranging between 8-32°C support larval growth with the ideal range being 12-28°C where survival rates exceed 70%; high mortality rates are common at 34°C. The development threshold may lie between 6 and 8°C (Guppy and Harcourt 1978).

Larvae are more damaging than adults and consume plant biomass one to 10 times their body weight (Livia 2006). The fourth-instar larva is photopositive, globose and has a characteristic fecal coat. It enters a prepupal stage before forming a pupa. The prepupa is elongate, lacks a fecal coat, and secretes adhesive material to form a cocoon using earthen material (Wellso et al. 1973). Prepupae of *O. melanopus* enter the soil at the base of the host plant and form pupal cases near the roots at a preferred depth of about 5 cm (Dysart et al. 1973). High temperatures negatively affect prepupae while variations in humidity are undesirable for adults (Wellso and Hoxie 1981b). First-generation adults emerge in about three weeks and feed on various grasses before overwintering until March-April (Grant and Patrick 1993). Preferred overwintering sites include edges of crops and woodlots, fence rows, sparse woods and dense woods (Casagrande et al. 1977). Within these sites, the beetles prefer field debris, crevices of bark and rolled leaves for overwintering (Piesik and Piesik 1998; Ulrich et al. 2004). Greater numbers of overwintering *O. melanopus* adults near

edges of stubble fields have also been reported in some instances (Sawyer and Haynes 1978).

Oulema melanopus has a single generation per year (Wellso et al. 1973). However, a short second generation was reported in Virginia, U.S.A. in spring cereals (McPherson 1983b). Factors such as late planting, lack of nitrogen fertilization and poor quality of soil can reduce field populations of *O. melanopus* (McPherson 1983b). *Oulema melanopus* attains pest status in the areas where a short spring is followed by dry summer spells (Stilmant 1995).

In general, with higher summer temperatures *O. melanopus* adults undergo a period of aestivation. Phenomena like diapause and aestivation are more prominently observable in female *O. melanopus* than the males (Wellso 1972). Attempts to understand *O. melanopus* reproductive physiology during aestivation have been made (Hoopingarner et al. 1965; Teofilovic 1969; Conin and Hoopingarner 1971; Wellso 1972). Female beetles aestivate under field conditions from mid-July to mid-September at prevailing high temperatures and then remain quiescent until the next spring, a period marked by undeveloped reproductive systems. The male reproductive physiology during aestivation and diapause is not completely understood and it is speculated that it depends primarily upon the physiological state and maturity of the females (Wellso 1972; Connin and Hoopingarner 1971). In support of these findings, Wellso (1972) found that there was no true reproductive diapause in *O. melanopus* males as presumably aestivating males had larger testes, sperm availability, and were capable of fertilizing females at any age.

In southern Alberta, beetle activity in the field begins from mid-April to May with the emergence of overwintered adults (Kher, unpublished data). Adults disperse to winter wheat fields, mate and begin ovipositing. Peak oviposition occurs in May. Larvae are active from May until July. Larvae are less mobile and do not usually move from one plant to another. Pupation occurs beneath the soil in July and teneral adults emerge in about three weeks. The adults feed for a short time on crop plants in late summer before dispersing to overwintering sites. Greater infestation levels have been observed in winter wheat than in spring cereals including spring wheat, oat and barley (Kher, unpublished data) (Fig. 1.2).

1.6. Host range

Oulema melanopus attacks many wild and domesticated grasses (Gutierrez et al. 1974). One of the early host range accounts of *O. melanopus* by Hodson (1929) considered wheat, oat and barley as primary hosts of the beetle. Venturi (1942) further suggested that *O. melanopus* is polyphagous within the family Gramineae with potential to feed on most grasses. However, Wilson and Shade (1966) identified some members of the Gramineae that exhibit antibiosis against *O. melanopus.*

The host plants of *O. melanopus* are categorized based on average larval survival as: “superior” (barley, oat and wheat), “favorable” (rye, timothy), “intermediate” (fescue), and “unfavorable” (grain sorghum, dent corn). Non-food plants were recognized as those on which there was no survival (Sudan grass, green foxtail, wild cane) (Wilson and Shade 1966). Further, metabolic pathways

of plants also contribute to host preferences of *O. melanopus*. For example, differences in feeding on plants with C3 and C4 photosynthetic pathways have been reported; higher preferences for C3 plants (wheat, barley, triticale) than for C4 plants (maize) are known (Wellso 1978).

Host physiological condition at certain developmental stages also influences host preferences of the beetle, particularly in oat and barley (Wellso 1973). For example, when plants of the same developmental stage of oat, wheat and barley were offered, the beetle adults and larvae preferred oat for feeding compared to wheat and barley. Preferences of *O. melanopus* for younger oat stands compared to older stands planted earlier in the season are reported (Hoffman and Rao 2010). Late-planted oat in summer is attractive to beetle populations for oviposition due to the succulent canopy late-emerging plants provide compared to that of early-planted stands. Tissue toughness of leaves is an important determinant of *O. melanopus* oviposition site selection, particularly in oat (Hoffman and Rao 2011). Leaves of higher insertion levels (position of leaves from base to apex) in oat with higher thickness and silica content are less attractive for oviposition compared to leaves with lower insertion levels that are succulent (Hoffman and Rao 2011).

Varied host preferences of *O. melanopus* across different geographical regions have been reported. For example, the beetle showed a preference for oat over barley and spring triticale in Poland (Piesik and Piesik 1998), for corn in Hungary (Pozsgai and Saringer 2006), and for soft red winter wheat and spring oat in some parts of the U.S.A. (Bailey et al. 1991). Variations in host preferences

over the geographic range may be influenced by local phenology of the beetle and the host plants. Local agronomic practices can significantly influence host preference of *O. melanopus*. For example, late-planted spring cereals are preferred for oviposition and feeding due to succulent canopy they produce compared to early-planted cereals in many regions (Hoffman and Rao 2011). Similarly, due to high early availability of winter wheat in southern Alberta, more beetle population hot-spots have been recorded in winter wheat fields than spring cereals. However, beetle populations were also recorded in some parts of the province where only spring cereals were available (Kher, unpublished data). Despite variations in host preferences, oat, wheat and barley are highly preferred hosts across the geographical range over which the beetle has established (Philips et al. 2011).

1.7. Damage potential

1.7.1. Direct damage

Both larvae and adults are the damaging life stages. Wheat seedlings are most prone to attack (Wilson and Shade 1966). Adult feeding is characterized by uniform longitudinal incisions on cereal leaves (A'Brook and Benigno 1972); however, this does not affect yield (Philips et al. 2011). Larvae are defoliators and feed on parenchymatous tissue and chlorophyll material (Buntin et al. 2004). Larval feeding is marked by elongated "windowpanes" in the leaves (Grant and Patrick 1993). Larval feeding leads to significant losses in crop yield quantity and quality due to reduced photosynthetic activity (Haynes and Gage 1981; Grant and Patrick 1993; Kostov 2001). Most crop damage is caused by the late larval instars

with the fourth instar alone responsible for about 70% of all damage. Greater infestations in spring grains compared to fall grains are reported (Ruppel 1972); however, patterns of infestations change from region to region. Plant growth stage and age also influence larval and adult damage. Higher population densities of *O. melanopus* are recorded at the early seedling stage than at later stages (Wilson et al. 1969).

Larval feeding at the flag leaf stage is most damaging to crop yield (Wilson et al. 1969). Area of the flag leaf and its succulence influence larval feeding of *O. melanopus* (Dimitrijević et al. 2001). Higher larval population densities on flag leaves with large surface areas are known. Similarly, higher feeding on succulent first and second flag leaves is observed compared to third flag leaves (Dimitrijević et al. 2001). The flag leaf is an important site for photosynthesis and determines grain filling and the plant's adaptability to stress in cereals, besides being a rich source of nitrogen for herbivorous arthropod pests (Dimitrijević et al. 2001). Hence, feeding at the flag leaf stage results in high yield reduction and crop losses. Also, transformation from the vegetative to the reproductive phase in wheat is critical to *O. melanopus* attack as this phase determines stem length and vigour (Webster et al. 1972).

Grain yield reductions primarily from flag leaf damage in Europe ranged from 3 to 8% in Poland (Ulrich et al. 2004) to 95% in The Netherlands (Daamen and Stol 1993) and 70% in central Europe (Stilmant 1995; Dimitrijević et al. 2001). In North America, yield losses of 55% in spring wheat, 23% in winter wheat, and 38 to 75% in oat and barley have been documented due to *O.*

melanopus infestations (Webster and Smith 1979; Royce 2000). In western Canada, the beetle has not yet reached pest densities that affect yield in most infested fields.

In Europe, the economic threshold level (ETL) is two to three larvae per tiller (Stilmant 1995). In North America, the threshold level is three eggs or larvae per plant at the boot stage and one larva per flag leaf at the flag leaf stage (Webster and Smith 1983). Further, ETLs of 25 eggs or small larvae per 100 tillers are considered for timing insecticidal sprays in some areas of the U.S.A. (Philips et al. 2011). No specific ETLs have been determined for *O. melanopus* management in Canada. Nominal ETLs are prescribed in different provinces. For example, an ETL of one *O. melanopus* adult or larva per stem is prescribed in Ontario (OMAFRA 2011), while the ETL is one larva per flag leaf after the boot stage in British Columbia (Government of British Columbia 2011).

1.7.2. Indirect damage

A'Brook and Benigno (1972) reported that *O. melanopus* and *O. lichenis* can carry and transmit cocksfoot mottle virus and phleum mottle virus to its host. They further suggested that longer acquisition periods could result in more effective virus transmission and if ingested together, only one of the two viruses could be effectively transmitted by the beetle. This highlights possible indirect damage that the beetle can cause to its hosts. However, there have not been any other reports that suggest any instances of such transmissions causing economic losses.

1.8. Plant responses to *O. melanopus* damage

Host plant responses to herbivore attacks by biochemical means and chemical signaling by production of volatile compounds are known (Karban and Baldwin 1997; Piesik et al. 2010). Wheat plants respond to the mechanical injury caused by adult feeding damage of *O. melanopus* and *O. cyanella* by production of green leaf volatiles such as linalool and a terpene compound, β-carophyllene (Piesik et al. 2010). *Oulema melanopus* and *O. cyanella* adults are attracted to plant volatiles such as (Z)-3-hexanal and (Z)-3-hexanyl acetate from wheat at low concentrations that incite feeding; however, higher concentrations of these compounds mixed with linalool result in deterrence (Piesik et al. 2010). Further research indicated that such a response of production of volatile compounds was not limited to wheat alone but also observed in oat and barley (Piesik et al. 2011). Barley and wheat both produced higher concentrations of β-linalool oxide compared to oat. The array of other volatile compounds produced as a response to *O. melanopus* adult feeding damage included compounds such as: (Z)-3-hexenal, (E)-2-hexenal, (E)-2-hexenol, (Z)-3-hexenyl acetate and (Z)-1-hexenyl acetate, and (E)-β-farnesene (Piesik et al. 2011). However, these compounds were produced in different amounts. The release of these volatile compounds from infested plants due to herbivore injury induces production of volatiles from neighbouring uninfested plants as a defense mechanism (Piesik et al. 2010). The extent of volatile production by undamaged plants depends on their distance from injured plants (the greater the distance the less the production). A recent report

indicates that *O. melanopus* adults are repelled by production of high concentrations of *cis*-jasmones, terpenes and indoles (Delaney et al. 2013). However, further research attention is needed to understand the effects of manipulation of chemical signals released by plants and their potential role in managing *O. melanopus* populations.

1.9. Overview of *O. melanopus* control strategies

Tactics for controlling *O. melanopus* in North America include quarantine, chemical control, cultural control, plant resistance, and classical biological control using its native natural enemies from Europe (Webster et al. 1978; Philips et al. 2011). Attempts to sterilize males with radiation were not successful due to high mortality from the irradiation and low beetle survival on artificial media. No effective attractants are known (Haynes and Gage 1981). Integrated management strategies including biological control and plant resistance, among others, were implemented (Haynes and Gage 1981; Bailey et al. 1991; Grant and Patrick 1993). Initial efforts to control the pest included detection, eradication, containment and implementation of host plant resistance programmes (Haynes and Gage 1981). However, after successful introduction and establishment of the parasitoid complex from Europe in various parts of the U.S.A. and Canada, *O. melanopus* has been controlled very effectively with biocontrol strategies (Haynes and Gage 1981; LeSage et al. 2007; Philips et al. 2011).

1.9.1. Chemical Control

Oulema melanopus management initially relied heavily on the use of insecticides (Anonymous 1963). Wellso (1982) reported that chemical control was a must for the control of *O. melanopus* within six years of its introduction, especially in the eastern U.S.A. where the spread and crop damage were extensive. For example, an area of about 39,000 acres was under insecticide-based management in Michigan, U.S.A., where the beetle was first discovered (Anonymous 1963). A similar pattern of heavy insecticidal use was reported in the newly infested western regions of the U.S.A. upon rapid range expansion by *O. melanopus* (Ruppel 1972).

Chemicals used for *O. melanopus* management included compounds like carbofuran (soil application) and endosulfan (foliar sprays) (Merritt and Apple 1969; Webster et al. 1972). In the absence of insecticides such as carbofuran, yield reductions in oat of up to 62% were observed (Merritt and Apple 1969). Synthetic pyrethroids such as permethrin, cypermethrin and fenvalerate were effective at low doses and were biodegraded by the plant, but were found to be lethal to the natural enemies of the pest, mainly the parasitoid wasp, *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae) (Coats et al. 1979). Use of chemicals may adversely affect the survival and development of natural enemies thus hampering the process of natural control of the pest, and such reliance on chemical insecticide might favour pest outbreaks. Seed treatment in barley with imidacloprid caused about 40% mortality of *O. melanopus*, while foliar sprays caused about 90% mortality in the cereal leaf beetle population (Tharp et al. 2000). In view of the negative effects of insecticides on *O. melanopus* natural

enemies, it is recommended that insecticides should be used only when necessary (Philips et al. 2011).

1.9.2. Cultural Control

In North America, few studies have focused on effects of cultural and agronomic practices on the management of *O. melanopus*. General guidelines for beetle management recommend early planting and maintaining uniform crop stands to mitigate damage by *O. melanopus* (Philips et al. 2011). Here I review a few approaches for *O. melanopus* management using cultural practices.

1.9.2.1. Seeding rate manipulation

Lower seeding rates in oat to mitigate *O. melanopus* attack were successful in some regions on a limited scale (Webster et al. 1978). This might be due in part to the resultant sparse crop stand, the capacity of oat to compensate for beetle damage, and differences between temperature thresholds of oat and the beetle. In colder growing seasons, oat can develop well and compensate for any damage even at low temperatures between 3 to 4^{o}C while the temperature threshold for the beetle is about 9^{o}C. This difference favours oat over the pest. Sparse crop stands result in fewer eggs and larvae per unit area and thus less damage. However, due to the limited success of this approach it is generally not recommended to seed at rates lower than recommended (Webster et al. 1978).

1.9.2.2. Mixed cropping

The effect of mixed cropping vs. mono cropping of cereal hosts on *O. melanopus* damage has been studied in Europe. No studies on effects of cropping diversity on *O. melanopus* damage to host crops were found from North America. Mixed cropping of oat with barley in Poland lowered yield losses by about 9% in oat by providing the opportunity for complementary feeding by the pest while the host plant compensated for the damage (Piesik and Piesik 1998). Fields seeded to oat alone suffered substantial losses of up to 25%; a mixture of barley and oat reduced the yield loss to 16%. Mixing barley with other cereals and crops like pea reduced *O. melanopus* damage significantly (Piesik and Piesik 1998).

1.9.2.3. Plant nutrition

Plant nutrition and judicious fertilizer use also affect pest development. Adding a combination of nitrogen and potassium fertilizers in spring wheat proved to be an effective cultural practice against *O. melanopus*. Nitrogen contributed to high crop vigour while potassium induced early crop maturity before the larvae had attained their peak activity. Potassium also imparted unpalatability to host plants that reduced pest damage (Dimitrijević et al. 1999).

1.9.3. Host Plant Resistance

The importance of resistance breeding against *O. melanopus*, in view of the economic and ecological consequences of chemical control, is well documented (Papp and Masterhazy 1996). Two potential mechanisms in cereals against *O. melanopus* infestation are antixenosis and antibiosis (Gallun et al.

1966; Schillinger 1966; Wellso 1973; Hoxie et al. 1975; Wellso 1979). Trichomes or pubescence (plant hairs) deter feeding, oviposition or both, resulting in "non-preference" (Price et al. 1980). Mechanisms of resistance in wheat have been widely explored (Everson et al. 1966; Gallun et al. 1966; Ringlund and Everson 1968; Smith et al. 1971; Wallace et al. 1974), and wheat demonstrates strong resistance mechanisms compared to oat and barley (Hahn 1968). Leaf pubescence in wheat can deter oviposition and affects hatchability, larval survival and adult feeding on resistant wheat varieties (Gallun et al. 1966; Wellso 1973; Hoxie et al. 1975; Papp et al. 1992). The source of resistant germplasm for *O. melanopus* is concentrated mainly in Asia Minor and south-eastern Europe (Ringlund and Everson 1968; Hahn 1968) and initial efforts to control the pest focused on exploration of resistant germplasm in small grain host crops, such as wheat (Gallun et al. 1966; Wellso 1973) and barley (Hahn 1968). Trichomes of pubescent wheat varieties contain silica which imparts indigestibility (Wellso et al. 1973). Narrow-leaved cereal varieties also resist larval feeding by limiting the space for feeding and larval activity (Shade and Wilson 1967).

Mechanisms associated with resistance in barley could be non-preference by *O. melanopus* larvae and differential egg laying by adult females (Hahn 1968). In their review of the diversity of germplasm in small grains, Reitz and Craddock (1969) noted that resistant varieties of barley to *O. melanopus* were reported from Poland, Russia, Turkey and Iran. In North America, extensive screening of barley germplasm for *O. melanopus* resistance involving approximately 8500 genotypes indicated resistance in less than one percent of genotypes. Two improved resistant

lines of barley, namely CI15820 and CI15821, were produced but never released commercially (Porter et al. 1998).

Antibiosis mechanisms for *O. melanopus* are known in wheat (Schillinger 1966; Ringlund and Everson 1968; Wellso 1979), oat (Steidl et al. 1979), barley (Hahn 1968), and corn (Wellso 1981). Glandular trichomes in certain wheat genotypes exert antibiotic effects on *O. melanopus* eggs and larvae (Wellso 1979). Reduced feeding and fitness of *O. melanopus* larvae on plants with very low trichome density are indicative of the presence of associated mechanisms of antibiosis (Ringlund and Everson 1968). The genes controlling leaf pubescence in wheat genotypes may be linked with genes responsible for chemical antibiosis (Ringlund and Everson 1968); however, such associations have not been explored. Cereal varieties with greater trichome density may also exert antibiosis effects on *O. melanopus* in addition to antixenosis. For example, lower fitness and feeding of the beetle larvae on some pubescent wheat varieties were reported in the U.S.A. and were attributed to antibiosis rather than to antixenosis (Smith and Webster 1974). Strong biochemical antibiosis resulting in low larval weight gains and reduced fitness is known in oat genotypes (*Avena sterilis* L.) (Steidl et al. 1979). There are no studies elaborating biochemical antibiosis in wheat genotypes.

Production of volatile compounds by some host species can have antibiotic effects on *O. melanopus* larvae. For example, a secondary volatile chemical, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (also called DIMBOA), in corn negatively affects the growth of overwintered adults (Wellso

1978). Genetically engineered varieties expressing genes of *Bacillus thuringiensis* Berliner (Bt) producing Cry3Bb1 crystal proteins did not lower *O. melanopus* damage (Meissle 2012). When feeding and development of *O. melanopus* larvae and adults on Bt corn cultivars were studied, the expression of Cry3Bb1 proteins affected to some extent only the neonate larvae; the adults and older larval instars did not show mortality or developmental failures (Meissle 2012).

Associations of seed-borne fungal endophytes such as *Neotyphodium* sp. (Ascomycota: Hypocreales: Clavicipitaceae) are known to induce resistance in plant hosts to pest species by deterrence or production of antibiotic substances (Popay 2009; Clement et al. 2011). However, the results of a study testing the effects of inoculation of wild alpine timothy grass, *Phleum alpinum* L., with the fungal endophyte, *Neotyphoidium* sp., indicated that survival and development of *O. melanopus* larvae was not affected by host association with the endophyte, while the populations of bird cherry oat-aphid, *Rhopalosiphum padi* (L.), were suppressed (Clement et al. 2011).

Some important constraints in exploiting resistant germplasm for cereal leaf beetle control include difficulties in locating resistance sources, low success with crossing, narrow germplasm adaptation and more importantly, the association of resistance with lower yields (Kostov 2001). Nonetheless, host plant resistance deserves consideration as a component of integrated pest management.

1.9.4. Biological Control

Biological control with introduced natural enemies has been the most successful strategy for managing *O. melanopus* (Wellso 1982). The fauna of natural enemies of the cereal leaf beetle includes insect predators, parasitoids, mites and some bird species (Schmitt 1988).

Early attempts of biocontrol included screening native bioagents in Europe for their possible introduction to North America (Wellso 1982). The Plant Protection Division of the United States Department of Agriculture (USDA) initiated a biological control program for screening, culturing and colonization of imported natural enemies of *O. melanopus* at Niles, Michigan (Maltby et al. 1969, 1971; Anderson and Paschke 1970). Research on biological control of *O. melanopus* has focused mainly on conservation biocontrol rather than inundative releases of natural enemies. Economic considerations make the conservation approach more suitable than mass field releases based upon artificial parasitoid production in the laboratory (Barton and Stehr 1970).

The species of larval parasitoids introduced for biological control include a eulophid, *T. julis,* and two other species, *Diaparsis carinifer* (Thomson) and *Lemophagus curtus* (Townes) (Hymenoptera: Ichneumonidae), and an egg parasitoid, *Anaphes flavipes* (Foerster) (Hymenoptera: Mymaridae) (Haynes and Gage 1981; LeSage et al. 2007). Mass multiplication of *T. julis* in the laboratory for field release is difficult due to limited success in parasitoid reproduction and development (Dysart et al. 1973). This gave rise to the successful concept of "field nurseries" in which the parasitoids are reared in a protected field area with *O. melanopus* infestations to support parasitoid growth under natural conditions;

parasitized larvae are then relocated to infested fields (Dysart et al. 1973; Logan et al. 1976; Harcourt et al. 1977). *Tetrastichus julis* remains the most successful parasitoid of *O. melanopus* and the wasp is well established in North America due to its high synchronization with the host and capacity to track its host as the host range expands geographically (Haynes and Gage 1981).

1.9.4.1. The principal parasitoid: *Tetrastichus julis*

Tetrastichus julis was initially introduced as part of an *O. melanopus* biocontrol program in 1967 in Michigan and has successfully established in the U.S.A. and Canada since then (Dysart et al. 1973; Evans et al. 2006). After its introduction, *T. julis* was first recovered in 1969 from relocated fields (Gage and Haynes 1975). It is a host-specific, bivoltine, gregarious larval endoparasitoid of *O. melanopus* (Dysart et al. 1973; Haynes and Gage 1981; Evans et al. 2006). The parasitoid lays about four to six eggs per host larva and attacks all instars, but the young larvae are preferred (Dysart et al. 1973). It overwinters in the larval stage within the pupal cell of its host at a soil depth of 5 cm (Leibee and Horn 1979). Overwintered females parasitize mostly early-developing beetle larvae at the beginning of the season (Evans et al. 2006). The parasitized host larva dies after pupation (Dysart et al. 1973; Gage and Haynes 1975). Parasitization is high in spring with a peak in mid-May to June. However, the second generation of adults also parasitizes late-maturing host larvae (Dysart et al. 1973; Haynes and Gage 1981; Staines 1984). *Tetrastichus julis* is equally active in oat, wheat and barley (Evans et al. 2006). High temperatures during mid-June can induce a period of

quiescence (called diapause) until favourable temperatures return (Nechols et al. 1980).

High temperatures have a role in diapause maintenance during late summer and early fall, while short photoperiods affect diapause advancement and termination. Termination of diapause follows post-diapause dormancy. Temperature also determines spring and summer emergence of parasitoid adults (Gage and Haynes 1975). The development threshold of *T. julis* is 8.99^{o}C (=48 ^{o}F). Thus, diapause mechanism in *T. julis* is governed by two factors unlike in many other hymenopteran parasitoids in which either of the two factors regulate diapause maintenance (Nechols et al. 1980). As synchronization of adult parasitoids with suitable host stages determines parasitization success (Evans et al. 2006), mechanisms like diapause play a major role in synchronization of the *T. julis* life cycle with the host (Nechols et al. 1980).

In eastern Canada, *T. julis* has established, and parasitism rates range from 14 to 95% (Harcourt et al. 1977). In western Canada, the parasitoid was first introduced to wheat fields in the Creston Valley, British Columbia by relocating parasitized larvae from Missoula, Montana, U.S.A. (WCCP 2002). Since its introduction, the parasitoid has dispersed naturally along with *O. melanopus*. Especially in Alberta, the parasitoid is established and reduces *O. melanopus* populations to varying degrees depending on site and year (Dosdall et al. 2011). Parasitization occurs from mid-late May and continues until July. Peak parasitization in June is the usual trend. Second-generation *T. julis* parasitize late-maturing *O. melanopus* larvae and such parasitoid larvae overwinter inside

infested larval cocoons and start the cycle again (Kher, unpublished data) (Fig. 1.3).

Several factors can determine parasitization success. Early studies on *T. julis* emergence suggest that field plowing and the depth to which the cocoons containing overwintering parasitoid larvae are placed influence emergence of parasitoid adults. It is suggested that minimum tillage operations be followed in the regions undergoing annual tillage and disking (harrowing) to avoid damage to overwintering parasitoid populations (Leibee and Horn 1979). Intensive tillage in tobacco-cereal rotations in Ontario, Canada resulted in the absence of parasitism of *O. melanopus* larvae by *T. julis*, and tillage killed about 95% of overwintering parasitoids in the soil (Ellis et al. 1988).

Further, provision of nectar/sugar sources (such as sucrose) early in the season after *T. julis* emergence can significantly influence *T. julis* activity. However, time of targeted provision of sugar is critical and late applications do not increase parasitization success (Evans et al. 2010). Annual variations in climate, particularly temperatures, can significantly influence population dynamics and parasitization success of *T. julis* (Evans et al. 2013). Warmer springs cause phenological mismatch between *O. melanopus* and *T. julis*, and can reduce parasitization success (Evans et al. 2013).

1.9.4.2. The egg parasitoid: *Anaphes flavipes*

The second major parasitoid of *O. melanopus* is the egg parasitoid, *A. flavipes*, which was first discovered from *O. melanopus* eggs in Pandino, Italy in

1964 (Dysart 1971). It is a minute species measuring less than 1 mm, and was first released in the U.S.A. in 1966 (Maltby et al. 1971). *Anaphes flavipes* has a Europe-wide distribution including Spain, France, Italy, Germany and Yugoslavia (Anderson and Paschke 1970). The parasitoid lays varying numbers of eggs in host eggs (with a maximum of eight eggs observed per host egg) which develop in about 10 to 11 days at 21^{o}C (Barton and Stehr 1970). The optimum temperature range for parasitoid development is 10-35^{o}C with temperatures above 35^{o}C being lethal and below 10^{o}C arresting growth (Anderson and Paschke 1969). The females emerging from parasitized eggs start active host searching, parasitize new host eggs within one hour of emergence, and deposit both fertilized and unfertilized eggs inside host eggs. Fertilized eggs develop into females whereas unfertilized eggs developed into males, and the males always emerge earlier than females. Despite its establishment in some parts of North America, the parasitoid is not as successful as *T. julis* due to its asynchrony with peak oviposition activity of the beetle (Dysart 1971).

1.9.4.3. Other introduced parasitoids

Brief accounts of the life histories of other introduced parasitoids, *L. curtus* and *D. carinifer*, were presented by Dysart et al. (1973). *Lemophagus curtus* is a solitary, multivoltine, larval endoparasitoid of *O. melanopus*. It is present in most parts of Europe where it is abundant in southern Europe compared to northern Europe. The overwintering behaviour is not completely understood but the adults are most likely the overwintering stage, overwintering inside the

host cocoons. Females generally lay one egg per *O. melanopus* larva; in cases where higher numbers of eggs are laid only one adult parasitoid emerges (Dysart et al. 1973).

Diaparsis carinifer is a univoltine, solitary larval endoparasitoid of *O. melanopus* (Dysart et al. 1973), and is present throughout Europe across the range of *O. melanopus*. Unlike other larval parasitoids, it forms its own cocoon within the host pupal cell and overwinters inside the cocoon as a final-instar larva. The females cannot discriminate between already parasitized larvae by conspecific females and also by the females of other parasitoid species.

In Europe where all species are present in *O. melanopus*-infested areas, the activity of *T. julis* and *D. carinifer* coincide but precede that of *L. curtus* by one week. *Lemophagus curtus* continues to oviposit after the other parasitoids have ceased their activity (Dysart et al. 1973).

1.9.4.4. Other natural enemies

Other natural enemies of *O. melanopus* include parasitoids, predators, pathogens and nematodes. The parasitoid of adult beetles, *Hyalomyodes triangulifer* (Loew) (Diptera: Tachinidae), was reported from Michigan (Wellso and Hoxie 1969) and North Dakota (Anonymous 2002). *Trichogramma* sp. is an egg parasitoid in Michigan (Maltby et al. 1969). *Meigenia mutabilis* (Fallén) (Diptera: Tachinidae) is a larval-pupal parasitoid of *O. melanopus* in Russia (Bjegovic 1967, 1968). Sedivy (1995) reported over 10 species of hymenopterous parasitoids in the Czech Republic, including the eulophid species, *Necremnus*

leucarthros (Nees), as a gregarious pupal parasitoid. Coccinelid predators like *Hippodamia parenthesis* (Say), *H. tredecimpunctata* Linneaus and *Coccinella novemnotata* (Herbst) are egg predators, while *Coleomegilla maculata* (De Greer) and *Hippodamia convergens* Guerin prey on eggs and larvae (Shade et al. 1970; Bragg 2009). A predatory neuropteran, *Chrysopa* sp., has also been recorded to attack eggs and larvae (Speyer 1954 cited in Schmitt 1988, page 484). An egg predator, *Nabis feroides* Remane (Hemiptera: Nabidae), is known from Russia (Bjegovic 1968).

Pathogens include fungi such as *Alternaria alternata* Keissler, *Isaria farinose* (Holmsk.) and *Verticillium lecanii* (Zimmerman) Viegas (Machowicz-Stefaniak and Miczulski 1985). The entomopathogenic fungus, *Beauveria bassiana* Vuillemin, is known to impact beetle populations (Paschke 1965). *Oulema melanopus* adult beetles treated with conidia of *B. bassiana* showed a significant reduction in activity and feeding 48 h after the treatment at 26^{o}C (Paschke 1965). However, little research has been conducted to optimize efficiency of this pathogen, probably because the crop value is too low relative to the cost of these alternative biopesticides. The nematode species, *Steinernema carpocapsae* (Weiser), is also reported as a biocontrol agent of *O. melanopus* (Laznik et al. 2010). *Steinernema carpocapsae* strain C101 resulted in greater than 80% mortality when overwintered *O. melanopus* adults were treated with juveniles at varying doses from 250-1000 juveniles/adult (Laznik et al. 2010) under laboratory conditions.

1.9.5. Other control approaches for O. melanopus

Mass trapping of *O. melanopus* has received some attention in capturing post-overwintering beetle populations dispersing to fields. Wilson and Shade (1967) studied the relative effectiveness of luminescent colours in attracting *O. melanopus* adults to develop a survey method to detect the presence of the beetle in cereal fields. Boards painted lemon yellow attracted more *O. melanopus* adults compared to orange-yellow with intermediate attraction to red and green and low to blue, pink and white (Wilson and Shade 1967). However, further reports are not available on utilization of colour traps in *O. melanopus* scouting.

The role of semiochemicals in *O. melanopus* adult communication is known (Cossé et al. 2002). A male-specific compound, (*E*)-8-hydroxy-6-methyl-6-octen-3-one, elicits strong responses in both males and females (Cossé et al. 2002). Field evaluations further confirmed that this compound produced by males attracted both males and females of *O. melanopus* (Rao et al. 2003). The mean emission rates of 6.7 ng/day have been observed in males with some males releasing quantities as high as 20 ng/day (Cossé et al. 2002). In field studies, traps baited with (*E*)-8-hydroxy-6-methyl-6-octen-3-one attracted three times more beetles at the dose of 500 μg than control traps, and acted as an aggregation pheromone for *O. melanopus* males and females migrating to spring cereal fields from overwintering sites (Rao et al. 2003). The study suggested using higher concentrations of the synthetic compound for baiting in field studies with a need for development of suitable traps. However, commercial use of this technique for trapping *O. melanopus* populations has not been reported.

1.10. Objectives of the study

The current overview of *O. melanopus* as a significant insect pest in North America underlines the fact that using only a single control strategy cannot guarantee population control; rather, a management system with judicious integration of possible approaches can optimize pest management. In the western Canadian context, the recent invasion by *O. melanopus* provides an opportunity to study and understand the initial dispersal characteristics of the cereal leaf beetle. This is fundamentally important for understanding community assembly dynamics, and initial colonization and dispersal of a pest is the best stage during which to implement management efforts (Crooks and Soule 1999).

The natural occurrence of *T. julis* in southern Alberta with its range expansion along with its host provided an added advantage in that no importations for release of the principal parasitoid were required. Management of *O. melanopus* can therefore be directed toward strengthening biological control efforts by augmenting the activity of *T. julis*. However, this will be facilitated by an enhanced understanding of the interactions between the beetle and its parasitoid in this new eco-region. In view of this need to enhance knowledge relating to this pest management opportunity, the overall goal of my study was to develop an understanding of factors influencing the dynamics of tritrophic interactions between cereal hosts, *O. melanopus* and *T. julis* through studies on their life histories, host preferences, and their field dynamics on a spatio-temporal scale. I further expanded my studies to include the component of host plant

resistance as a potential underexploited tool within the integrated management framework.

In Chapter 2, host preferences and fitness attributes of *O. melanopus* were tested on different cereal crops that can act as potential hosts of the beetle in western Canada. Western Canadian cereal agro-ecosystems provide a wide variety of hosts for the beetle and it is of particular interest to evaluate the performance and fitness of the beetle on these hosts. The study was designed to quantify life history parameters of the beetle on major cereal crops (wheat, oat, barley, corn, rye and triticale), and so assess the potential suitabilities of different hosts. I tested the hypothesis that *O. melanopus*, being an oligophagous pest of cereals, would feed on all major cereal host species and cultivars equally, and each major crop host would confer equivalent fitness to its offspring.

Biological control of *O. melanopus* in North America using *T. julis* is perhaps the most successful example of a classical biological control programme implemented for a major pest of field crops in North America. *Tetrastichus julis* has contributed significantly to the natural suppression of beetle pest populations and it mitigates crop losses. Despite five decades of research on the *O. melanopus-T. julis* host-parasitoid system, relatively little is known about host affinities and host adaptability strategies of *T. julis*. Although the general life history of the parasitoid is known, it is not known whether *T. julis* employs certain strategies to adapt to its host or whether host-specific preferences can help the parasitoid to gain fitness in terms of its gregariousness. Also, the cues associated with the host-finding behaviour of *T. julis* for the beetle are not known. The fecal

coat of *O. melanopus* larvae may have a role in orienting parasitoids to the host (Wellso and Hoxie 1988); however, such a role has not been investigated. In Chapter 3, my experiments were designed to understand host-specific preferences of *T. julis*, and the role of the olfactory cues associated with *O. melanopus* larvae in the host-finding behaviour of *T. julis*. Specifically, my objectives were to investigate developmental parameters of *T. julis*, clutch size characteristics, *T. julis* preferences for different host instars, the influence of host instar on gregariousness of the parasitoid, and cues involved in parasitoid host-finding. I tested the hypothesis that *T. julis* is capable of discriminating between life stages of its hosts and can adjust its clutch size depending on the larval host stage, thereby maintaining its populations in synchrony with the host. With regard to the cues associated with host-finding, I tested the hypothesis that olfactory cues associated with the larval fecal coat of *O. melanopus* contribute to host-finding by *T. julis*.

In Chapter 4, I investigated within-field distribution dynamics and tritrophic interactions between a cereal host, *O. melanopus* and *T. julis* in relation to host plant nutrition and plant vigour metrics. The major goal of my investigation was to understand how the host-parasitoid interactions take shape in a particular agroecosystem at an early phase of invasion on a spatio-temporal scale. Specific objectives were to investigate the distribution dynamics of *O. melanopus* and *T. julis* in winter wheat crops in southern Alberta and to test whether plant vigour and nutrition had bottom-up effects on *O. melanopus* and its principal parasitoid. Understanding such interactions is crucial for developing

site-specific management strategies. My hypothesis is that *O. melanopus* colonizes areas with vigourous plant stands and areas of high nutritional availability within fields, thus forming larval population hot spots that in turn influence the population structure of *T. julis*.

In Chapters 5 and 6, I explored resistance mechanisms in exotic wheat genotypes procured from central Asia. The genotypes I tested were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), Syria and originated from Uzbekistan and Kyrgyzstan. This region is known to have well established *O. melanopus* infestations, and certain wheat genotypes are reported to possess putative resistance against *O. melanopus* oviposition and feeding. However, resistance of these wheat genotypes to the North American biotype of the beetle is not known, and the underlying resistance mechanisms of these wheat genotypes have not been explored. An understanding of such mechanisms can provide potential sources for future resistance breeding programmes for cereals for *O. melanopus* management not only in Canada but also in other regions where beetle infestations pose serious problems.

Resistance in wheat to *O. melanopus* is extensively studied and non-preference is the major modality of resistance. Hence, I first explored whether antixenosis is the underlying modality of resistance in the genotypes procured (Chapter 5). In Chapter 5, I reported the results of oviposition and feeding behaviour of *O. melanopus* adults on six genotypes with putative antixenosis resistance. I tested the hypothesis that host plant resistance exists in some

genotypes and non-preference for feeding and oviposition will be the underlying mechanism as previously observed in the most resistant genotypes of wheat.

I then tested the role of antibiosis as a resistance mechanism in the exotic genotypes (Chapter 6). Antibiosis upon feeding by adults and larvae negatively influences pest physiology. However, little research attention has focused on antibiosis in wheat genotypes. In several crops, mechanisms of antixenosis and antibiosis can overlap. Understanding antibiotic effects requires detailed studies on the biology of the herbivore on resistant hosts. Here I reported results of my laboratory assessment of effects of exotic genotypes on the development and survivorship of *O. melanopus* life stages. I tested the hypothesis that some genotypes exert negative effects on the developmental physiology of *O. melanopus* larvae, indicating the presence of antibiosis as a resistance modality.

In Chapter 7, I present a general discussion and synthesis of observed results from a series of laboratory- and field-based experiments from the different research themes discussed above. I discuss implications of my results for each experimental objective for the management of *O. melanopus* in the Prairies and provide a general framework of management for future consideration.

Figures.

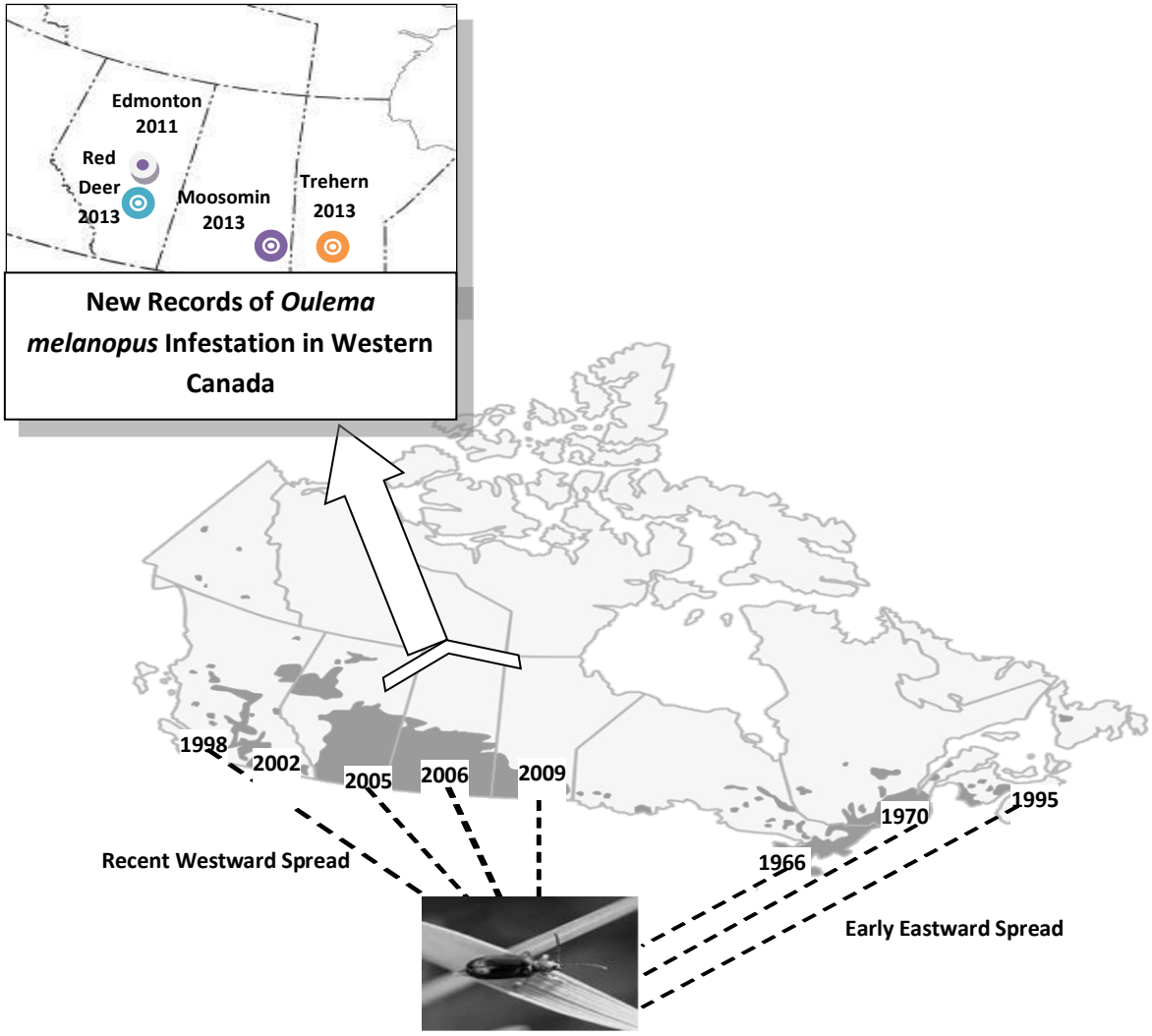

Figure 1.1. Historical trajectory of *Oulema melanopus* in western Canada

Illustration: Swaroop Kher (Source: Kher, unpublished data)

Photo credit: Cereal leaf beetle adult: Dr. L. M. Dosdall

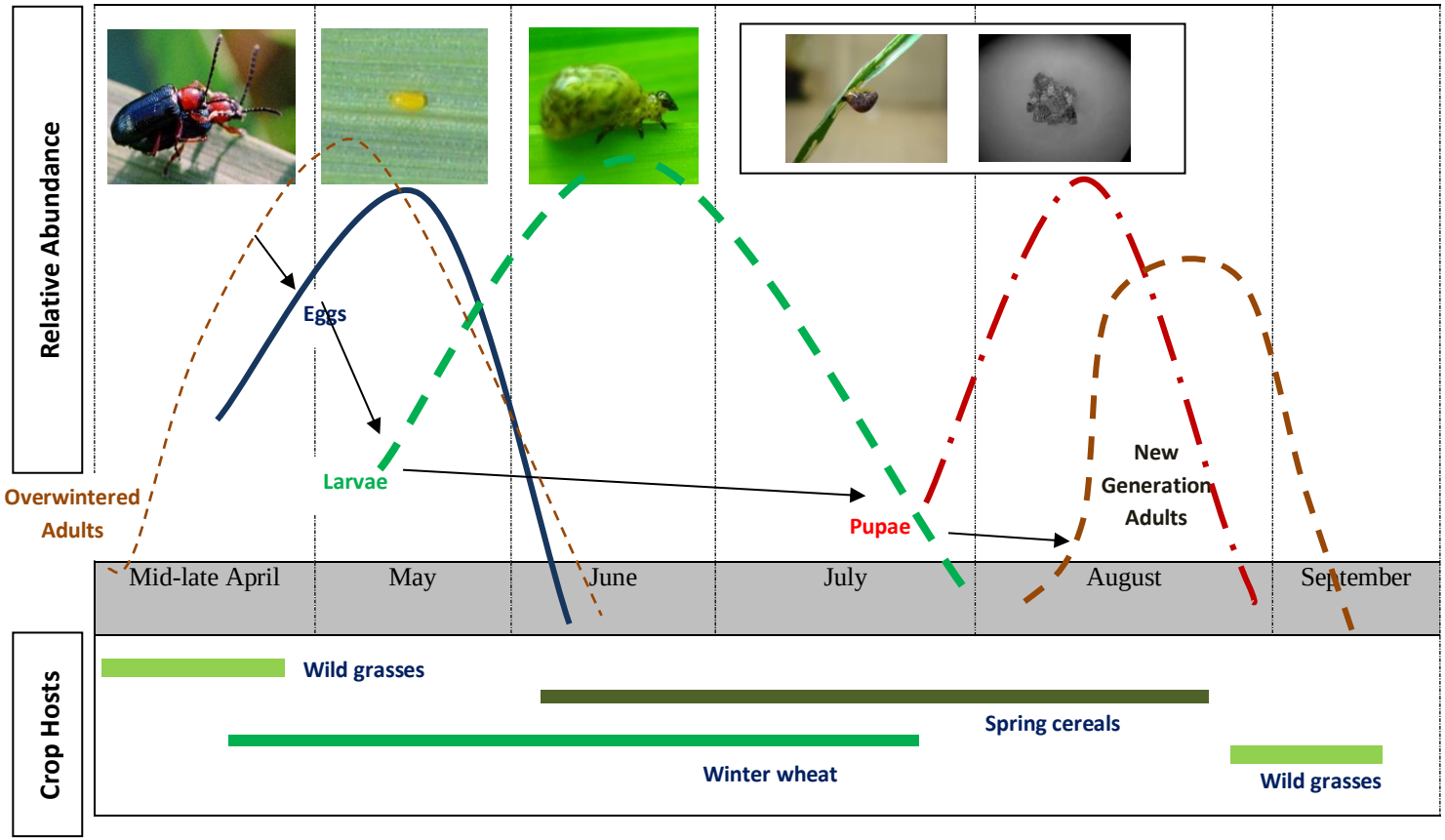

Figure 1.2. Phenology of *Oulema melanopus* in southwestern Canada (Alberta and Saskatchewan)

Illustration and photos: Swaroop Kher (Source: Kher, unpublished data)

Photo credit: Mating cereal leaf beetle adults on leaves by Dr. L. M. Dosdall

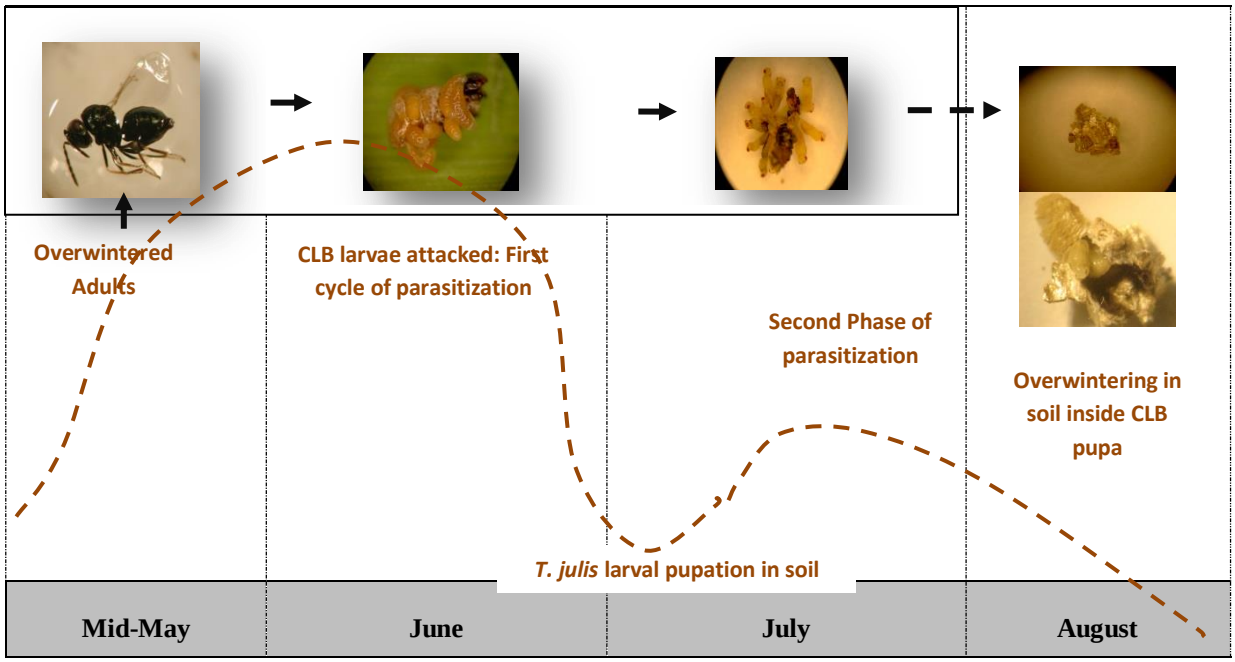

Figure 1.3. Phenology of *Tetrastichus julis* in southwestern Canada

Illustration and photos of developmental stages of *T. julis*: Swaroop Kher (Source: Kher, unpublished data)

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Chapter 2: Biology and host preferences of the cereal leaf beetle, *Oulema melanopus* (Coleoptera: Chrysomelidae), in western Canada

2.1. Introduction

Since its discovery in North America in Michigan, U.S.A. in 1962 (Dysart et al. 1973), the cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), has expanded its geographic range significantly, encompassing most regions of cereal production in the U.S.A. and Canada (Ihrig et al. 2001; Buntin et al. 2004; Dosdall et al. 2011). The beetle is native to Europe and Asia where it is an important pest of cereals (Kostov 2001). Its invasion of western Canadian provinces is recent (Dosdall et al. 2011; Kher et al. 2011). Within a short time period following its discovery, the beetle expanded its geographic range over a vast area, invading portions of the western Canadian provinces of British Columbia, Alberta, Saskatchewan, and Manitoba (CFIA 2008; Dosdall et al. 2011).

Adult and larval feeding of *O. melanopus* can cause yield losses as high as 55% in spring wheat, 23% in winter wheat and 38-75% in oat and barley (Webster and Smith 1979; Royce 2000). In Canada, the pest is predicted to spread across all cereal-growing regions (Olfert et al. 2004). Establishment of this pest thus has several economic implications for grain production, trade and export.

Oulema melanopus is univoltine and active in the field from May to August with the peak oviposition period being late May to mid-June in western Canada (Kher et al. 2011). Female fecundity ranges from 50 to 275 eggs (Schmitt

1988), and eggs are laid on upper surfaces of leaves either singly or in multiple clusters (Piesik and Piesik 1998; McPherson 1983). An incubation period of four to six days (Barton and Stehr 1970) is followed by a larval period consisting of four instars that feed on adaxial leaf surfaces (Smith et al. 1971). Larval feeding leads to significant losses in crop quantity and quality due to reduced photosynthetic activity (Haynes and Gage 1981; Grant and Patrick 1993; Kostov 2001), particularly at the flag leaf stage (Wilson et al. 1969). Pupation occurs in the soil by forming earthen cocoons (Dysart et al. 1973); teneral adults emerge in about three weeks and feed on various monocotyledonous plants before overwintering until late April of the following spring (Grant and Patrick 1993; Kher et al. 2011).

Oulema melanopus attacks many wild and domesticated members of the family Gramineae (Gutierrez et al. 1974). Venturi (1942) considered *O. melanopus* to be "polyphagous" within the Gramineae; however, Wilson and Shade (1966) showed that larval survival and overall performance differed among cereal species. Broadly, wheat, oat and barley are recognized as "superior" hosts while corn is an "unfavourable" host; grasses such as Sudan grass and green foxtail exert antibiosis on beetle larvae, and therefore are "non-host" plants (Wilson and Shade 1966). However, variation in host preferences of *O. melanopus* in different eco-regions is known (Hodson 1929; Wilson and Shade 1966; Philips et al. 2011). For example, preferences were reported for oat, wheat and barley in the UK (Hodson 1929), for oat over barley and spring triticale in Poland (Piesik and Piesik 1998), for corn in Hungary (Pozsgai and Saringer

2006), and for soft red winter wheat and spring oat in some parts of the U.S.A. (McPherson 1983; Bailey et al. 1991). However, the fitness consequences of making such host choices remain to be elucidated (Philips et al. 2011), particularly for the commercially cultivated species.

Diet as determined by host plant quality significantly influences fitness of phytophagous insects (Ishihara and Suzue 2011; Dosdall and Ulmer 2004; Awmack and Leather 2002). Availability of suitable host plant species and performance on such hosts can influence the population dynamics of an alien invasive species (Kim and Lee 2002), and determine its spread and establishment in a new eco-region (Philips et al. 2011). Studies on preference and performance of *O. melanopus* on small grain hosts are limited to major hosts such as wheat and oat, and fitness was measured in terms of larval survivorship and weight gains on hosts (Wellso et al. 1973; Casagrande et al.1977). Other measures of fitness such as realized fecundity (Perez and Wang 2004), adult weight, and associations between insect age, body weights and fecundity (Tisdale and Sappington 2001) on different hosts have not been explored in detail on plants within the potential host range of *O. melanopus.*

Currently, *O. melanopus* is in its early phase of range expansion and establishment in western Canada (Kher at. 2011). Commercially cultivated cereal crops in the region include wheat, oat, barley, triticale, and rye (Canadian Grain Commission 2013), and are potential hosts for *O. melanopus*. Detailed studies on preferences and fitness of the beetle on these hosts are currently lacking, in spite of the need for understanding *O. melanopus* host preferences and adaptation in

this new eco-region. Understanding biological parameters of *O. melanopus*, fitness and survivorship on potentially available host species can lead to designing strategies for effective management of the beetle (Haynes and Gage 1981; Ihrig et al. 2001; Kim and Lee 2002). Fitness is a relative measure of genotypic performance, and can be defined as "*per capita* rate of increase of a genotype with reference to the population carrying associated genes" (Sibly and Smith 1985). Fitness of an individual can be measured using several proxies that determine individuals' performance. For example, the most commonly used proxies include progeny survivorship, development, longevity and fecundity to name a few (Roitberg et al. 2001). In this context, laboratory studies were undertaken to determine the fitness proxies including survivorship, developmental time, adult weight, and fecundity of *O. melanopus* on commercially important cereal hosts in western Canada to identify those hosts that impart greater fitness. Experiments were conducted on both intact hosts and excised leaf tissues from selected plant species to compare host suitability and fitness.

2.2. Materials and Methods

2.2.1. Host plants and Insects

Six commercially grown cereal species that can act as potential hosts for *O. melanopus* were included in the study. I tested representative cereal cultivars that included wheat (*Triticum aestivum* L.) of both its winter (cv. AC Radiant) and spring (cv. CDC GO) types, oat (*Avena sativa* L.; cvs. Morgan and Waldern), barley (*Hordeum vulgare* L. cv. Champion), corn (*Zea mays* L. cv. UT 12813),

rye (*Secale cereale* L. cv. AC Remington), and triticale (*x Triticosecale* Wittm. cv. Pronghorn). Seed of these cultivars was obtained from the Agriculture and Agri-Food Canada Research Center, Lethbridge, Canada. Plants of each host were grown in the greenhouse using plastic containers (15 cm diameter) filled with sterilized potting mixture to avoid confounding in replicates by soil conditions. Plants were maintained under natural light augmented with high intensity sodium vapor lamps to maintain a photoperiod of 16:8 (L:D).

Recently emerged, overwintered *O. melanopus* adults were collected using insect sweep nets from a winter wheat field (49^{o} 41' 49" N, 112^{o} 46' 59" W) designated as the cereal leaf beetle nursery at the experimental farm of the Lethbridge Research Centre of Agriculture and Agri-Food Canada, and other commercial winter wheat fields near Lethbridge (49^{o} 41' 39"N, 112^{o} 49' 85" W). The adult colonies were maintained under standard laboratory conditions of 21^{o} C and 16L: 8D (L:D) regime on wheat plants and starved for 24 h before conducting the tests.

The laboratory studies were conducted on excised leaf tissues as well as on intact, live host plants in the summers of 2010, 2011 and 2012. In 2010, biological parameters were studied on excised and live hosts recognized as "preferred/superior" (Wilson and Shade 1966) and included wheat (spring wheat), oat (cv. Waldern) and barley. In 2011, the studies were expanded to include winter wheat, corn, rye, and triticale. The studies in 2010 indicated unexpected developmental patterns of larvae on Waldern oat. Hence, to reassess this effect, an additional cultivar of oat (cv. Morgan) was tested in 2011. In 2012, further studies

were conducted to validate the patterns observed during the previous years in the oat and wheat cultivars.

2.2.2. Developmental biology on excised leaf tissues of cereal hosts

Pre-imaginal developmental parameters on excised leaves of each host were determined by placing a leaf strip of a given host plant with a newly laid egg of *O. melanopus* in the centre of a plastic Petri dish (10 cm diameter). This was lined with a Whatman No. 4 filter paper moistened with distilled water to maintain adequate moisture. Each Petri dish with its specimen served as a replicate, and a minimum of 30 such replicates were maintained for each host species or cultivar. Leaf clippings of the given host were placed in the Petri dish to provide a food source to newly eclosing larvae, and incubation periods on each host were recorded. Upon hatching, the larvae were fed daily with fresh leaf clippings of the test cereal host, and observations were recorded on the developmental times for individual larval instar stages, pupal stage and the total development time from eclosion to adult emergence. Cast exuviae of larval instars after each molt were used to identify larval stages and to note the developmental times for each stage. As the fourth-instar larvae turned into pre-pupae, the base of the Petri dish was covered with a thin layer of vermiculite as a substrate for pupation. Once the pupae were formed, they were maintained individually in each Petri dish and observed for adult emergence.

2.2.3. Developmental biology on intact, live plants of cereal hosts

Developmental biology of *O. melanopus* was also investigated using potted plants of each host. For each host, a plastic greenhouse potting container (15 cm diameter) was used to grow seedlings. In each container, five seedlings of a given genotype were maintained. Plants in pots were allowed to grow for about eight weeks and were caged in BugDorm^{TM} insect rearing cages. The plants of a given genotype in each cage were then exposed to five mating pairs of *O. melanopus* adults for 96 h. Eggs were then counted and allowed to hatch to record developmental progress.

Observations were recorded daily to determine larval, pupal, and total developmental periods on each test genotype. Due to the small sizes of early instars, it was difficult to record the observations on the developmental periods of individual instar stages. Hence, I measured larval period as a whole on each host. The base of each container and the entire cage was lined with a 4 cm layer of vermiculite to provide a substrate for pupation. A minimum of six cages were maintained for each host.

2.2.4. Fitness and survivorship on excised and intact hosts

Adult fitness was measured in terms of live weights of freshly emerged adults and survivorship on each host species from intact and excised hosts. Survivorship was calculated as the total number of adults emerging from a given cohort of eggs laid on plants of each replicate, and expressed as percentages.

2.2.5. Oviposition preference and performance: choice test

The oviposition preferences of *O. melanopus* were investigated on two wheat types (winter and spring wheat), and two types of oats (Morgan and Waldern) to determine species and cultivar effects on oviposition. Four potted plants, one of each host genotype, were set in an insect rearing cage (Bugdorm, Megaview, Taiwan), and were exposed to five pairs of *O. melanopus* for 96 h. Oviposition on each host was recorded and the eggs were maintained intact to study developmental parameters as described in the studies with intact plants. I also quantified the damage caused by *O. melanopus* adults to host plants during the oviposition period under a choice scenario. The damage was assessed visually for plants of each host and a rating was given on a scale of zero to five based on the percentage of leaf area consumed by adults. The ratings were defined as: 0= no damage, 1= 1-10% leaf area consumed, 2= approximately 11-20% consumed, 3= 21-40%, 4= 41-60%, and 5 = > 60% of the leaf area consumed. Each leaf of each plant of the given host was assessed to assign the numeric damage rating and mean ratings were compared among hosts.

Finally, I compared biological parameters of *O. melanopus* on Waldern oat with those on Morgan oat using intact host plants of these hosts. Live plants of each host were caged independently in plastic insect rearing cages as before and exposed to five mating pairs of *O. melanopus* for 96 h. The eggs laid on the plants of each host were maintained intact and allowed to hatch. Observations were recorded on incubation time, larval period, pupal period and adult emergence, and compared between the two host cultivars.

2.2.6. Oviposition behaviour, realized fecundity, and oviposition trends on wheat and oat

Realized fecundity was measured in terms of total number of offspring actually produced (Awmack and Leather 2002) on different host plants by *O. melanopus* females in their lifetimes. I observed total oviposition on a given host from the initiation of oviposition to its cessation. As wheat and oat are commonly available as the principal hosts of *O. melanopus* in western Canada, the fecundity studies were restricted to these two hosts only and comprised winter and spring wheat as well as Morgan and Waldern oat.

Fecundity was estimated from a minimum of 10 adult pairs (1M :1F) of *O. melanopus,* with each pair confined with a potted host plant in a plastic rearing cage. Plants were replaced every five days until oviposition ceased. Based on the average oviposition calculated on a weekly basis, I analyzed the trend of oviposition on a given host to understand whether advancement in the female age influenced oviposition performance on a given host.

I also measured the daily oviposition of *O. melanopus* over its total lifetime on excised leaves of Morgan oat - a preferred host. Excised studies were conducted using plastic rearing containers (Polar^{TM} 240mL) lined with moist filter papers. In each container, one pair (1M : 1F) of *O. melanopus* adults was released and provided with fresh foliage on a daily basis. Each container with its adult pair was considered a replicate; there were 10 replicates in total. The number of eggs laid was counted daily until oviposition ceased or the females died. Age of the females was recorded in weeks as described before. The relationship between *O.*

melanopus daily oviposition and female age was determined. In a similar manner, the values for fecundity on all hosts and the respective initial body weights recorded for females for all hosts were pooled to analyze whether fecundity was linearly dependent on the body weight of the female.

2.2.7. Statistical analyses

The comparisons of incubation time, developmental times of larval instars, pupae and adults developing on excised tissues of cereal hosts did not follow a normal distribution according to Shapiro-Wilk and Kolmogorov-Smirnov tests. The data transformations did not help to achieve normal distributions. Hence, I fitted Generalized Estimating Equations with Poisson distributed error functions to the data using PROC GENMOD (SAS Institute 2008a). The parameters assessing goodness of fit indicated that the Poisson distribution appropriately fitted the data. The treatment means among hosts for individual developmental parameters were then compared using the PDIFF statement in PROC GENMOD using Tukey's *post hoc* test for multiple comparisons.

The data on biological parameters of *O. melanopus* in tests involving live hosts followed a normal distribution and hence the differences in larval period, pupal period and total developmental period were compared using analysis of variance in PROC MIXED (SAS Institute 2010). Host was treated as a fixed effect and differences among hosts for biological parameters of interest were compared using Tukey's test. Similarly, survivorship and adult weight gains on excised and live hosts were compared using analysis of variance. In a choice test,

the differences in oviposition, developmental parameters of *O. melanopus*, and adult damage to different hosts during oviposition were analyzed using analysis of variance as described above.

Comparisons of developmental parameters between Waldern and Morgan oat involved mean comparisons between two independent samples. However, the data did not follow a normal distribution and hence a non-parametric alternative, the Wilcoxon-Mann-Whitney two sample test, was used to compare the median values of the biological parameters. As the sample size for each host tested exceeded 20, probabilities based on z-approximation were reported.

In studying realized fecundity, measurements were taken on each *O. melanopus* replicate female over the oviposition activity period (expressed in weeks), and hence the differences in oviposition time between hosts were compared using analysis of repeated measures with PROC MIXED (SAS Institute 2010). The goal here was to understand the differences between hosts over the weeks in terms of mean oviposition. Total daily oviposition was compared among hosts using host, week and their interaction as fixed effects. The repeated effect was "week" while the replicate nested within host and week combination was considered as the subject effect. The variance-covariance structure used was compound symmetry (TYPE=CS; PROC MIXED). Oviposition rates over time among hosts were compared using a Tukey's test (SAS Institute 2010).

The effect of age of the female on oviposition on a given host was compared using regression analysis (PROC REG, SAS Institute 2008b). The average daily oviposition was used as a dependent variable while the age in weeks

was used as an independent variable. Similarly, the relationship between fecundity and initial body weights of the females was explored. Fecundity (expressed as average number of eggs laid in a lifetime) was used as the dependent variable and the initial body weight as the independent variable. The graphs of linear relationships between oviposition rates, female age and initial body weights were generated using XLSTAT v. 2013.2 (Addinsoft, NY, USA).

2.3. Results

2.3.1. Development on excised leaf tissues of cereal hosts

Mean egg incubation periods differed significantly among hosts (χ^2 = 404.36, df = 7, 232; $P < 0.0001$). The shortest incubation period occurred on the oat and wheat cultivars evaluated, with the longest on corn, rye and triticale (Table 2.1).

Significant differences in the developmental times of first instars were observed (χ^2 = 305.8, df = 7, 232; $P < 0.0001$). The neonates developed fastest on wheat, oat and barley and slowest on corn (Table 2.1). The two wheat cultivars differed in durations of neonate development with a longer developmental period recorded on winter wheat than spring wheat.

Development of second instars was more prolonged on corn, rye and triticale (χ^2 = 158.09, df = 7, 232; $P < 0.0001$) compared to spring wheat and Waldern oat. Second-instar larvae developed more rapidly on barley, spring wheat and Waldern oat than on corn, rye or triticale. Barley favoured rapid development of second instars (Table 2.1).

As larval growth advanced, prolonged growth patterns were observed on certain hosts. For example, third-instar larvae completed their development at a faster rate on barley, Morgan oat, and wheat (about 4-4.5days) than on other host plants including corn, rye and triticale (5-6 days). Among the preferred hosts, the duration of third-instar larvae was longer on Waldern oat than on other hosts (Table 2.1).

Fourth-instar larvae also developed most rapidly on barley, winter and spring wheat, and on Morgan oat (4-4.5 days). However, development was prolonged on Waldern oat (about 5.5 days) among favoured hosts, which was comparable to that on corn, rye and triticale (Table 2.1).

Duration of the prepupal stage was similar among the host plant genotypes investigated, although its shortest duration occurred on corn. Significant differences were observed among cereal hosts in terms of pupal development (χ^2 = 30.89, df = 7, 232; $P < 0.0001$). The pupation period was significantly shorter in duration on wheat, oat and barley than on corn and rye (Table 2.1).

The changes in developmental periods of larval instars influenced the time required for total development from eclosion to adult emergence. Significant differences were observed in the total developmental periods on different hosts (χ^2 = 26.72, df = 7, 232; $P < 0.0004$). Development was completed in the shortest time on wheat, oat and barley (43-48 days), while the longest developmental period occurred on corn (54 days). The total developmental times on winter wheat, Morgan oat and barley were shorter (43-45 days) compared to rye (54 days) and triticale (51 days). Among the favored hosts, the developmental time on

Waldern oat was longest (48 days) when compared to Morgan oat, and spring and winter wheat types (43-44 days) ($P < 0.001$ for all comparisons) (Table 2.1).

2.3.2. Development on intact, live plants of cereal hosts

Mean oviposition on living plants of different cereal hosts did not differ significantly ($F = 2.08$, df = 7, 40, $P > 0.05$). The developmental parameters of *O. melanopus* differed significantly on live hosts of different genotypes. There were significant differences among hosts in incubation period ($F = 52.92$, df = 7, 40; $P < 0.0001$), larval period ($F = 97.20$, df = 7, 40; $P < 0.0001$), pupal period ($F = 92.57$, df = 7, 40; $P < 0.0001$), and total developmental period ($F = 193.64$, df = 7, 40; $P < 0.0001$) (Fig. 2.1). Egg incubation time was shortest on barley (about 5 days) and longest on rye (about 11 days). The two wheat and the two oat types did not differ in terms of incubation times. Corn, rye and triticale differed significantly in terms of mean incubation periods from each other and from other host species, and longest incubation times were recorded on these three hosts (9-11 days) (Fig. 2.1).

Host genotype significantly influenced the development of *O. melanopus* larvae. The shortest duration for larval development was recorded on Morgan oat and spring wheat (20-23 days) while the longest occurred on corn (28 days) (Fig. 2.1). Among the hosts considered preferred, feeding on Waldern oat prolonged larval development (25 days) compared to that on spring wheat, Morgan oat and barley (20-23 days). Among other hosts including corn, rye and triticale, larvae

developed in a shorter time on triticale (23 days), and longer development was recorded on rye (25 days) and corn (28 days) (Fig. 2.1).

Pupal period was shorter on wheat, oat and barley (23-25 days) than on corn, rye and triticale (29-34 days) (Fig. 2.1). This consequently prolonged total development of *O. melanopus* on corn, rye and triticale (57-63 days) compared to wheat, oat and barley (49-56 days) (Fig. 2.1).

2.3.3. Fitness and survivorship on excised and live hosts

Cereal hosts affected adult weights and survivorship in the live ($F = 54.21$, df = 7, 439, $P < 0.001$) and excised ($F = 63.98$, $F = 7, 242$, $P < 0.001$) assays (Fig. 2.2).

Adult weights were numerically higher for beetles reared on live plants than on excised tissue. The exception was for triticale where adult body weight of beetles reared on excised leaves exceeded that for beetles feeding on living plant tissue (Fig. 2.2). In both live and excised studies, greatest weight gains were recorded on winter wheat and Morgan oat and the lowest on Waldern oat and corn. Wheat, oat (Morgan) and barley thus favored better development of *O. melanopus* adults in terms of weight gains compared to Waldern oat, corn, rye and triticale.

Oulema melanopus larval survival was highest on spring and winter wheat, Morgan oat and barley and lowest on Waldern oat and corn (Fig. 2.3). On Waldern oat, development was prolonged and the death of early-instar larvae was observed. On corn, larval death was not common. However, the number of non-

viable eggs was very high compared to those deposited on any other host. Among less preferred hosts, triticale showed higher survival rates that were comparable with spring and winter wheat and Morgan oat. Survivorship on triticale was also higher than on rye, corn and Waldern oat (Fig. 2.3).

2.3.4. Oviposition preference and performance: Choice test on live hosts

Oviposition did not differ significantly among live plants of the preferred hosts (spring and winter wheat, Morgan and Waldern oat) under a choice scenario ($F = 1.11$, df = 3, 16; $P > 0.05$) (Fig. 2.4). Eggs laid on the hosts did not differ significantly in terms of the mean incubation time ($F = 1.62$, df = 3, 16; $P > 0.05$) (Table 2.2). Similarly, the damage caused by *O. melanopus* adults did not differ significantly among the hosts ($F = 19.21$, df = 3, 16; $P > 0.05$) (Fig. 2.5). However, significant differences were noted in larval development ($F = 21.53$, df = 3, 16; $P < 0.0001$), pupal development ($F = 7.06$, df = 3, 16; $P < 0.0100$), and the total developmental time ($F = 29.14$, df = 3, 16; $P < 0.0001$) on different cultivars of wheat and oats.

The larval period was more prolonged on Waldern oat compared to Morgan oat, and winter and spring wheat types ($P < 0.0001$ for all comparisons). Similarly, Waldern oat differed significantly in terms of pupal development from Morgan oat and spring wheat. In terms of total developmental time, the spring and winter wheat types did not differ from each other, but developmental period was prolonged on Waldern oat when compared to Morgan oat, and spring and winter wheat types. The developmental period on Morgan oat was comparable to spring

wheat ($P > 0.05$) but significantly more rapid than on winter wheat ($P < 0.0001$). Significant differences were observed among the different cereal hosts in terms of weights of newly eclosed adults developing as larvae on the various hosts ($F = 29.14$, df = 3, 16; $P < 0.0100$). Comparatively lower adult weights were observed on Waldern oat when compared to spring and winter wheat types, and Morgan oat (Table 2.2).

Further exploration to evaluate the performance of *O. melanopus* between the two oat cultivars indicated significant differences in terms of developmental parameters. Incubation time did not differ between the cultivars ($Z = 0.34$, $P > 0.05$) (Fig. 2.6a). However, the larval period on Waldern oat was significantly longer than on Morgan oat ($Z = 6.23$, $P < 0.0001$) (Fig. 2.6c). Similarly, pupal development on Waldern oat was longer than on Morgan oat ($Z = 8.44$, $P < 0.0010$) (Fig. 2.6b). This resulted in a longer total developmental period on Waldern oat compared to Morgan oat ($Z = 7.88$, $P < 0.0001$) (Fig. 2.6d). Hence, significant differences in Waldern versus Morgan oat were observed in all developmental parameters of *O. melanopus*.

2.3.5. Oviposition behaviour, realized fecundity, and oviposition trends of O. melanopus on wheat and oat

The rate of oviposition on the two wheat and two oat types was compared over time (expressed as activity period in weeks) (Fig. 2.7). The interaction between host genotypes and activity time was significant ($F = 5.64$, df = 33, 387; $P < 0.0001$). This indicated that the net egg load harbored by different hosts over

time was different and that the oviposition trends differed among hosts. The mean number of eggs laid by *O. melanopus* females on different hosts showed periods of higher oviposition activity followed by a lowered activity period over time thus indicating fluctuations in ovipositional activity as the age of the females advanced (Fig. 2.7). In general, greater numbers of eggs were deposited during Weeks 1 to 5 upon initiating the egg laying activity. The two wheat genotypes and Morgan oat generally showed periods of peak egg laying activity during this window of oviposition. Waldern oat, on the other hand, indicated a gradual increase in oviposition. A gradual decline in the mean numbers of eggs laid on live host plants was observed from Week 6 onward. While a gradual reduction in numbers of eggs laid was evident for Waldern oat, and spring and winter wheat, oviposition on Morgan oat increased from Weeks 6 and 7 to Week 8, and then declined over the remainder of the oviposition period (Fig. 2.7).

I compared the total period of oviposition activity on different hosts and the total fecundity over the active oviposition period. The cereal hosts differed significantly in terms of active oviposition period ($F = 4.28$, df = 3, 36; $P < 0.0100$), and total fecundity ($F = 69.26$, df = 3, 36; $P < 0.0001$) (Table 2.3). A significantly shorter oviposition activity period was noted on Waldern oat relative to the other host plants evaluated (Table 2.3). The greatest number of eggs was laid on spring wheat which was comparable with the eggs laid on Morgan oat, but exceeded those laid on winter wheat and Waldern oat. Realized fecundity was lowest on Waldern oat than on other hosts evaluated (Table 2.3).

On live hosts, the rate of oviposition was linearly dependent on the age of the female, and as age progressed, oviposition activity decreased (Fig. 2.8). The amount of variation in oviposition as explained by age was as follows: spring wheat (r^2 = 0.81; Fig. 2.8c), Waldern oat (r^2 = 0.74; Fig. 2.8a), Morgan oat (r^2 = 0.72; Fig. 2.8b), and winter wheat (r^2 = 0.65; Fig. 2.8d). I also tested the fecundity on excised leaves of Morgan oat and observed a similar linear relationship between fecundity and female age (r^2 = 0.80; Fig. 2.9). However, on excised hosts, the oviposition period lasted for eight weeks whereas it continued for 12-14 weeks on live hosts. The rate of daily oviposition was lower on excised leaf tissues compared to live hosts.

Finally, I tested the effect of initial body weight of newly eclosed *O. melanopus* females on fecundity. A positive linear relationship was noted between female body weight and total number of eggs laid by the female (r^2 = 0.67; Fig. 2.10). The number of eggs laid in the lifetime significantly increased as the female body weight increased.

2.4. Discussion

The total of all host species on which an insect can successfully complete its life cycle comprises its host range (van Klinken 2000). However, differences in the host ranges and host preferences of insect species over their geographic ranges are known (Hodkinson 1997). Such variations in preferences can be attributed to the concepts of fundamental and realized host ranges (van Klinken 2000). While the fundamental host range comprises all host species that can be exploited by an

insect, the realized host range indicates how the hosts in the fundamental range are actually utilized under given conditions (Nechols et al. 1992). Hence, the host range can be broad but the actual utilization of hosts within it may vary in different eco-regions. This can lead to differences in host suitability and performance on different hosts (Hodkinson 1997). Knowledge of such preferences is of great value in predicting the initial establishment phase of an invasive insect pest species. Experiments conducted here on the biology of *O. melanopus* on major cereal crops of western Canada provide new insights on how the beetle can exploit some of the host species in its fundamental host range. Differences in beetle performance were observed when specimens were reared on the various hosts investigated in this study. Developmental parameters of the beetle differed significantly among different hosts, and there was significant variation in the levels of fitness attained. This result concurs with previous studies that indicate that the beetle exhibits specific host preferences (Wilson and Shade 1966; Casagrande et al. 1977), and that the preferences vary in different geographic regions (Piesik and Piesik 1998; Pozsgai and Saringer 2006; Philips et al. 2011).

Differences were observed in developmental patterns when larvae were reared on excised leaf tissues *versus* live, intact host plants. Egg incubation time was shorter on live hosts compared to excised hosts, while the durations of pupal and total developmental periods were longer on live hosts than on excised hosts. Adult weight gains on live hosts were generally higher compared to those on excised hosts. Such differential responses in developmental parameters in response to the state of the host plants are documented in species such as the

bertha armyworm (*Mamestra configurata* Walker) (Dosdall and Ulmer 2004), and differences in fitness gains are also known in species such as the crucifer flea beetle (*Phyllotreta cruciferae* (Goeze)) (Palaniswamy et al. 1997). Such differences in *O. melanopus* performance may be associated with intrinsic factors associated with the host species and cultivar (Hoffman and Rao 2010; Philips et al. 2011). Excised leaf tissue can have markedly different chemical characteristics than living plants (Schmelz et al. 2001), and differing chemical and physiological properties of excised and intact plant material of the same plant species can impact larval feeding and development (Dosdall and Ulmer 2004).

Several consistent patterns of development on the different hosts included in this study were observed. In excised studies, barley and spring wheat were the most preferred hosts for larval development, and development was fastest on spring wheat but prolonged on corn. In a similar manner, intact plants of spring wheat favoured fastest development whereas development on Morgan oat occurred more rapidly than on barley and winter wheat when live host plants were assessed. Corn slowed larval growth while extended developmental periods were also common on rye and triticale. Interestingly, the oat cultivar, Waldern, exhibited developmental periods in both live and excised host studies that were significantly more prolonged than on the Morgan oat cultivar. Hence, in terms of relative time to complete pre-imaginal development, wheat, oat and barley were favourable hosts compared to corn, rye and triticale in both studies. As far as developmental patterns of *O. melanopus* on different hosts are concerned, longer

developmental times on hosts such as rye and corn are reported (Wilson and Shade 1966).

Metabolic pathways of host plant species and the presence of antibiotic components in hosts are known to hamper performance of *O. melanopus*. For example, differences exist in feeding and adaptation to plants exhibiting the C4 pathway (e.g., corn) compared to plants of the C3 pathway (e.g., oat, wheat, barley) (Caswell et al. 1973). In corn, a secondary volatile, 2, 4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), negatively affects longevity of overwintered *O. melanopus* adults (Wellso 1978). As a result, negative effects of feeding of pre- and post-aestival adults of *O. melanopus* on corn are known, and greater mortality in pre-aestival adults was reported (Wellso 1978). Similarly, feeding on oat with antibiosis expression affected larval fitness and survivorship of *O. melanopus* adults (Steidl et al. 1979), and the weights of larvae and adults were lowest on plants of a certain *Avena sterilis* L. cultivar in studies conducted in the U.S.A. However, prior to my study detailed comparative assessments of the performance of individual life stages of *O. melanopus* on a range of cereal host species were lacking, and information was not available on other fitness parameters such as survivorship and realized fecundity.

Body weight or size determines insect physiological development and fitness (Klingenberg and Spence 1997). Higher fitness of *O. melanopus* in terms of weights of adults developing from larvae fed on different hosts was achieved on Morgan oat and winter wheat in both the excised and intact plant studies (Fig. 2.2). Spring wheat and barley ranked next in imparting higher fitness in both types

of studies. The beetle consistently performed poorly in terms of adult weight gains on corn and Waldern oat in both studies. Larval feeding on high quality hosts translates into greater adult fitness as reflected through higher body weight or size (Ishihara and Suzue 2011). I found consistently higher body weights of adults reared on winter wheat, spring wheat and Morgan oat than on the remaining hosts. Although adult weight gains on triticale were high in excised studies, they were low on live plants of triticale. Such patterns have not been previously reported. However, the performance on live plants suggests that the fitness gain was lower on triticale. Living plants, unlike excised tissue, can release toxic compounds (Schmelz et al. 2001), and this may explain the different weight gain and fitness results for this host. Within the two wheat types, greater fitness was attained on winter wheat in terms of adult weights compared to spring wheat. Winter wheat is the first available crop host in southern Canada for newly emerged, overwintered *O. melanopus* adults for feeding, mating and oviposition (Kher et al. 2011). Current population hotspots have been observed mainly in winter wheat fields, and the beetle populations shift to spring cereals including spring wheat and oat once these hosts are available (Kher et al. 2011). However, such shifts depend on availability of spring cereals, and the beetle populations currently rely on winter wheat as a stable host (Kher, unpublished data).

Survivorship is another major indicator of species fitness (Crone 2001). In our studies, survivorship was highest on winter and spring wheat types with adult recoveries of 100%. In terms of survivorship, the hosts can be ranked from highest to lowest survival rates as: wheat (winter and spring) > barley, oat (cv.

Morgan) > triticale > rye > corn, oat (cv. Waldern). Despite lower adult weight gains noted on triticale, survivorship was very high and was statistically comparable with survival rates on Morgan oat, wheat and barley. This indicates that *O. melanopus* can perform well on triticale despite low fitness gains. Triticale is a commercially successful hybrid between wheat and rye developed to combine the quality traits and uniformity of wheat, and the vigour, resistance properties and hardiness of rye (Lorenz and Pomeranz 1974). Higher survivorship on triticale may have greater association with the quality traits of wheat, rather than rye.

The oviposition preferences of phytophagous insects are considered to be oriented for fitness maximization (Gillespie and Wratten 2011). However, the lack of such a relationship was noted in some systems. Ovipositional preferences may not be accurately predicted under field conditions as a result of differences in host availability, and the chances of females finding hosts of equal abundance and quality (Thompson 1988). Also, insect fitness alone may not be the best predictor of insect success and studies on realized fecundity can provide a better estimation of insect performance (Awmack and Leather 2002).

In the current studies, *O. melanopus* females did not show particular preferences for oviposition on different host plants under choice and no-choice scenarios. However, developmental success and fitness on various hosts differed significantly. Low fitness and survivorship was evident on hosts like corn and Waldern oat. A relatively high proportion of eggs laid on corn was nonviable. Eggs are considered nonviable if they remained in place on the leaf surfaces

without apparent embryonic development, turned brown or black or both (Shade et al. 1970). In our studies, eggs on corn turned brown to black, crumpled and desiccated eventually. Up to five to 20 percent of *O. melanopus* eggs may be nonviable in laboratory studies (Shade et al. 1970); however, we did not observe nonviable eggs on other host species. Nonviability of *O. melanopus* eggs on corn has not been reported before, and nonviable eggs on corn may be attributed to a general nonviability attributed to laboratory rearing (Shade et al. 1970), or a cultivar-specific effect. Further studies will be required to determine whether nonviability is caused by cultivar-specific effects exerted on eggs. In subsequent studies, Waldern oat differed from Morgan oat, and from spring and winter wheat types in terms of development and fitness and the duration of active oviposition by *O. melanopus* females. Although the cultivars Morgan and Waldern were equally attractive for oviposition, the fitness and survivorship was greater on Morgan. In terms of realized fecundity, the rankings were as follows: spring wheat > Morgan oat > winter wheat > Waldern oat. The decline in oviposition activity was reached earlier on Waldern oat compared to other hosts. This emphasizes that different cultivars of the same host species can vary significantly in how they affect the performance of their associated herbivores.

Under laboratory conditions, peak oviposition lasted for five weeks from the commencement of egg laying. A similar activity span was observed for oviposition under field conditions, with peak oviposition in the field lasting about four to six weeks (Kher et al. 2011). The oviposition pattern showed alternate periods of peak egg laying and gradual declines in activity on different hosts. The

cyclic nature of *O. melanopus* populations is known with frequent peaks and gradual declines in the oviposition and larval populations of the beetle (Kodosca 1916: in Shade et al. 1970, pp. 52). Periods without egg laying are common in the life history of *O. melanopus* (Hodson 1929). Generally, the females of phytophagous insect species prefer to oviposit on host plants that maximize the fitness of their larval populations (van Klinken 2000), and such clear preferences are known in insects like the diamondback moth, *Plutella xylostella* (L.) (Sarfraz et al. 2011). However, prior studies also indicate that host quality and nutritional status may not be the criteria for oviposition choice in some insects (Awmack and Leather 2002). A negative relationship between host choice and progeny performance may be driven by ecological factors and selection forces (Gillespie and Wratten 2011). Insect oviposition strategies may differ based on host quality. Higher fecundity on poor quality hosts can be observed; the eggs of progeny, however, may be of poor quality (Rossiter 1991). Other factors such as information processing and discriminatory abilities to distinguish among hosts, host availability and abundance, and time available for oviposition and resource utilization can determine insect host range (Janz and Nylin 1997). More importantly, a lack of discrimination between hosts can be an adaptive strategy to adjust to newer environments (Mayhew 1997). Our results indicated that *O. melanopus* female preferences for oviposition were not associated with fitness gains of the progeny. This needs further investigation to better understand the mechanisms underlying oviposition choice.

Ovipositional activity (duration of oviposition) can be hampered by several factors. This study focused, in particular, on the effects of host plant, female body weight, and female age on the duration of oviposition and realized fecundity. The decline in oviposition activity over time was explained by age of females; initial body weight also significantly affected oviposition. Indices such as adult size and potential fecundity are highly correlated (Leather 1988), and we observed a similar pattern for *O. melanopus*.

Certain differences in terms of oviposition behaviour were observed. Eggs were laid not only on upper leaf surfaces but also on lower leaf surfaces. Eggs were also laid close to the leaf base, and inside leaf folds and curls. Generally, females of *O. melanopus* lay eggs on upper leaves compared to lower leaves, and this could be due to their strongly phototactic response and tendency to move upwards to seek light (Shade et al. 1970). However, we did not observe this pattern.

Based on the developmental and fitness parameters, results of this investigation indicate that among the hosts tested, the favourable hosts for *O. melanopus* development are winter and spring wheat, oat and barley. Hosts such as oat, barley and wheat were previously considered "superior" and no significant differences were found in terms of development on these hosts (Wilson and Shade 1966). However, although these hosts favoured beetle development, this investigation found differences between these hosts and within their cultivars.

In terms of suitability, triticale can be a potential host species and can help sustain beetle populations. Rye can serve as an intermediate host. Corn is

recognized as an "unfavourable" host (Wilson and Shade 1966), and the current pattern of development confirms this result. Pre-imaginal rearing on Waldern oat indicated significant deviation from the normal developmental pattern and further studies are needed to confirm and validate these results. This study indicates that the preference for and performance on host plants within the fundamental host range can change in a new eco-region.

This investigation on development and performance of *O. melanopus* on different cereal hosts was laboratory-based. The results need to be validated through extensive field studies. Currently, *O. melanopus* is in its early establishment phase in western Canada and the populations are scattered with localized activity hot-spots in the cereal-growing provinces. Current population structures and irregular patterns of pest activity set limitations for extensive field studies to understand host preferences and performance parameters. However, factors such as availability of potential hosts, lack of barriers for spread and favourable climate create potential avenues for further range expansion and establishment of this pest (Olfert et al. 2004; Dosdall et al. 2011; Kher et al. 2011). Hence, the understanding of host preferences can help to predict host suitability (Xue et al. 2010), and the future spread of this pest. The early invasion phase is an appropriate stage to investigate such preferences, and so help to contribute to the design of strategies to mitigate risks associated with the spread and expansion of *O. melanopus* as done in the U.S.A. (Wilson and Shade 1966; Philips et al. 2011). Knowledge of host preferences and underlying mechanisms for such preferences has significantly contributed to integrated pest management

strategies for *O. melanopus*. For example, extensive studies on *O. melanopus* performance on major small grain crops helped to discover pubescent genotypes of wheat exerting antixenosis for oviposition and feeding (Wellso et al. 1973), and oat genotypes with antibiotic properties (Steidl et al. 1979). Hosts that are attractive for oviposition but significantly hamper beetle fitness and survivorship may be used effectively to design strategies such as trap cropping as part of an integrated pest management framework. Contribution to the knowledge on biology of *O. melanopus* in western Canada can thus help to strengthen efforts for the sustainable management of the beetle.

Tables.

Table 2.1. Mean developmental times in days (± SE) of different pre-imaginal life stages of *Oulema melanopus* reared on excised leaf tissues of various cereal hosts.

Host and Cultivar	**Incubation Time**	**1st Instar**	**2nd Instar**	**3rd Instar**	**4th Instar**	**Prepupa**	**Pupa**	**Total Development**
Wheat-Spring **(CDC GO)**	6.40 ± 0.18 [a]	4.33±0.20[a]	4.40±0.20 [a,d]	4.53±0.23 [a,b]	4.23±0.16 [a]	1.46±0.10[a]	25.30±0.44[a]	42.76±0.68[a,d]
Wheat-Winter **(AC Radiant)**	6.60 ± 0.13 [a]	5.26±0.14[b,h]	5.06±0.13[b,c,f]	4.83±0.11[a]	4.63±0.11[a]	1.30±0.08[a,b]	25.06±0.19[a]	44.03±0.45[b,d]
Oat **(Waldern)**	6.06 ± 0.11 [a,b]	5.40±0.13[c,b]	5.03±0.13[c,d]	5.16±0.16[a,c]	5.53±0.09[b]	1.23±0.07[a,b]	28.00±0.24[a,b]	47.66±0.35[b,a,d]
Oat **(Morgan)**	5.80 ± 0.24 [a,b]	4.70±0.18 [d,b]	4.83±0.18[c,f]	4.80±0.16 [a,b]	4.63±0.15 [a]	1.23±0.07[a,b]	24.16±0.30[b,c]	43.23±0.65[c]
Barley **(Champion)**	6.83 ± 0.24 [c]	4.26±0.19[a,e,d]	4.16±0.15[d]	4.20±0.14[b]	4.16±0.15[a]	1.46±0.09[a]	27.23±0.43[c]	44.80±0.65[d]
Corn **(UT 12813)**	9.23 ± 0.22 [d, c]	7.63±0.10[f]	6.50±0.10[e]	5.83±0.09[c]	5.53±0.09[b]	1.06±0.04[b]	29.33±0.20[d]	54.23±0.39[e]
Rye **(AC Remington)**	8.73 ± 0.16 [d, c]	6±0.11[g]	5.66±0.10[f]	6.13±0.09 [d,c,e]	5.43±0.10[b]	1.18±0.06[a,b]	30.36±0.21[d]	53.80±0.35[f,e]
Triticale **(Pronghorn)**	8.43 ± 0.11 [e, c, d]	5.93±0.13[h]	5.26±0.12[f]	5.60±0.11 [e,c]	5.26±0.11[b]	1.10±0.05[a,b,c]	28.83±0.39[e,b,d]	51.23±0.33[g]

Means in the columns followed by the same letter indicate no significant difference by using ANOVA and Tukey's studentized range test.

Table 2.2. Mean developmental parameters (days ± SE) of *Oulema melanopus* life stages developing from eggs laid on different living plants of wheat and oat cultivars under a choice scenario, and mean adult weights (mg ± pooled SE) following rearing on the host plants.

Host	**Incubation Time**	**Larval Period**	**Pupal Period**	**Total Developmental Period**	**Adult Weight (mg)**
Spring Wheat	5.60 ± 0.40 [a]	19.20 ± 0.37 [a]	27.60 ± 0.40 [a]	52.40 ± 0.50 [a, b]	5.90 ± 0.37 [a]
Winter Wheat	5.60 ± 0.24 [a]	19.20 ± 0.37 [a]	28.40 ± 0.24 [a,b]	53.20 ± 0.48 [a]	6.33 ± 0.15 [a]
Oats (Morgan)	5.00 ± 0.31 [a]	18.20 ± 0.37 [a]	26.80 ± 0.73 [a]	50.00 ± 0.83 [b]	6.34 ± 0.18 [a]
Oats (Waldern)	6.00 ± 0.31 [a]	22.80 ± 0.58 [b]	30.20 ± 0.66 [b]	57.20 ± 0.89 [c]	5.78 ± 0.12 [b]

Means in the columns followed by the same letter indicate no significant difference by using ANOVA and Tukey's studentized range test

Table 2. 3. Mean (± SE) realized fecundities and oviposition activity periods of *Oulema melanopus* on living plants of two wheat and two oat genotypes.

Host	**Realized Fecundity (Mean eggs/host/12 weeks)**	**Oviposition Activity Period (Total active days of oviposition)**
Spring Wheat	275.45 ± 6.54^{a}	65.30 ± 1.16^{a}
Winter Wheat	198.50 ± 3.16^{b}	63.60 ± 0.83^{a}
Oat (Morgan)	263.20 ± 7.48^{a}	64.80 ± 0.96^{a}
Oat (Waldern)	180.50 ± 4.29^{c}	61.00 ± 0.68^{b}

Means in the columns followed by the same letter indicate no significant difference by using ANOVA and Tukey's studentized range test

Figures.

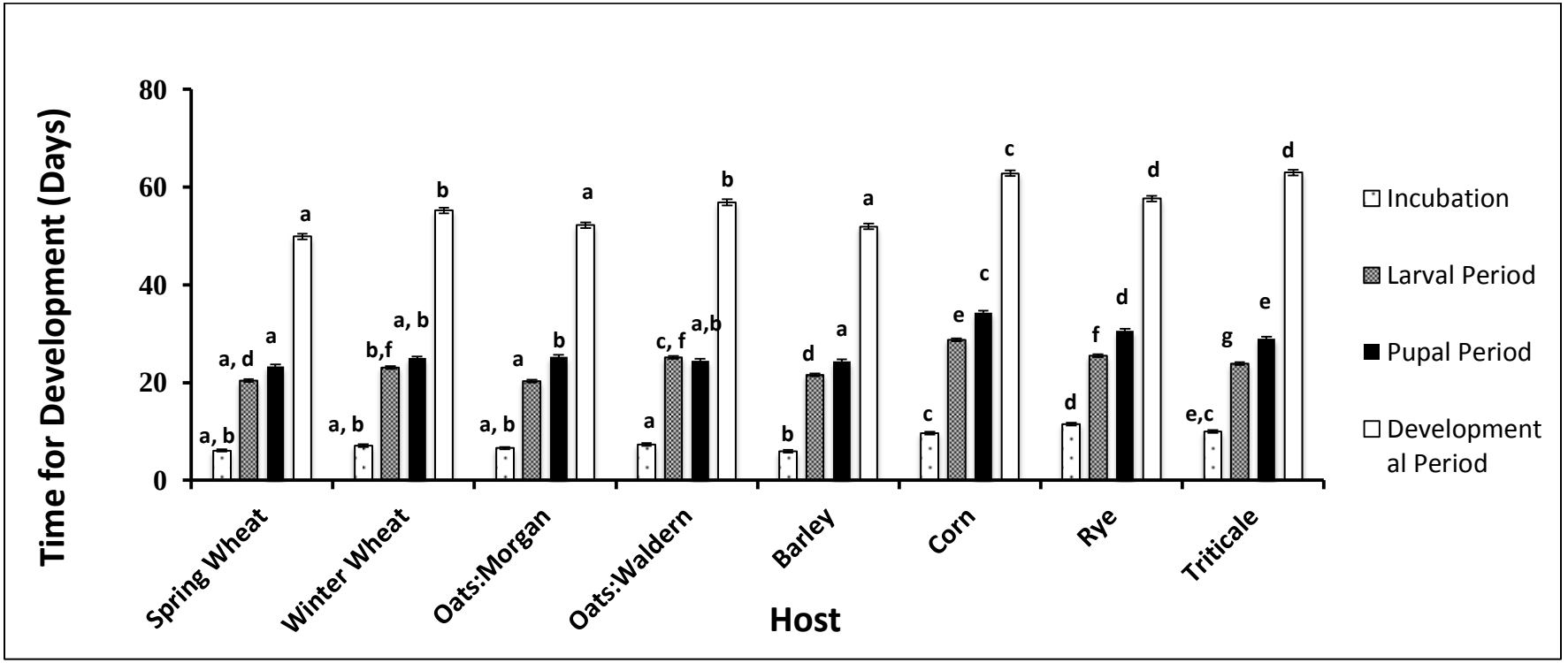


Figure 2.1. Mean developmental times (days ± SE) of pre-imaginal *Oulema melanopus* reared on live plants of various cereal hosts in Lethbridge, Canada in 2010 and 2011. Bars for the various hosts for a given pre-imaginal life stage and for the total development period sharing different letters indicate significant treatment differences.

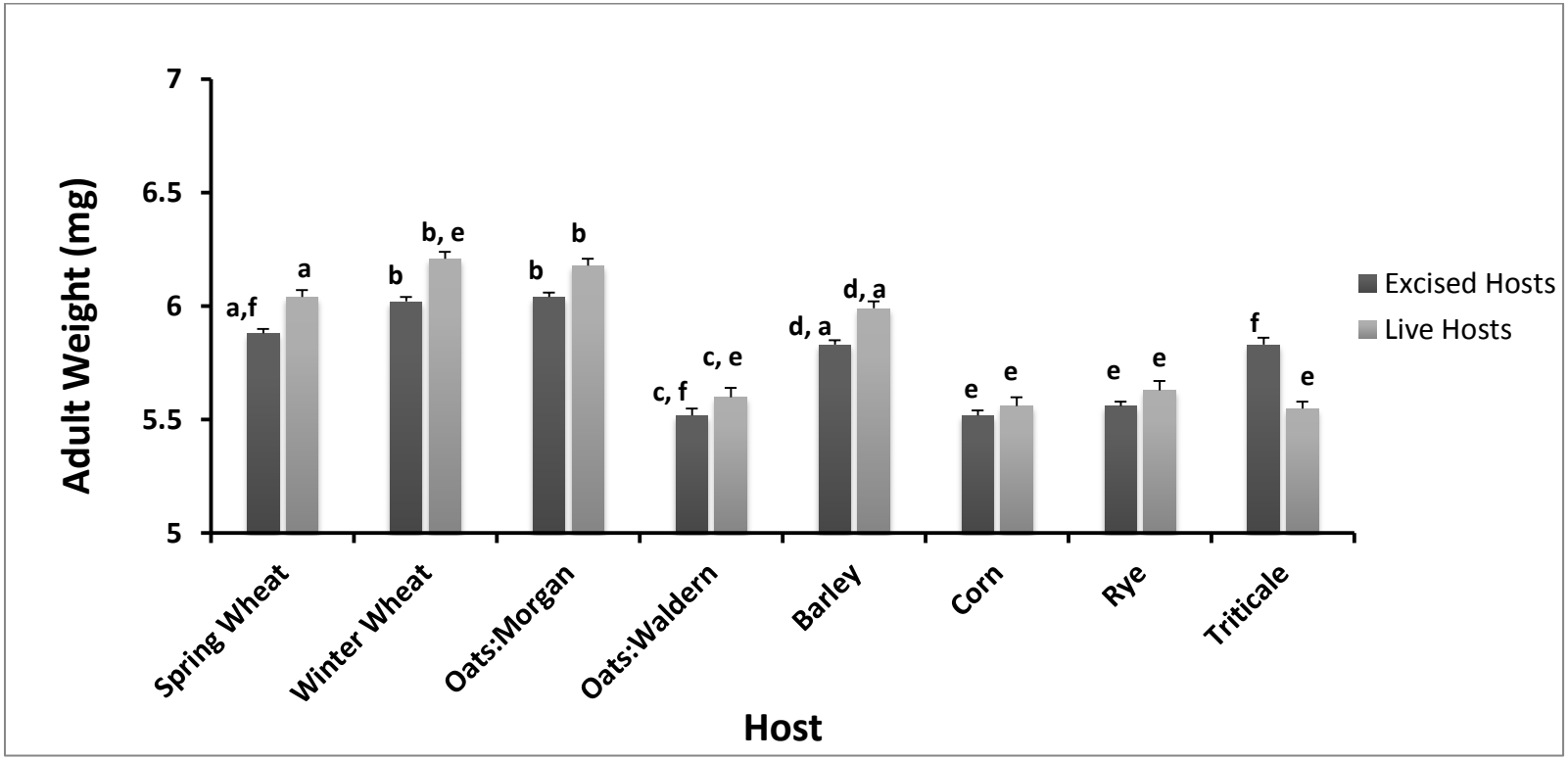


Figure 2.2. Mean adult weights (± SE) of *Oulema melanopus* reared on excised leaf tissues and live plants of various cereal hosts in Lethbridge, Canada in 2010 and 2011. Bars for hosts for a given rearing method sharing different letters indicate significant treatment differences.

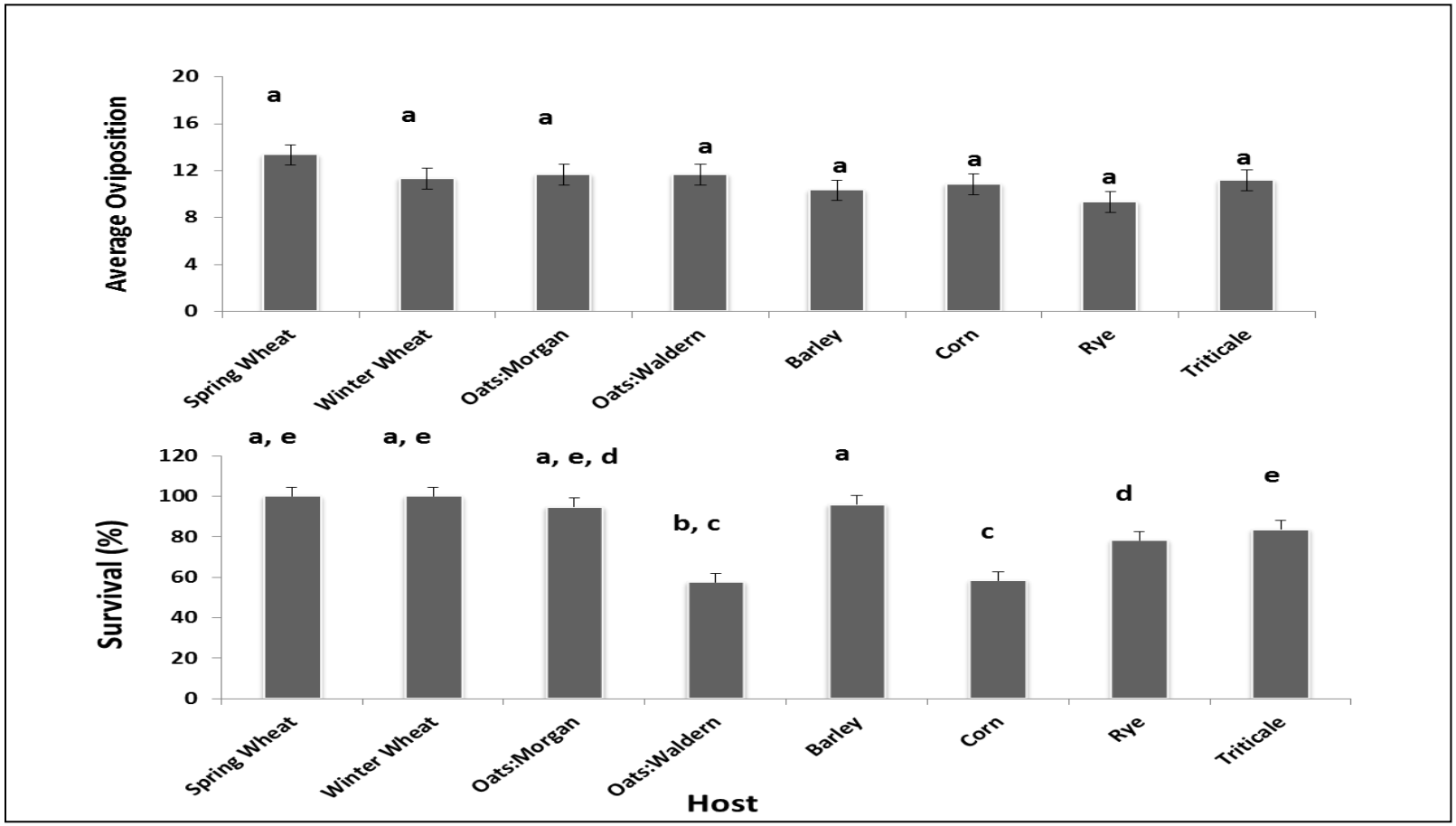


Figure 2.3. Mean eggs/replicate deposited over 96 h (± SE) and survival of *Oulema melanopus* from hatching to adult emergence on various cereal hosts. Bars sharing different letters indicate significant treatment differences.

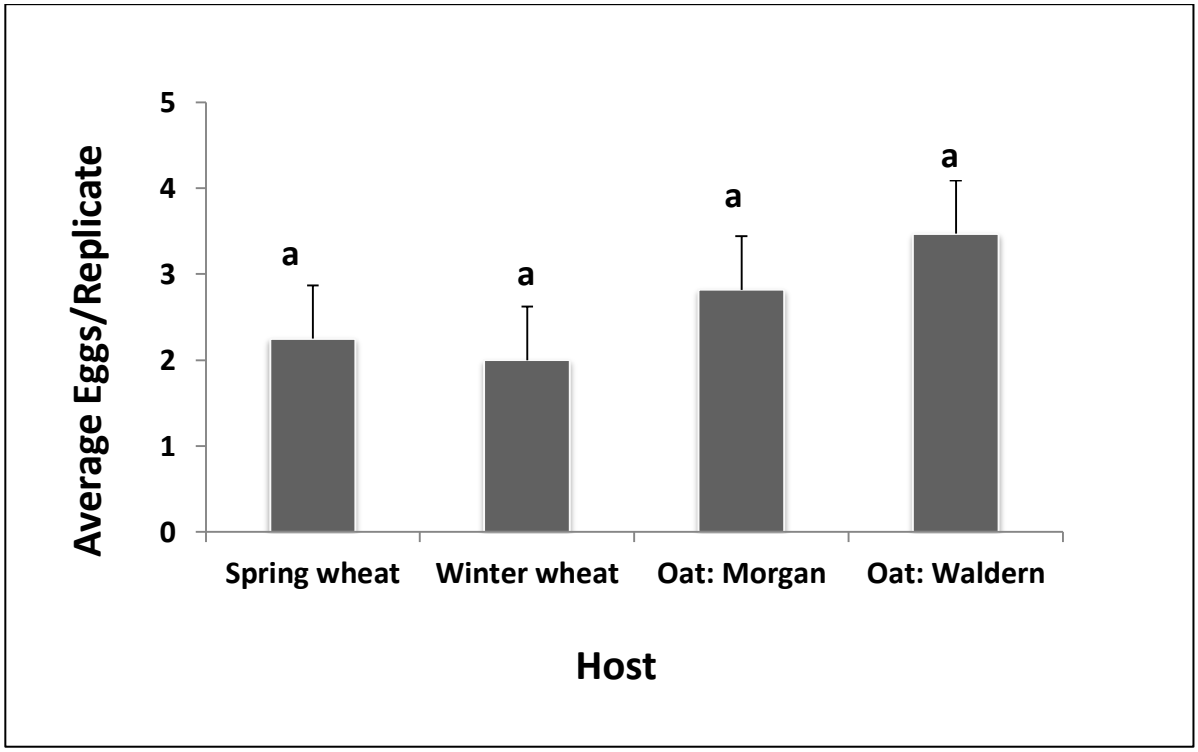


Figure 2.4. Mean oviposition (eggs ± SE) of *Oulema melanopus* on live plants of cereal hosts under a choice scenario. Bars sharing different letters indicate significant treatment differences.

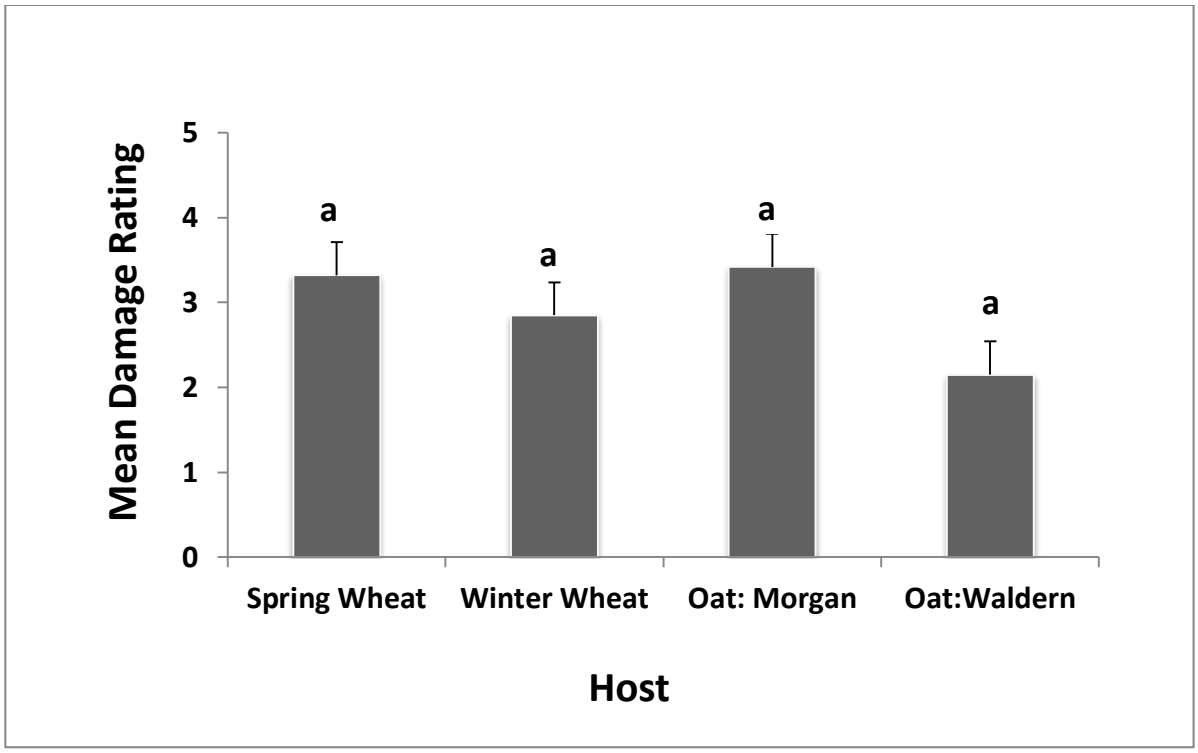
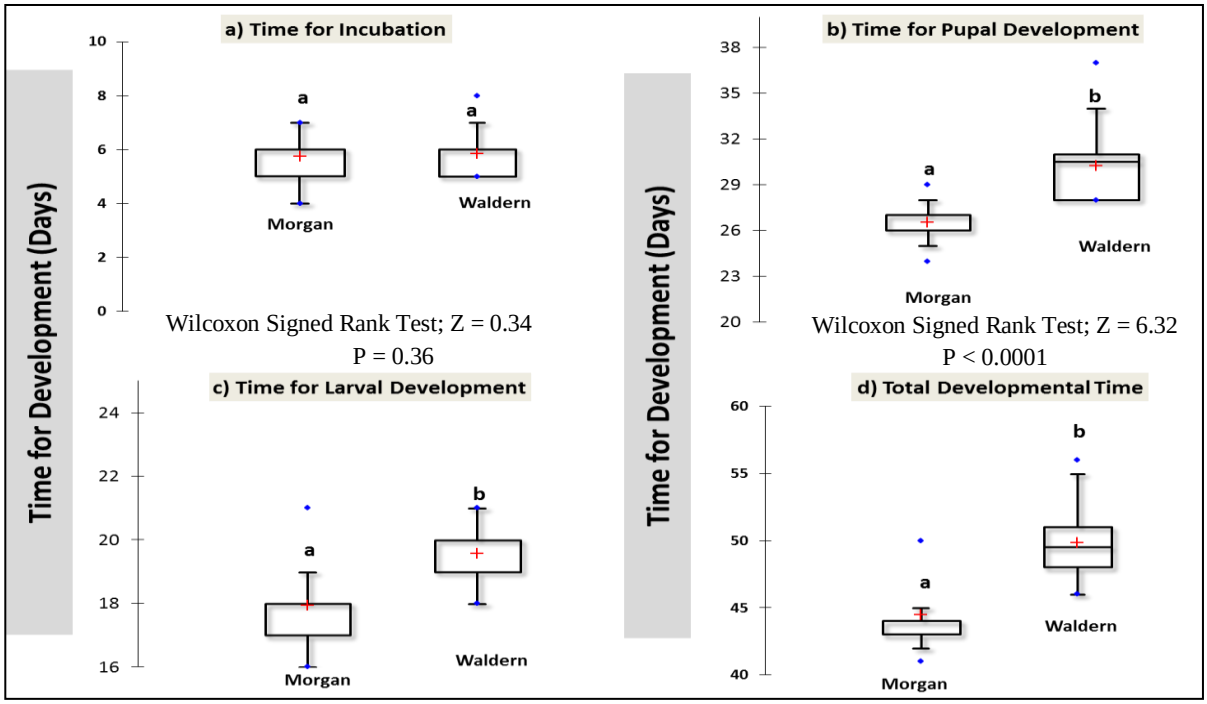


Figure 2.5. Mean damage ratings (± SE) of *Oulema melanopus* reared on live plants of various cereal hosts. Bars sharing different letters indicate significant treatment differences.



Wilcoxon Signed Rank Test; Z = 0.34
P = 0.36

Wilcoxon Signed Rank Test; Z = 6.32
P < 0.0001

Wilcoxon Signed Rank Test; Z = 8.44
P < 0.0001

Wilcoxon Signed Rank Test; Z = 7.88
P < 0.0001

Figure 2.6a-d. Box-plots showing developmental parameters (median and 5th and 95th percentiles) of *Oulema melanopus* on two oat cultivars: a) incubation time; b) time for pupal development; c) time for larval development; and d) total developmental time. Boxes sharing different letters indicate significant differences in developmental times for a given life stage.

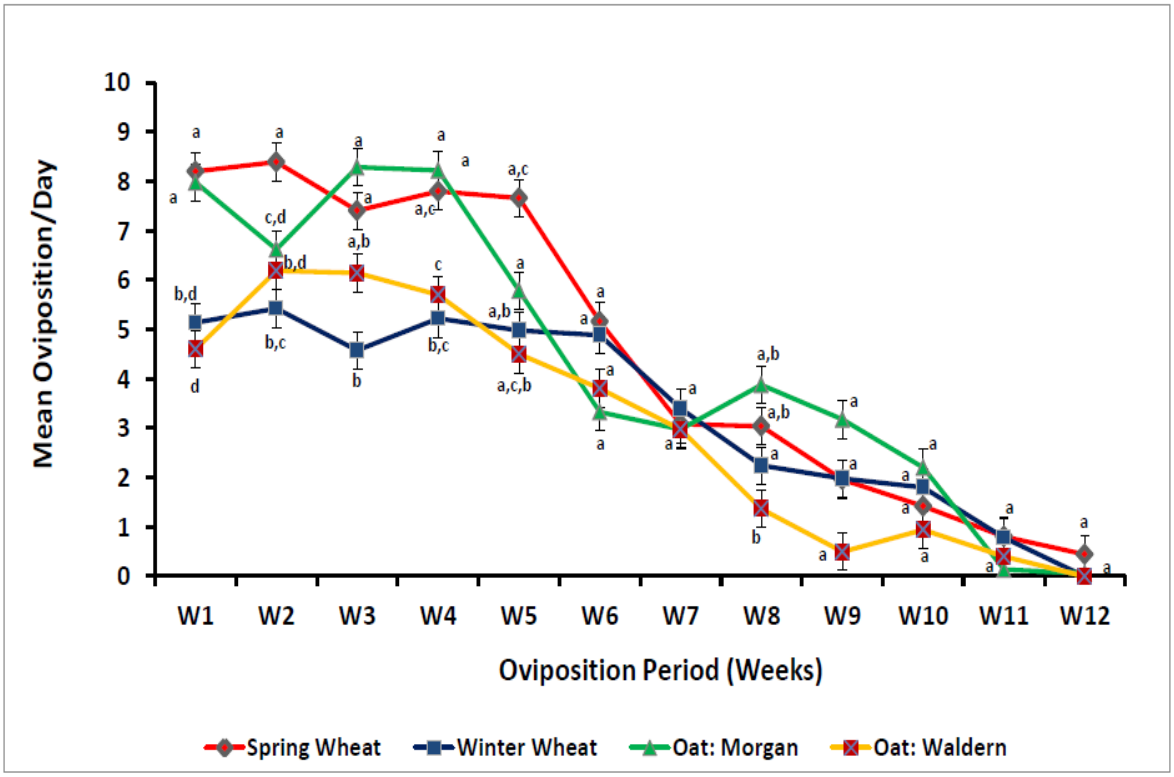


Figure 2.7. Mean oviposition per week (mean eggs ± SE) of *Oulema melanopus* on live plants of cereal hosts over 12 weeks. Data points within a given oviposition period (week) sharing different letters indicate significant differences in oviposition between hosts for that period.

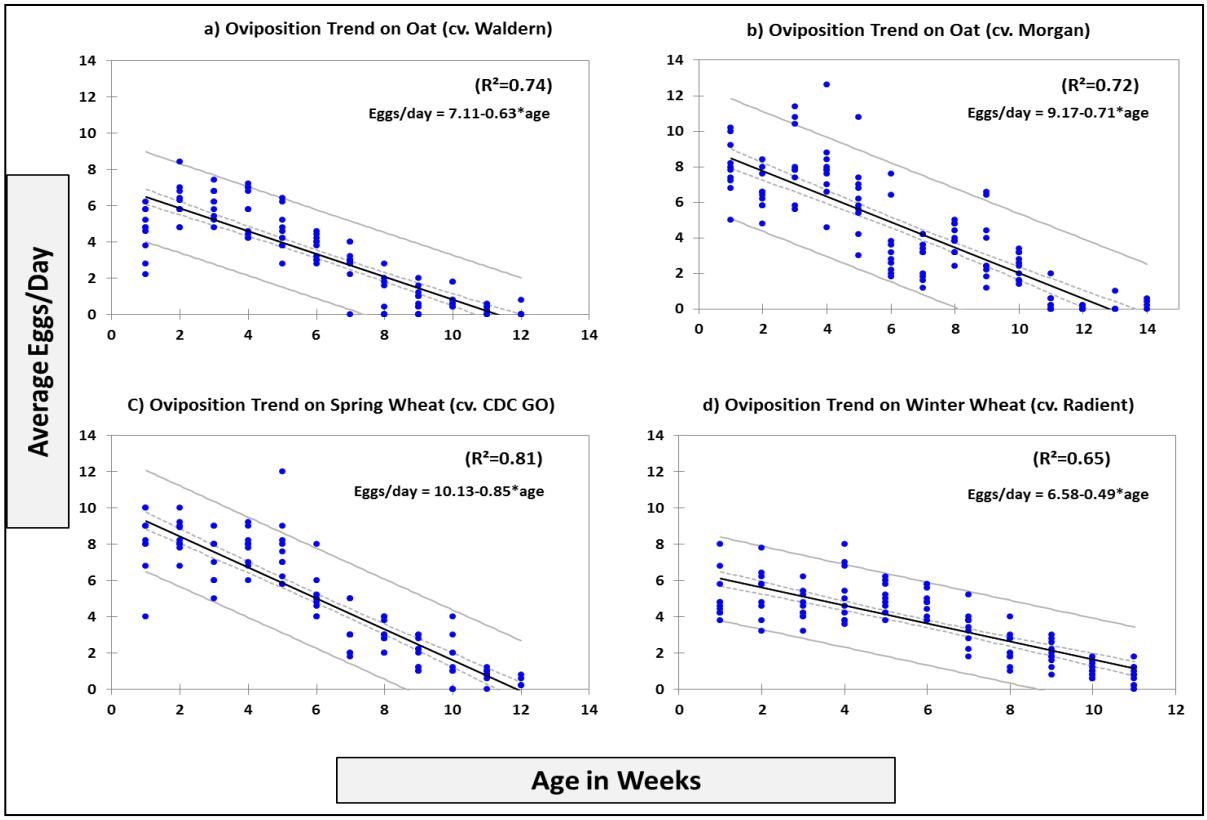


Figure 2.8a-d. Linear relationship between mean oviposition per day of *Oulema melanopus* on live plants of various cereal hosts and the age of females (weeks). 95% Confidence intervals around the fitted line are presented in the figure above.

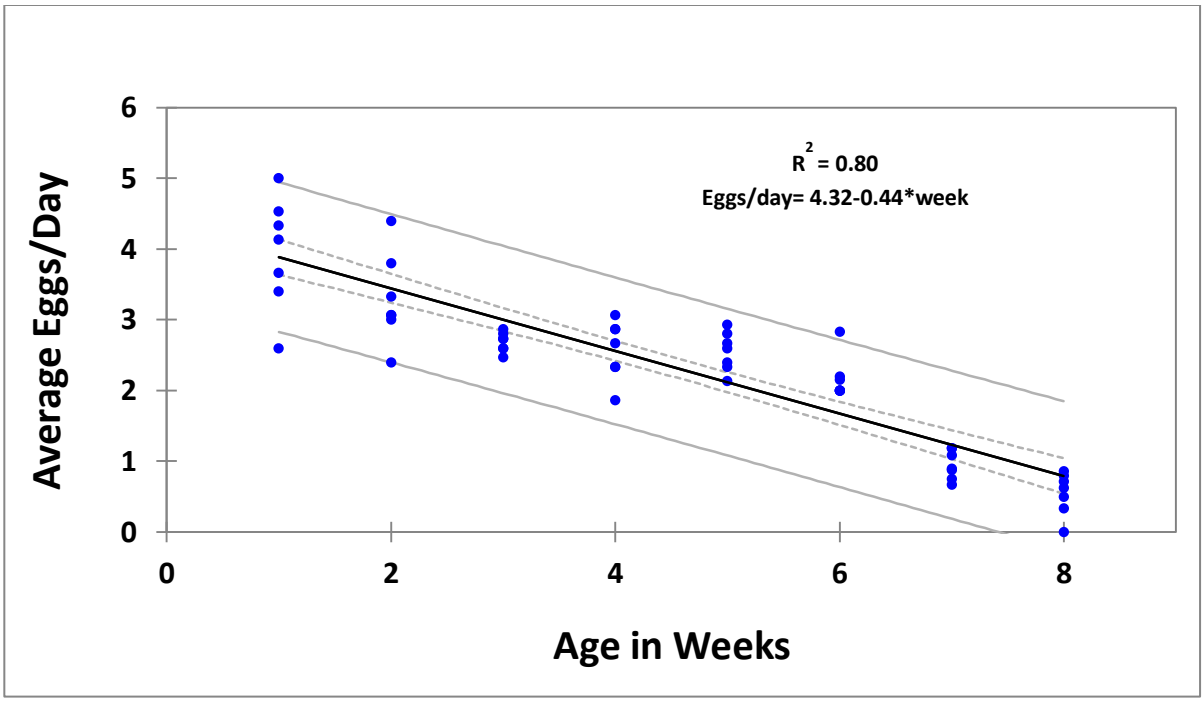


Figure 2.9. Linear relationship between mean oviposition per day of *Oulema melanopus* on excised leaf tissues of oat (cv. Morgan) and the age of females (weeks). 95% Confidence intervals around the fitted line are presented in the figure above.

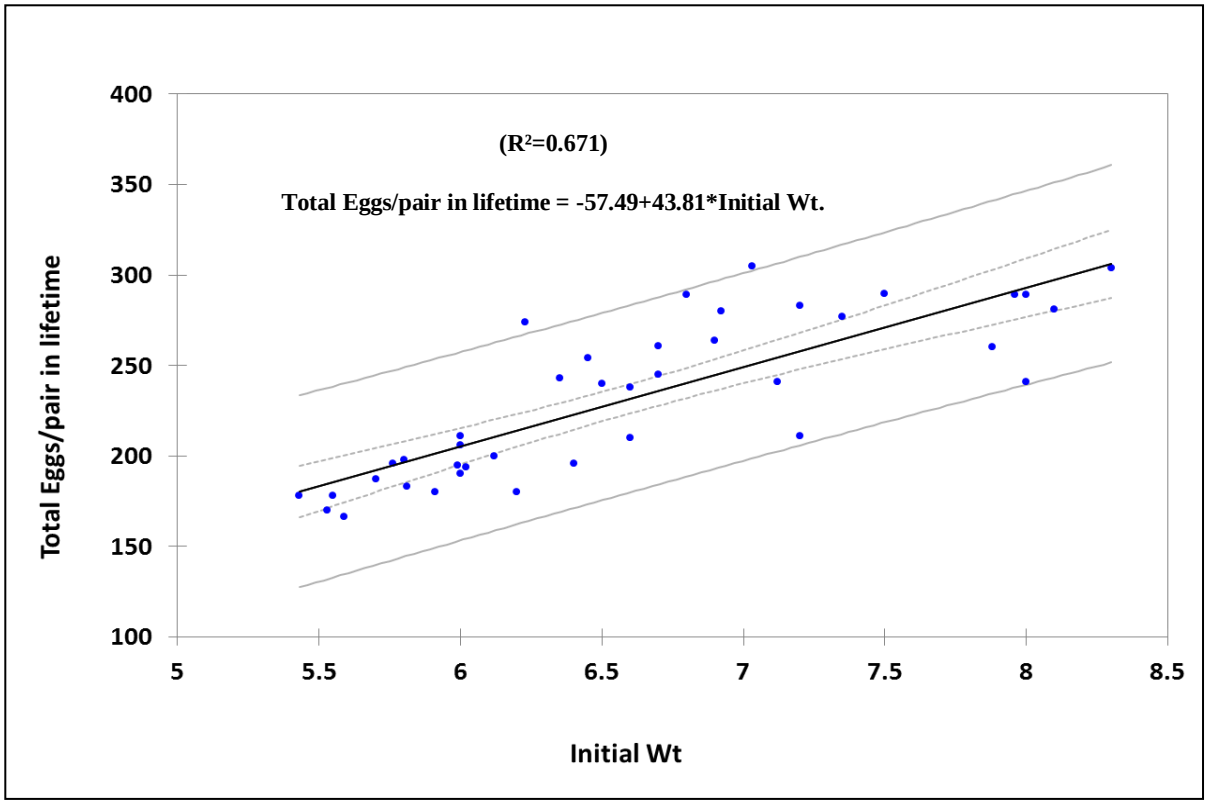


Figure 2.10. Linear relationship between fecundity of *Oulema melanopus* on live plants of cereal hosts and the female body weight (mg). 95% Confidence intervals around the fitted line are presented in the figure above.

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Chapter 3: Contributions to the life history, host preferences, and host-finding behaviour of *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), the principal parasitoid of the cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae)

3.1. Introduction

Tetrastichus julis (Walker) (Hymenoptera: Eulophidae) is the principal, gregarious larval endoparasitoid of the cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae) (Evans et al. 2006; Lesage et al. 2007). It is an important member of the natural enemy complex of *O. melanopus* consisting mainly of egg and larval parasitoids in Europe, and has been reported from about 15 European countries (Dysart et al. 1973). *Tetrastichus julis* is highly host-specific and is associated only with *O. melanopus* (Cárcamo et al. 2012).

In North America, *T. julis* was introduced as part of a classical biological control programme for the management of *O. melanopus*. The beetle was first reported from Michigan, U.S.A. in 1962, and substantial cereal crop losses were reported within a few years (Haynes and Gage 1981). The beetle expanded its geographic range significantly to encompass most regions of cereal production in the U.S.A. (Battenfield et al. 1982; Haynes and Gage 1981; Philips et al. 2011) and Canada (Harcourt et al. 1984; Dosdall et al. 2011; Kher et al. 2011). Initial management efforts relied mainly on chemical insecticides (Merritt and Apple 1969; Webster et al. 1972), but did not result in efficient management. Hence, an extensive programme for identification and introduction of natural enemies of *O. melanopus* from Europe and their establishment in North America was initiated

(Wellso 1982). This resulted in the introduction and subsequent relocation of *T. julis* in North America. Since its inception, classical biological control of *O. melanopus* has been the most successful management strategy for the beetle (Wellso 1982).

Adults of *T. julis* oviposit in field populations of larvae as soon as *O. melanopus* eggs start hatching (Dysart et al. 1973; Haynes and Gage 1981; Evans et al. 2006). The parasitoid lays eggs in all larval host stages (Dysart et al. 1973). Around four to six eggs are laid at a time and the eggs are deposited in the posterior abdominal region of the host by inserting the ovipositor through the fecal coating of the beetle larva (Dysart et al. 1973; Haynes and Gage 1981). Death of host larvae as a consequence of parasitization occurs when host prepupae form pupal cells within the soil. *Tetrastichus julis* larvae developing inside these dead *O. melanopus* then emerge and pupate within their host's earthen cocoon. Following pupation, adult parasitoids emerge from host cocoons by chewing through them, mate on the soil surface and disperse to grain fields (Gage and Haynes 1975). Parasitization of *O. melanopus* larvae by *T. julis* is high in spring as emergence of the parasitoid is well synchronized with that of early instars of *O. melanopus*, thus providing ample opportunities for parasitism. However, the second generation of adults also plays a role in parasitizing the population of late-maturing host larvae (Dysart et al. 1973; Haynes and Gage 1981; Staines 1984). Field parasitization rates are same among wheat, oat and barley (Evans et al. 2006). The parasitoid exhibits facultative diapause under

unfavourable environmental conditions (Haynes and Gage 1981; LeSage et al. 2007).

The wasp is well established in North America due to its gregariousness, host specificity, high synchronization with the host and capacity to track its host as it expands its geographic range (Haynes and Gage 1981; Evans et al. 2006; Philips et al. 2011; Kher et al. 2011). Parasitization rates range from five to 90 percent and help to maintain beetle populations at low levels (Haynes and Gage 1981).

In eastern Canada, relocation of *T. julis* facilitated management of beetle populations beneath economic threshold levels for many years (Harcourt et al. 1984), especially in fields with reduced or no tillage (Leibee and Horn 1979). In the Prairies western Canada, *T. julis* occurs adventively (Dosdall et al. 2011), and annual surveys in Alberta, Canada, have indicated that parasitization activity has increased in recent years with levels in the range of 5-30% in portions of southern Alberta (Kher et al. 2013). However, *O. melanopus* activity has increased substantially and its populations show localized hot-spots of high densities (20-40%) across the Prairie Ecozone. Although sample sizes are small, *T. julis* has not been collected naturally in recent beetle-infested portions of Saskatchewan (Carcamo and Dosdall, unpublished Data). In the absence of the parasitoid and appropriate management practices, adult and larval damage by *O. melanopus* may cause yield losses as high as 55% in spring wheat, 23% in winter wheat and 38-75% in oat and barley (Webster and Smith 1979; Royce 2000).

In Canada, the beetle is predicted to spread across all cereal-growing regions (Olfert et al. 2004), and its establishment has economic implications for grain production, trade and export. Establishment of *T. julis* in western Canada and its relocation in newly infested areas is therefore very important to mitigate potential economic losses. An understanding of the biology of *T. julis* and its interactions with the beetle in the new eco-region will contribute to improving strategies to augment the activity of the parasitoid.

The general biology of *T. julis* is known and studies on local phenology from various parts of Europe and North America have contributed to the current knowledge of its life history patterns. Nevertheless, there are still knowledge gaps on life history traits that can perhaps be exploited to improve the effectiveness of this parasitoid, particularly in relation to host affinities, behaviour and interactions between the beetle host and the parasitoid. Much research on *T. julis* has focused on various applied aspects associated with field dynamics on a broader scale such as field efficacy in managing beetle populations (Dysart et al. 1973; Harcourt et al. 1977; Harcourt et al. 1984), effects of cultural practices and tillage on population dynamics of *T. julis* (Leibee and Horn 1979), factors influencing diapause (Nechols et al., 1980), seasonal phenology (Evans et al. 2012; Evans et al. 2006), and tactics to enhance field parasitization levels (Evans et al. 2010). However, basic questions such as whether *T. julis* prefers specific host stages, and whether these preferences influence clutch size and population structure remain unanswered.

The aim of this investigation was to enhance understanding of the association of *T. julis* with the beetle, and to develop insights into host-parasitoid interactions that strengthen the success of *T. julis* as an effective biological control agent of *O. melanopus*. I studied developmental parameters, clutch size characters, *T. julis* ovipositional preferences for larval stages and their influences on gregariousness of the parasitoid, and cues involved in parasitoid host-finding.

3.2. Materials and Methods

3.2.1. Host and parasitoid culture

A laboratory colony of the host species, *O. melanopus*, was developed by collecting recently emerged, overwintered *O. melanopus* adults using insect sweep nets from a winter wheat field (49^o 41' 49" N, 112^o 46' 59" W) designated as the cereal leaf beetle nursery at the experimental farm of the Lethbridge Research Centre of Agriculture and Agri-Food Canada, and other commercial winter wheat fields near Lethbridge (49^o 41' 39"N, 112^o 49' 85" W). The adult colony was maintained under standard laboratory conditions of 21^o C and 16L: 8D (L:D) on plants of winter wheat (*Triticum aestivum* L. cv. Radiant). The plants were checked daily for oviposition and once eggs were noted, such plants were transferred to separate cages to maintain larvae. The larval colonies were maintained by providing fresh, intact host plants of winter wheat weekly and removing old ones.

The parasitoid colony was initiated each year by collecting adult parasitoids emerging from colonies of parasitized *O. melanopus* larvae collected

from the field. Parasitized larvae captured were maintained in the laboratory at 21^{o} C and 16L: 8D (L:D) in groups of three to five larvae in plastic rearing containers (Polar®; 240 mL capacity) lined with moist filter paper (Whatman No. 4) and a thin layer of vermiculite as a base for pupation of the larvae. The cocoons formed were observed daily for parasitoid emergence and newly eclosed adult parasitoids were confined to Plexiglas cages. In each colony cage, plastic rearing containers lined with moist filter paper and vermiculite containing five *O. melanopus* larvae were set up as hosts for *T. julis* females using an exposure period of 48 h. Exposed larvae were then removed, observed for parasitization and the emerging parasitoids were used to maintain the colony.

Oulema melanopus larvae exposed to second generation *T. julis* females formed cocoons; however, no parasitoids emerged in the same season as *T. julis* is bivoltine. These cocoons were maintained in plastic containers and were overwintered at 4^{o} C until the following spring when *T. julis* adult eclosion occurred. The overwintered parasitoids formed the colony the following year, along with newly collected specimens from the field.

3.2.2. Biological parameters of T. julis and effective exposure period

The following biological parameters of *T. julis* were investigated using parasitized larvae of *O. melanopus* under laboratory conditions: total pre-imaginal developmental time, clutch size/gregariousness, longevity of adults, sex ratio of emerging parasitoids, and duration of oviposition event (oviposition time taken by a female for one instance of oviposition). The studies were conducted using three

O. melanopus larvae (late second instars) confined to plastic rearing containers lined with moist filter paper and vermiculite and provided with fresh leaf cuttings of winter wheat. In each container, one M : F pair of freshly emerged *T. julis* adults was released and exposed to the *O. melanopus* larvae for 48 h. I did not test whether or not virgin females could oviposit.

Developmental time was calculated as days from parasitization to emergence of adult parasitoids from a larva (in instances where *O. melanopus* larvae failed to pupate due to parasitization) or a cocoon. The clutch size or gregariousness of the parasitoid was measured in terms of numbers of *T. julis* adults emerging from each cocoon/parasitized larva. Numbers of male and female parasitoids emerging in each clutch were recorded to determine sex ratio. The newly eclosed adults were maintained in individual containers to calculate longevity and were provided with 10% honey solution. Longevity was calculated for *T. julis* females that were never exposed to the beetle larvae for parasitization, and for females that were exposed to the beetle larvae. The females that were exposed to *O. melanopus* larvae were maintained in individual rearing containers (Polar®; 240 mL capacity) and provided with three to five fresh larvae after every 48 h. The exposure of females to larvae was continued until the females died. Longevity was calculated as the time between eclosion of female adults to their death.

Levels of parasitization achieved by *T. julis* females when exposed to *O. melanopus* larvae for different time periods were calculated. The objective was to determine the effective exposure period and to understand if differences exist in

degree of parasitization at various exposure periods. Four exposure periods were investigated: 24, 48, 72 and 96 h. For each period, replicate containers (Polar® 240 mL capacity, 9 cm diameter x 5 cm deep) each with five third-instar larvae placed on fresh leaves with a vermiculite base were used in which a pair of adult *T. julis* (1M : 1F) was released. Fifteen replicate containers were maintained for each exposure treatment and arranged on a laboratory bench in a completely randomized design. Following exposure, the parasitoids were removed and larvae were observed daily for visual symptoms of parasitization (crumpled larvae with recognizable growth of immature stages of *T. julis* inside the bodies). The proportion of larvae parasitized in each replicate container for a given exposure period was then calculated and compared among treatments. The replicate containers were held at 21^{o} C and 16L: 8D (L:D) throughout the experiment.

3.2.3. Host-instar preference of T. julis

I investigated whether *T. julis* females exhibit specific preferences for parasitizing certain larval stages, and whether such preferences influence gregariousness and sex determination. Host-instar preferences of fecund *T. julis* females were studied using choice and no-choice tests. In a choice test, a Petri dish (20-cm-diameter) was compartmentalized using thin strips cut out of plastic transparency sheets held in place with an adhesive tape. Each plate was partitioned into four compartments; each compartment was assigned randomly to represent one of four instars of *O. melanopus* larvae. In each compartment, five larvae of a given instar were set with fresh leaf clips of wheat plants such that

each Petri dish provided a choice to *T. julis* of all four instars of *O. melanopus*. One pair of *T. julis* (1M : 1F) was released into the middle of the choice arena and the Petri dish was closed. After a 48 h exposure period, the parasitoids were removed from the Petri dish and the larvae of each instar were removed and maintained in separate individual containers. Larvae were fed with fresh leaf cuttings of winter wheat until death or formation of cocoons and were observed for parasitoid emergence. The Petri dishes were held at 21^{o} C and 16L: 8D (light: dark) throughout the experiment. Observations were recorded on the proportions of larvae parasitized for each instar, the number of parasitoids emerging from each larva, and sex ratio. Each choice arena was considered as a block representing four treatments; the experiment was repeated four times with five blocks each.

In a no-choice test, the larvae of each instar were exposed to *T. julis* females independently. Five larvae of one particular instar were confined to a plastic rearing container (Polar® 240 mL capacity) lined with a thin layer of vermiculite and moist filter paper. Each container thus acted as a replicate in which a pair of *T. julis* (1M : 1F) was added for an exposure period of 48 h. Host stage (larval instar) was the treatment, and a minimum of 20 replicate containers were maintained per larval instar stage in a completely randomized design. Upon completion of the exposure period, parasitoids were removed and larvae were observed daily for parasitization. Once cocoons were formed, they were maintained individually to observe gregariousness and sex ratio. The replicate containers were held at 21^{o} C and 16L: 8D (light: dark) throughout the

experiment. The proportions of larvae parasitized per replicate were calculated to determine percent parasitization for each instar.

3.2.4. Host-instar preference and seasonal activity in the field

To understand host-instar preferences of *T. julis* females under field conditions, choice arenas representing all instar stages were set up in the field. Each choice arena consisted of a plastic tub (60.96 x 40.54 x 33.02 cm; 62 L capacity) partitioned with thin cardboard sheets to make four compartments held in place with adhesive tape. The base of the tub was lined with a 5 cm layer of vermiculite covered with moist industrial strength paper towel. In each compartment, a live potted winter wheat plant was held. Each of the plants within the tub was assigned randomly to be infested with one of the instar stages of *O. melanopus* such that all four larval instars were represented. Depending upon larval availability, three to five larvae of each instar were set up on the assigned host plant. The plants were fastened to stakes to minimize wind damage and positions of leaves infested with larvae were marked by coloured tapes at the base of tillers. The paper towel layer lining the vermiculite was placed to detect any larvae that fell from their host plants. The tubs were taken to an experimental winter wheat field with known parasitoid activity where five such buckets were set up separated from each other by 10 m to represent the field plot. Each tub thus served as a block representing four instars (treatments) and there were five such blocks; the entire experiment was repeated four times. Following an exposure period to field populations of *T. julis* of 96 h, the buckets were returned to the

laboratory, and numbers of larvae observed feeding on the plants were counted. The plants with live larvae of a given instar were separated and maintained independently in separate bucket containers with vermiculite and observed for development. Plants that hosted advanced instars were checked to determine whether cocoons had been formed in the vermiculite. Cocoons in vermiculite were extracted and maintained in individual containers to record emergence. Any missing larvae were excluded from the analysis.

A second experiment was conducted in a similar manner to understand the choice for larval instars but experimental treatments were altered. First- and second-instar larvae were classified as "small" and third- and fourth-instar larvae as "large". The choice arena consisted of plastic tubs as before that were portioned into two compartments using thin cardboard sheets. In each compartment, two pots (15 cm diameter) each with five plants were placed to set *O. melanopus* larvae on the foliage. Eight to 10 larvae of small instars (4 to 5 larvae each of first and second instars) were set on the plants in one compartment while the same number of larvae of large instars (4 to 5 larvae each of third and fourth instars) were set on plants in the other compartment thus forming a paired design structure with the tub as a replicate. Five such replicate tubs were set in the field in one run of the experiment with an exposure period of 96 h. The experiment was replicated three times. The larvae from each group were reared in the laboratory as described before and rates of parasitization were recorded.

I determined seasonal activity of *T. julis* by observing rates of parasitization for each sampling period in the above experiments by calculating

the proportions of larvae parasitized from the larvae exposed to *T. julis* populations. Furthermore, I set up additional buckets with plants and sentinel larvae to collect *T. julis* adults to stock laboratory colonies and supplement phenological studies of parasitism. These observations were taken from 25 May through 5 August 2012.

3.2.5. Role of olfactory cues in host-finding

To investigate whether olfactory cues associated with *O. melanopus* larvae, particularly with the larval fecal coat, had a role in attracting *T. julis* females, I conducted laboratory bioassays using a four-chambered olfactometer (Analytical Research Systems, Gainesville, Florida, USA; Model #OLFM-4C-2440PE). The main choice arena of the olfactometer was 30.48 x 30.48 x 2.54 cm with a removable lid (Fig. 3.1). It consisted of four outlet ports laterally connected to four odour source chambers, and an insect inlet port ventrally to introduce the test insect. Each lateral outlet port was connected to the internal odour source (IOS) with a glass insect isolation trap (IIT) that prevented re-entry into a choice arena (Fig. 3.1). The odour source was connected to an air delivery system that pumped moist air through the odour sources to the choice arena, and a vacuum to the insect inlet chamber to centralize the airflow throughout the choice arena. The air from all the odour sources was directed to the insect inlet chamber using a vacuum suction mechanism and this exposed the test insect in the insect chamber to odours emanating from different chambers and allowed it to make a choice. The insect inlet chambers allowed the test insect to walk or fly into the main

choice arena through a circular opening. Upon entering the arena, the test insect could track the odour source of its choice and walk/fly to the respective chamber. For my experiments, all the odour treatments were connected in the internal odour source.

To test *T. julis* olfactory preferences, one female was introduced at a time in the insect chamber and given 20 min to orient to odours emanating from different treatments and to make a choice by tracking the odour of preference. The behavior of each female was observed during orientation and choice selection. If a female orientated itself to a particular odour source and entered the insect isolation trap connected to that odour source without retreating into the main arena, this odour source was considered as the female's choice. If the females did not show any orientation behavior or movement in the olfactometer arena, or if their movement represented random walks or flights without any particular choice confirmed, such females were considered to not exhibit any specific odour preferences and were eventually eliminated from data analysis. At the end of 20 min, each test female was removed from the olfactometer arena and a new female was introduced.

In the first bioassay, each replicate fecund *T. julis* female was exposed to four different odour sources emanating from the source chambers. The four treatments were: a) a beetle larva with a fecal coat on a filter paper, b) a beetle larva without a fecal coat on a filter paper, c) only a fecal coat on a filter paper, and d) blank filter paper without any larva or fecal coat (control/check). Larvae without fecal coats for one of above treatments were obtained by gently rinsing

larval bodies with distilled water using a wash bottle. Once the traces of fecal smear were removed, larvae were placed on filter paper (Whatman No. 4). To ensure that larvae remained without fecal coat, rinsing was done after every few runs.

The four treatments were assigned randomly to the odour chambers and the treatment structure and positions of the chambers were changed after every 10 runs to avoid biases associated with directionality or odour sources. The number of *T. julis* females entering any particular choice chamber was noted. Data were recorded on a minimum of 100 females over 2010 and 2011. Internal odour sources and insect isolation traps were rinsed with 70% ethanol and dried between runs to avoid residual effects of odours.

In the second bioassay, the olfactometer was converted to function as a two-chambered arena by limiting the air flow only through two chambers and by plugging the remaining chambers. To test whether olfactory cues associated with the fecal coat influence *T. julis* females, the fecal coat was collected from the larval body with a thin hairbrush, smeared onto a filter paper and set in one odour source. The other odour source chamber was kept blank by inserting just a filter paper without any fecal coat on it. Hence, any attraction to the chamber with a fecal coat in the absence of an individual larva indicated involvement of olfactory cues associated with the fecal coat in *T. julis* host-finding behavior.

3.2.6. Statistical Analyses

Data were analyzed using SAS statistical software (SAS Institute, Cary, NC). The data were transformed whenever necessary to achieve normal distributions. The assumptions of normality and homogeneity were tested using Shapiro-Wilk and Kolmogrov-Smirnov tests, and Levene's test, respectively. The biological parameters such as developmental time and parasitization time, and clutch characters including gregariousness, sex ratio and adult longevity were reported by calculating means and standard errors using PROC MEANS (SAS Institute 2010a).

I compared the following biological parameters using analysis of variance in PROC MIXED (SAS Institute 2010b): extent of parasitization among four exposure periods, host instar preferences of *T. julis* among larvae of *O. melanopus* in choice and no-choice tests under laboratory conditions, preferences for larval instars under field conditions using choice arenas, and differences in gregariousness and sex ratios of emerging parasitoids among different larval instars. Treatment means were compared using Tukey's studentized range test. In a laboratory and field choice test, each choice arena with four larval instar choices was considered a block and treated as a random effect. Larval instar as a treatment was treated as a fixed effect in both laboratory and field tests. Observations on clutch sizes and sex ratios from the above experiments and all related experiments were pooled to analyze the relationship between clutch size and female-biased sex ratio using correlation analysis with Spearman's rank correlation coefficient (PROC CORR, SAS Institute 2010a). In the experiment where only small and large instar larvae were exposed to field populations of *T. julis*, a non-parametric

Wilcoxon Signed Rank test was used to assess *T. julis* instar choice for oviposition (SAS Institute 2008a).

In the four-choice olfactory assay, each run consisting of 10 females was considered as one replication. Ten replications were performed (total of 100 females). In each replication, the proportions of *T. julis* females captured in each chamber of the olfactometer were compared by fitting generalized estimating equations with Poisson distributed error functions in PROC GENMOD (SAS Institute 2008b). Pair-wise comparisons of the proportions of females among odour choices associated with larvae were made using Wald chi-square tests (LS MEANS statement with the 'DIFF' option in PROC GENMOD) (SAS Institute 2008b).

In two-choice assays, the proportions of female *T. julis* responding to each choice were compared using two independent samples t-test (PROC TTEST, SAS Institute 2008c), to identify whether the treatments differed in terms of olfactory choice.

3.3. Results

3.3.1. Biological parameters of T. julis and effective exposure period

Developmental parameters of *T. julis* are summarized in Table 3.1. Upon encounter with an *O. melanopus* larva, a female inserted its ovipositor inside larval body near the anal region (Plate 3.1a). Once oviposition started, a single act of oviposition lasted, on average, 12 min (effective parasitization time). This was the time period between insertion of ovipositor into larval body and retracting the

ovipositor after laying eggs. Mean clutch size was about five eggs per larva (Plate 3.1b). A minimum of two to a maximum of 12 *T. julis* emerged from a parasitized larva. On average, the entire clutch of eggs laid in a single larva completed development in approximately 23 days.

Parasitized larvae appeared to feed less than nonparasitized larvae but they did not die during larval stages. Parasitized larvae formed cocoons and attempted to pupate within their earthen cocoons. However, death occurred before pupae had been formed, and *T. julis* larvae developing within the beetle larvae emerged from the cadavers of their hosts (Plate 3.1c). The larvae of *T. julis* formed naked pupae (Plate 3.1d) within their host cocoons. Pupae were initially yellowish to orange in colour and turned blackish as their development progressed. Eclosing adults cut open a tiny hole in the beetle cocoon and emerged through it. Both males and females emerged on the same day. The sex ratio was female-biased, and on average, a clutch yielded 74% females (Table 3.1). From each clutch, at least one male emerged. Mating occurred within a short time following emergence. One male mated with several females and each female mated multiple times during its life time. Females lived longer than males for up to three weeks while males died in about a week. When the females were not exposed to beetle larvae, mean longevity extended considerably and females lived for about 21 days (Table 3.1). Male longevity was short and it was not affected by the presence of hosts.

The number of larvae parasitized by newly emerged, naïve *T. julis* females increased significantly as time of exposure was increased (F= 36.53; df = 3, 42; $P < 0.0001$). Lowest parasitization was observed when larvae were exposed for 24 h

and it was significantly less than exposures of 48, 72 and 96 h ($P < 0.001$ for all comparisons) (Fig. 3.2). There were no significant differences between proportions of larvae parasitized in 48, 72 or 96 h periods. About 65% of exposed larvae were parasitized at an exposure period of 48 h. At 96 h, up to 86% larvae could be parasitized although such numerical differences did not result in statistically significant treatment differences.

3.3.2. Host-instar preference of T. julis

Females of *T. julis* preferred certain larval instar stages under both choice and no-choice scenarios. In choice tests, levels of parasitization differed significantly among larval instars ($F = 3.15$, df = 3, 42; $P < 0.05$; Fig. 3.3a). Highest parasitization was observed in fourth-instar larvae while the lowest was observed in first-instar larvae. First-instar larvae differed significantly in terms of parasitization from fourth-instar larvae ($P < 0.0001$), while parasitization of first instars did not differ from second and third instars. There were no significant differences in terms of parasitization among second and third instars, and also between third and fourth instars ($P > 0.05$) (Fig. 3.3a).

Fecund *T. julis* females adjusted clutch size based on the host instar selected for oviposition, and the number of eggs laid per larva differed significantly among instars ($F = 4.80$, df = 3, 42; $P < 0.05$; Fig. 3.3b). Most eggs were laid in fourth-instar larvae followed by second and third instars. Fewest eggs were laid in first-instar larvae. First-instar larvae differed significantly from fourth-instar larvae ($P < 0.01$), but did not differ significantly from second and

third instars. Similarly, second and fourth instars did not differ among each other statistically although numerically different clutch sizes were observed. However, change in clutch sizes among instars did not significantly influence the sex ratio. The sex ratio was generally female-biased (F = 0.32, df = 3, 42; P > 0.05; Fig. 3.3c).

In no-choice tests, levels of parasitization also differed significantly among different larval instars (F= 0.016, df = 3, 48; P < 0.05; Fig. 3.4a). The highest level of parasitization was observed in fourth instars while the lowest was in first instars. No significant differences in parasitization were observed among first-, second- and third-instar larvae. Significant differences existed between first and second instars and fourth instars in percent parasitization (P < 0.001). Mean numbers of eggs laid in larvae differed significantly among some instars (F = 15.53, df = 3, 48; P < 0.05; Fig. 3.4b). Both first- and second-instar larvae differed significantly from third- and fourth-instar larvae in mean numbers of eggs laid (P < 0.05). However, between first and second instars and between third and fourth instars, no significant differences were observed in terms of mean parasitoid eggs harboured.

The female-biased sex ratio was positively correlated with clutch size (ρ = 0.89; P < 0.001). As the clutch size increased, the number of female progeny emerging from the clutch also increased (Fig. 3.5).

3.3.3. Host instar preference and seasonal activity in the field

Females from field populations of *T. julis* exhibited specific preferences for host-instar parasitization; larval instars exposed to field populations differed significantly in percent parasitization (F= 3.57, df = 3, 32; $P < 0.05$; Fig. 3.6a). Highest percent parasitization was observed in fourth-instar larvae, while the lowest occurred in first-instar larvae. However, there were no significant differences between first-, second- and third-instar larvae in terms of proportions of larvae parasitized. Also, no significant differences were observed among second-, third- and fourth-instar larvae in proportions parasitized. Only first and fourth instars differed significantly in percent parasitization ($P < 0.05$). Field parasitization levels among first through third instars were in the range of 12-18%, but exceeded 30% in fourth-instar larvae (Fig. 3.6a).

The average clutch size differed among larval instars (F = 5.30, df = 3, 32; $P < 0.01$; Fig. 3.6b). Female parasitoids laid most eggs in fourth-instar larvae (mean = 8 eggs per larva). First-instar larvae harbored significantly fewer parasitoid eggs than fourth-instar larvae ($P < 0.01$). However, there were no significant differences among first, second and third instars in terms of clutch size. Similarly, second and third instars did not differ statistically from fourth instars based on clutch size ($P > 0.05$). As observed in the laboratory, females tended to lay more eggs in larger fourth-instar larvae. Differences in egg numbers laid did not influence the sex ratio. The proportion of *T. julis* females emerging from parasitized larvae of different instars did not differ among instars (F = 4.90, df = 3, 32; $P > 0.05$; Fig. 3.6c).

When the larvae were grouped as “small” and “big”, and exposed in choice arenas to field populations of *T. julis*, the levels of parasitization in larger instars (third and fourth) were slightly higher numerically than small instars (first and second). However, no difference in parasitization between large and small instars was evident statistically (Z= 7.0; $P > 0.05$) (Fig. 3.7).

The data on proportions of sentinel larvae parasitized over a sampling period of 11 weeks elucidated seasonal activity patterns of *T. julis* under local field conditions. Levels of parasitization differed significantly among sampling dates (F = 13.48, df = 6, 21; $P < 0.0001$; Fig. 3.8). No parasitization was observed in late May (25 to 30 May 2012). The onset of parasitization occurred in the first week of June (6 to 10 June) and there was a gradual increase in levels of parasitization of larvae exposed in the field. Peak parasitization was observed in the middle of June (15 to 20 June). Parasitization levels declined further in late June (22 to 26 June, 22% larvae parasitized) with little parasitization observed in July (7%). None of the larvae exposed were parasitized in the first week of August.

3.3.4. Role of olfactory cues in host finding

About 50% of females from the total replicates tested responded to either of the four odour sources (larvae with fecal coat, larvae without fecal coat, fecal coat only and no fecal coat). Once introduced in the insect chamber, the test females preferred to walk into the main arena of the olfactometer. Random movements in the main arena were observed in response to different odours. Once

the females had made a decision to orient to a particular odour source, they walked a short distance with their antennae held straight forward in the direction of the source. This behaviour was followed by bending the antennae forward to touch the floor of the choice arena. Females walked a short distance in the direction of the source of preference while tapping the chamber floor with their antennae. Tapping frequency was faster close to the source inlet valve. The females then entered the glass chamber connected to the odour source with antennae held either straight in a forward direction, or while tapping the chamber floor with antennae as they moved into the chamber.

The proportions of *T. julis* females responding to different odour sources associated with the fecal coat of *O. melanopus* larvae differed significantly among treatments ($\chi 2$ = 18.17, df = 3; $P < 0.05$) (Fig. 3.9). The proportions of females responding to the odours emanating from larvae with a fecal coat and the larvae without a fecal coat did not differ significantly ($P > 0.05$). However, the response rates to odours from the fecal coat only and control chambers were significantly lower than those recorded for both the larvae with a fecal coat and larvae without a fecal coat ($P < 0.05$). About 46% of females responding showed a preference to odours from the chamber containing larvae with a fecal coat followed by larvae without a fecal coat (response rate = 39%) (Fig. 3.9).

Two-choice olfactory bioassays indicated that the response rates to odours from a fecal coat vs. the control differed significantly (t =21.17; $P < 0.05$). The mean number of females responding to odours arising from a fecal coat was significantly greater than for odours arising from control chambers (Fig. 3.10).

3.4. Discussion

Parasitoids employ several strategies to adapt to their host and to synchronize their life cycle with the host to best utilize the host-available resources (Charnov and Skinner 1984; Godfray 1994). An understanding of such strategies involved in host-parasitoid interactions can enhance biological control efforts. The major strategies employed by parasitoid females for optimal resource utilization include: a female-biased sex ratio (Godfray 1994; Vet et al. 1994; Chong and Oetting 2006), adjustment of clutch size based on the host size (Godfray 1987), ability to select host stages of optimum quality (Lin and Ives 2003; Latham and Mills 2010; Amarasekare et al. 2010), and depositing eggs that produce female progeny in resource-rich areas and male eggs in resource-poor areas (Jones 1982; King 1987). The sex ratio of parasitoids emerging from a clutch is an important determinant of host quality in parasitoids, particularly for gregarious species (Bertschy et al. 2000; Harvey 2000). In the current investigation, some of the above strategies were observed in the life history of *T. julis* females.

Salient features of *T. julis* biology observed in this study were a short developmental time, a highly female-biased sex ratio, extended longevity of females, sibling mating, the absence of mechanisms such as gynandry or protandry, and more importantly, the capacity of females to live longer in the absence of hosts. Extended survival when the females were not exposed to hosts is an important fitness strategy to maintain the possibility of future encounters

with the host. Such life history patterns are known in other congeneric species. For example, production of a female-biased sex ratio, enhanced longevity and high reproductive rates have been reported for *Tetrastichus planipennisi* Yang, the introduced eulophid parasitoid of emerald ash borer (*Agrilus planipennis* (Fairmaire)) in the U.S.A. that make it a promising biocontrol agent (Duan et al. 2011).

I observed prolonged oviposition times in *T.* julis females (about 13 minutes) for a single act of oviposition. Shorter oviposition times in several parasitoid species are known (Godfray 1994). However, longer parasitization times are known in related eulophid species. For example, *Tetrastichus setifer* Thompson, a larval parasitoid of the lilly leaf beetle takes about 15 minutes for a single act of oviposition (Casagrande and Kenis 2004). The significance of longer oviposition times for *T. julis* life history has not been investigated. Newly emerged females of *T. julis* readily parasitized *O. melanopus* larvae upon mating; however, their parasitization rates were higher 48 h after their emergence. Such longer times observed for parasitization may be associated with host acquaintance and acquiring experience to parasitize the hosts (Segoli et al. 2009), or foraging nectar sources to increase fitness. However, readiness of the females to parasitize host larvae within a short time span suggests a high resource utilization capability of the parasitoid. *Tetrastichus julis* activity was relatively short-lived under field conditions; however, rates of parasitization achieved in a limited activity span were high.

The seasonal trend in parasitization of *T. julis* indicated that its greatest activity was concentrated in June with a peak observed in mid-June. Activity of *O. melanopus* begins in early to mid-May with high larval activity on winter wheat observed in mid-June to early-July (Kher et al. 2011). Onset of *T. julis* activity in early June therefore enables the parasitoid to synchronize its greatest activity with that of its host. The larvae parasitized late in the season may indicate the presence of second generation adults. The second generation adults emerge from the larvae parasitized in the first cycle of parasitization by overwintered *T. julis* adults. The second generation adults then can parasitize the remainder of *O. melanopus* larvae available in the field. Such parasitized larvae attempt to pupate inside the soil, but die inside their cocoons. *Tetrastichus julis* larvae then overwinter inside their host cocoons as fifth-instar larvae and emerge as adults in the following season (Stehr 1970; Kher et al. 2011). In the field area where I conducted my study, the availability of field populations of *O. melanopus* larvae was low late in the season. This may have restricted the activity of second generation *T. julis* adults and these adults may have parasitized the available host larvae and overwintered before the experimental arenas with laboratory-reared larvae were set out. Although bivoltine, some first generation *T. julis* larvae enter diapause in summer and continue to overwinter to emerge as adults in the following spring (Harcourt et al. 1977). Hence, a part of the population has two generations a year while the other has one generation (Harcourt et al. 1977). All these factors combined may have contributed to the absence of a second peak being observed in the field even though the parasitoid is bivoltine.

Previous studies have reported that parasitoid females choose host sizes that provide optimal resources to their progeny; selection of hosts of particular size by some parasitoid groups is a part of this strategy (Godfray 1994; Harvey et al. 2004; Bell et al. 2005). This investigation revealed that *T. julis* females possess the capacity to distinguish between developmental stages of the host (discrimination between host instars), and can adjust clutch size based on host stage. When a choice of larval instars was available, *T. julis* tended to lay more eggs in bigger instars compared to smaller ones. Nevertheless, all stages were accepted for parasitization but the levels of parasitization among instars differed. Given a choice of instars, *T. julis* females tended to oviposit in fourth-instar larvae compared to first-instar larvae. In terms of percent parasitization, the ranking for larval instars in order of highest to lowest parasitization was IV > II, III > I. A similar ranking in terms of clutch size was observed among host instars, where the instars preferred for oviposition were IV > III, II > I. Although the female-biased sex ratio was positively correlated with clutch size, the results of analysis of variance indicated that proportional female emergence from different instars did not differ. This discrepancy may be explained by differences in sample sizes for correlation analysis and analysis of variance. Correlation analysis used pooled data on female emergence from different trials resulting in a large sample size. Although the differences in female emergence among instars are not statistically significant using analysis of variance, numerical differences are indicative of a pattern of higher emergence as the instar size increases.

My discovery that generally all instars were accepted for parasitization corroborates the findings of Dysart et al. (1973). However, Dysart et al. (1973) suggested that young larvae are preferred for oviposition, which contradicts my results. I observed that despite acceptance of all host stages, percentage parasitization was highest in fourth instars. Such preferences for a particular host instar in the presence of a choice of host stages exist in several solitary parasitoid species parasitizing mealy bugs (Amarasekare et al. 2010).

Clutch adjustment based on host stage is reported elsewhere (Vet et al. 1994; Haeckermann et al. 2007; Wang et al. 2008; Sarikaya and Gülel 2011). For instance, specific preferences for host-instar size are known in the gregarious endoparasitoid *Anagyrus* sp. (Hymenoptera: Encyrtidae) and such preferences influence clutch size, sex ratio and development of the parasitoid progeny (Chong and Oetting 2006). Host developmental stage is among the important determinants of a parasitoid's developmental success and progeny allocation (Herbert and Cloutier 1990; Lykouressis et al. 2009). However, it must be noted that there are no consistent patterns in terms of host selection among parasitoid groups (Harvey 2005). Host choice of a parasitoid can be influenced by several factors including and not limited to: host specialization, local mate competition, parasitoid dispersal efficiency, longevity, lifetime reproductive success, parasitoid developmental pattern (gregarious vs. solitary), physiological state of host, host availability, and climate (Godfray 1994; Harvey 2005). Further, reproductive strategy of the particular parasitoid species can influence host choice (Jervis et al. 2001). For example, synovigenic females (can mature egg compliments throughout their

lifetime) and provigenic females (female adults with fixed egg compliment at eclosion) can differ in their host choice (Jervis et al. 2001). Similarly, life history patterns of parasitoids (idiobiosis vs. koinobiosis) also determine host preferences (Harvey 2005). Within idiobiont and koinobiont parasitoids, there are differences among species in terms of host affinities. For example, a koinobiont parasitoid of mealy aphids, *Aphidius transcaspicus* Telenga, is known to parasitize intermediate stages from second to fourth instars compared to first-instar nymphs (Latham and Mills 2010). In contrast, the parasitoid of pea aphid, *Monoctonus paulensis* (Ashmead), which is also a koinobiont, prefers early instars compared to late nymphal instars (Chau and Mackauer 2000). Similarly, an idiobiont parasitoid, *Sclerodermus harmandi* (Buysson), prefers advanced larval instars of *Monochamus alternatus* Hope (Liu et al. 2011), while another parasitoid, *Eriborus argenteopilosus* Cameron, prefers smaller larval instars of *Helicoverpa armigera* Hubner (Pascua and Pascua 2004). Hence, trends observed in my investigation are specific to *T. julis* and cannot be generalized. However, my investigation underlines wide host adaptability of *T. julis* as one of the traits contributing to its success as a biological control agent.

Another important fitness parameter is the sex ratio (Godfray 1994). Female-biased sex ratios are indicative of greater fitness than sex ratios that are not female-biased (Vet et al. 1994; Chong and Oetting 2006). *Tetrastichus julis* sex ratios were highly female-biased; however, clutches yielding only female progeny were never observed. Clutch sizes were highly correlated with female emergence indicating that large clutches resulted in greater emergence of female

progenies. At least one male emerged from most clutches (> 2) and sibling mating was common. Such extremely female-biased sex ratios are very commonly observed in gregarious parasitoids and consequently result in sibling mating (Godfray 1994). Such interactions among siblings emerging from the same brood are termed local mate competition (Hamilton 1967). The female-biased sex ratio and local mate competition help foster mating of potential males with females thus maintaining a progeny with higher fitness (Godfray 1994). In many hymenopteran parasitoids, progeny sex allocation is determined based on host quality and size, and high host quality results in high female emergence (Godfray 1994; Bell et al. 2005). However, we did not observe any such sex alterations among host stages by *T. julis*. The sex ratio of emerging parasitoids was comparable on all host stages. This indicates that females do not discriminate between hosts to determine sex allocation and are capable of utilizing hosts of any size/stage irrespective of their quality to produce female-biased sex ratios. Although many studies indicate that some parasitoids may lay female-producing eggs on larger and high quality hosts than in smaller hosts, the species studied mainly exhibited wide host ranges and such preferences were a result of availability of different hosts with variable quality (King 1987; Bell et al. 2005; Haeckermann et al. 2007; Sarikaya and Gülel 2011). In the case of *T. julis*, it is a highly host-specific parasitoid and does not show preferences for any other hosts (Dysart et al. 1973; Harcourt et al. 1977). This limits host choice preferences and foraging behaviour of this parasitoid under field conditions, especially in conditions where the host is rare, as is currently observed in some habitats in

western Canada. Hence, *T. julis* may have to employ strategies that do not take into account only the quality of the host but also utilize available host stages in the most efficient manner.

Apart from the preferences of female parasitoids for larval stages based on host quality, an important determinant for encountering a suitable host is related to the olfactory cues associated with the host. Host-finding is mediated by various cues associated with the host, of which olfactory cues play an important role (Charnov and Skinner 1984). The responses by *T. julis* females to various odour sources associated with the larval fecal coat of the beetle in an olfactometer arena indicated that olfactory cues from the fecal coat contribute to host-finding by *T. julis*. The two-choice bioassay, in particular, demonstrated greater response rates to odours associated with the larval fecal coat. Observations on the behaviour of *T. julis* females prior to and during parasitization of host larvae showed conspicuous antennal tapping on the fecal coat wherein the antennae were bent forward and downward to touch the fecal coat. Further, when the parasitoids were introduced into rearing containers containing beetle larvae, and when approaching the larvae for parasitization, the females tracked the trails of the fecal smears on the filter paper left by the beetle larvae. When a host larva was contacted, females tapped the larval fecal coat with their antennae. In certain cases, antennal tapping extended for several seconds to a minute; however, the females sometimes did not choose to parasitize a given larva upon tapping.

Although no specific reports describe host-finding behaviour of *T. julis*, it is speculated in the literature that the olfactory cues associated with the fecal coat

of *O. melanopus* larvae may have a role in orienting parasitoids to the host (Wellso and Hoxie 1988). This formed the rationale for conducting the olfactory bioassays. The observations recorded here on the importance of the *O. melanopus* fecal coat as an olfactory cue and the orientation behaviour observed in olfactory bioassays are reported for the first time and clearly indicate involvement of olfactory cues from the fecal coat in host finding by *T. julis*. Larvae of many species of Chrysomelidae including the cereal leaf beetle cover their bodies with their own excrement thus forming a fecal coat or shield (Schaffner and Muller 2001). While the fecal shield has been considered to protect the larvae from predators (Gómez et al. 1999; Bacher and Luder 2005; Chaboo et al. 2007), studies have also indicated that the olfactory characteristics associated with the fecal coat may be used as potential cues by parasitoids to locate larval hosts (Wellso and Hoxie 1988; Olmstead 1994; Schaffner and Müller 2001), and may contain kairomones that attract parasitoids (Steidle 2000) at least in their microhabitat (Fatouros et al. 2008). The role of cues associated with the fecal coat of a related species, the lily leaf beetle (*Lilioceris lilii* (Scopoli)), in the host-finding behaviour of its parasitoid *Lemophagus pulcher* Szepligeti is known (Schaffner and Müller 2001); both olfactory and contact bioassays indicated a greater behavioural response to cues emanating from the fecal coat. Similarly, a related species of *T. julis* and a biocontrol agent for the lily leaf beetle, *T. setifer*, responds to the fecal coat and fecal coat extracts of its host in olfactory bioassays (Casagrande and Kenis 2004). Egg parasitoids of elm leaf beetle, including two eulophid species, are also known to locate their host based on fecal chemical cues

(Fatouros et al. 2008). Parasitoid host-finding is a multi-step process and can involve olfactory, visual, tactile and gustatory cues (Vet et al. 1983). Hence, it is important to consider that olfactory cues associated with *O. melanopus* fecal coat alone may not be the only determinants of host-finding by *T. julis*. Cárcamo et al. (2012) studied whether larvae of the non-target chrysomelid beetle, *Cassida azurea* Fabricius, smeared with the fecal coat of *O. melanopus* can invoke a preference for parasitization in *T. julis* females. However, no parasitization behaviour in response to artificial smearing of the fecal coat was observed in the non-target chrysomelid. This suggests that the role of the fecal coat may be limited to host acceptance alone and other cues associated with *O. melanopus* larvae may be responsible for effective parasitization. Plant volatiles emitted by host plants and induced upon herbivore feeding can act as cues in the host-finding behaviour of several parasitoids (Cortesero et al. 2000). However, I did not test whether the host plant volatiles have a role in attracting *T. julis*. Research attention to involvement of other cues in *T. julis* host-finding should be undertaken.

This investigation of life history parameters and host size preferences encompassed data from both field and laboratory experiments. However, it is important to note that both *O. melanopus* and *T. julis* are currently in their early establishment phase in western Canada, with populations of *O. melanopus* scattered over a vast geographical area with localized hot-spots. The patchy distribution pattern of *O. melanopus* has presumably influenced population structures of *T. julis*. Although several studies described here were performed in

the laboratory under optimal conditions, it is important to note that field preferences of *T. julis* can depend on a variety of factors such as encounter rates of *T. julis* with its hosts, distribution and availability of various host stages (Weisser 2000), variations in life expectancy of host and parasitoid populations due to the dynamic nature of field interactions (Bezemer and Mills 2003), and physiological state of the host (Bell et al. 2005).

Nevertheless, this investigation examined a variety of life history traits such as developmental rates, adult longevity, the relationship between clutch size and sex ratio which determine intrinsic rates of increase of parasitoid populations in relation to their hosts. Such factors also determine successful establishment of parasitoids and the extent to which pest populations can be controlled using natural enemies (Latham and Mills 2010; Latham and Mills 2012). Prior studies indicated that the reasons for successful establishment of *T. julis* in North America included its host-tracking capacity, host-specificity, higher synchronization with the host and gregariousness (Haynes and Gage, 1981). However, I conclude that the mechanisms underlying host-tracking capacity and higher degree of host synchronization emanate from life history patterns and preferences of *T. julis* for its hosts, but have not been previously explored in detail. These mechanisms contribute to the success of *T. julis* as a biological control agent. This investigation provides novel information on *T. julis* host preferences and host-finding.

Information on the life history traits of *T. julis* developed in this study can contribute to designing efficient parasitoid rearing and mass relocation

programmes to strengthen classical biological control programmes. Understanding population structures of the natural enemy based on its host-specific preferences can help to develop insights into the dynamics of host-parasitoid interactions and factors influencing such interactions (Sandanayaka et al. 2009). This has implications for augmenting activity of *T. julis* in its new eco-region to manage cereal leaf beetle in a sustainable manner. In the U.S.A., *T. julis* was reared through development of field insectaries and by relocating parasitized larvae to other regions with beetle infestations (Dysart et al. 1973; Vail et al. 2001). Field insectaries are more successful and economical than laboratory rearing in the U.S.A. (Dysart et al. 1973). A similar approach of parasitoid multiplication and relocation can be adopted in western Canada. In view of patchy populations of *O. melanopus* over a vast geographic area in western Canada, establishment of field nurseries alone may not be a feasible approach. Hence, parasitoid mass rearing in laboratory can act as an effective way to mass-produce and relocate parasitoids in its early stage of beetle invasion. Results of this study indicate that mass rearing of *T. julis* can be enhanced by exposing mated *T. julis* females for at least 48 h to fourth-instar larvae of *O. melanopus*. Although female-biased sex ratios are common in the parasitoid, each clutch will contain at least one male, and this would enhance the gravid status of females. Mate competition among males would help maintain the fitness of *T. julis* progeny in mass rearings. Making field collections of *T. julis* would be most efficient in early to mid-June in western Canada when populations of both the host and parasitoid are most abundant. In laboratory rearing, care should be taken in handling and keeping

them at high humidity, to maintain the fecal coats on *O. melanopus* larvae that are being subjected to parasitization, as this should increase parasitization frequency by *T. julis*. Relocation of parasitized larvae to newly infested areas during peak larval activity season can help the parasitoids to disperse in the field and find the host.

Tables.

Table 3.1. Life history parameters and clutch size characters of *Tetrastichus julis* under laboratory conditions following parasitization of *Oulema melanopus* larvae

Parameter	**Mean ± S. E.**	**Salient Features of *T. julis* Life History**
Developmental time (days) (N=160)	22.75 ± 0.28	• Both males and females emerge on the same day: absence of protandry • Female-biased sex ratio • Sibling mating common; females mate multiple times in their life times • Superparasitism and multiple parasitism are common, females appear incapable of discriminating previously parasitized larvae
Average parasitization time (min) (N=100)	12.35 ± 0.69	
Clutch Size and Sex Ratio: (N=160)		
Mean clutch size	4.63 ± 0.19	
Mean females emerging	3.43 ± 0.14	
Mean males emerging	1.19 ± 0.07	
Percentage of females (%)	74.22	
Percentage of males (%)	25.77	
Mean male longevity (days)	6.42 ± 0.18	
Mean female longevity (days) with exposure to *O. melanopus*	16.37 ± 0.60	
Mean female longevity without exposure to *O. melanopus* (days)	20.62 ± 0.50	

Figures.

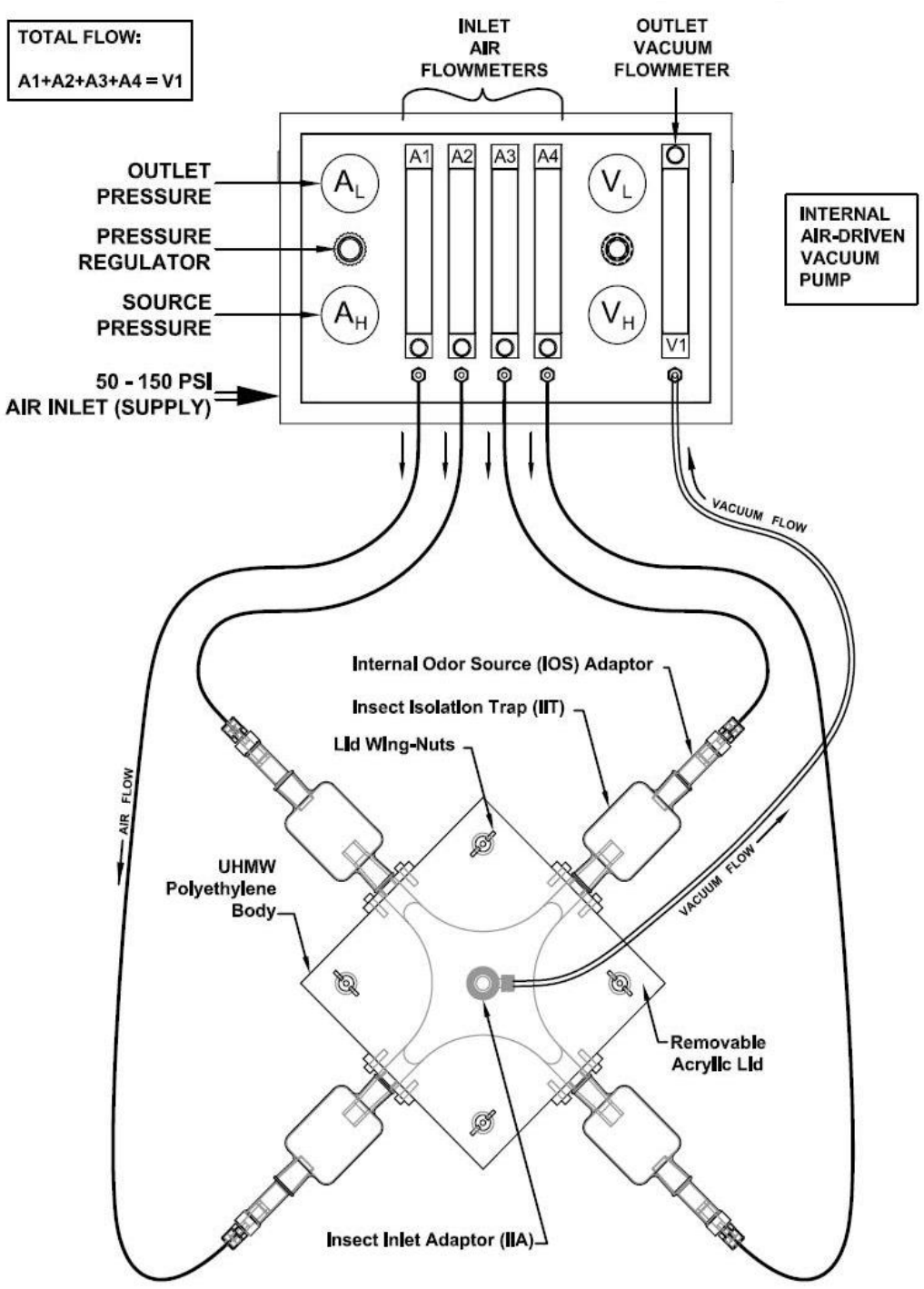

Figure 3.1. System diagram of the four-chambered olfactometer used in olfactory bioassays conducted to test whether olfactory cues associated with the fecal coat of *Oulema melanopus* larvae contribute to host-finding behaviour of *Tetrastichus julis* (Source of schematic: Analytical Research Systems Inc., Gainesville, USA).

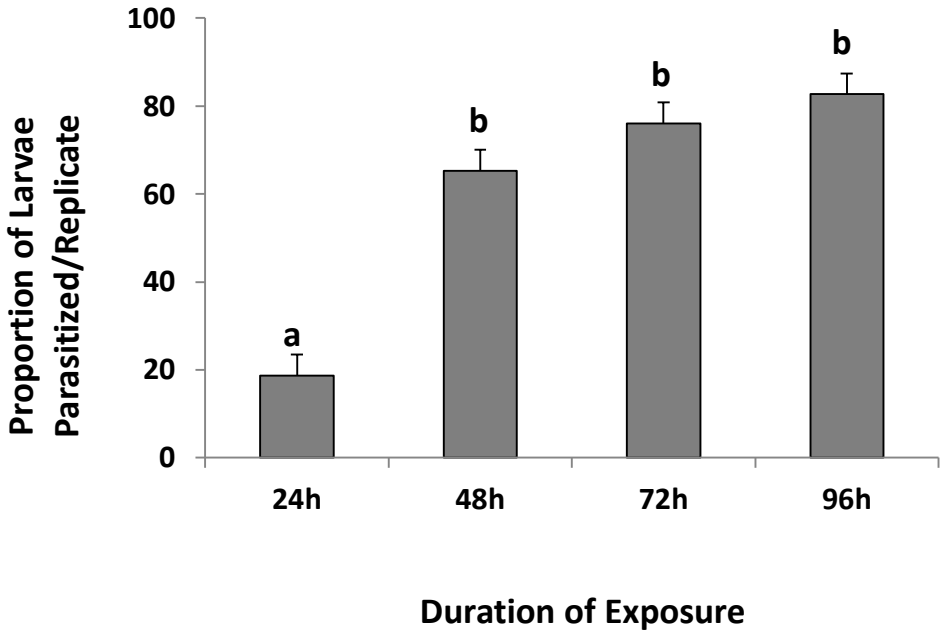

Figure 3.2. The proportion of *Oulema melanopus* larvae parasitized by *Tetrastichus julis* per replicate container at a given exposure time. Data points sharing different letters indicate statistically significant differences between exposure times in terms of larval parasitization by females using Tukey's studentized range test (α=0.05).

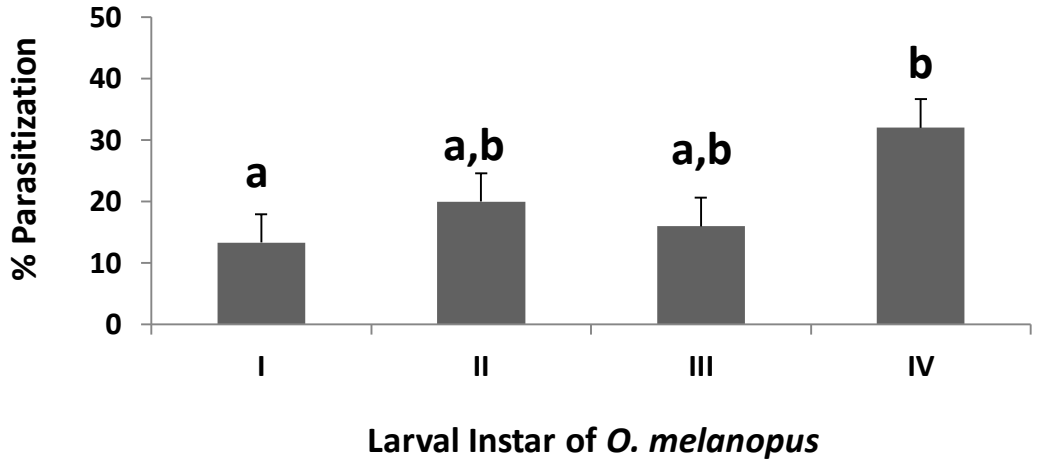

Figure 3.3a. Mean parasitization of different instars of *Oulema melanopus* larvae by *Tetrastichus julis* females in a laboratory choice test. Proportions of larvae parasitized in each replicate container are expressed in percentages (n = 20 replicates; 5 larvae/instar/replicate). Bars sharing different letters indicate statistically significant treatment differences using Tukey's studentized range test ($\alpha = 0.05$).

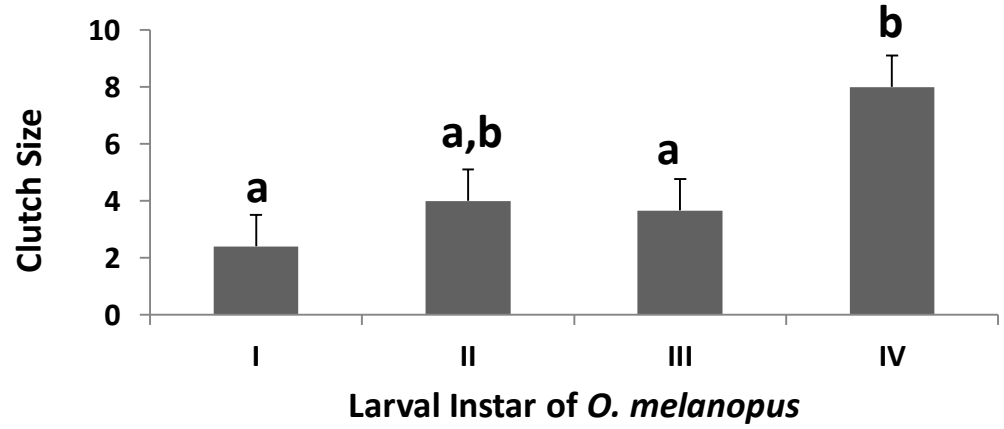

Figure 3.3b. Mean numbers of eggs oviposited (clutch size) in different instars of *O. melanopus* larvae by *T. julis* females in a laboratory choice test (n = 20 replicates; 5 larvae/instar/replicate). Bars sharing different letters indicate statistically significant treatment differences using Tukey's studentized range test ($\alpha = 0.05$).

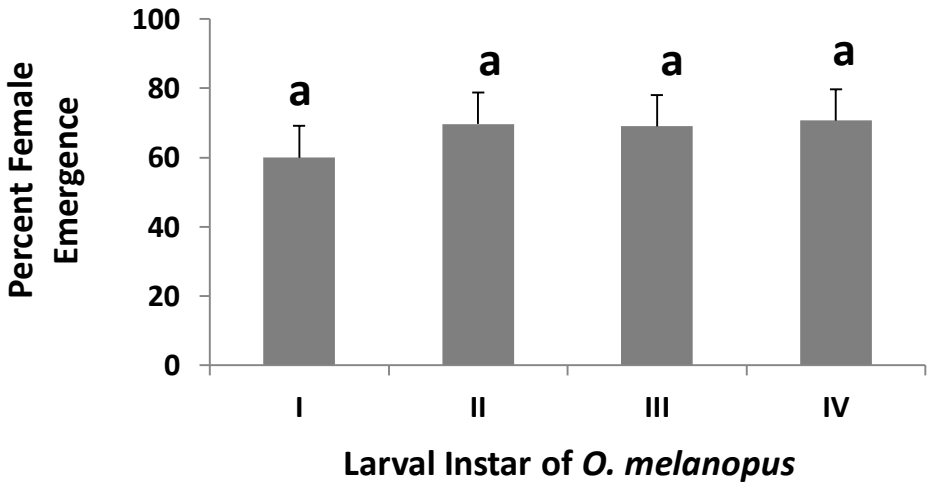

Figure 3.3c. Percent emergence of *Tetrastichus julis* female adults from eggs laid in different instars of *Oulema melanopus* by *T. julis* females in a choice test (n = 20 replicates; 5 larvae/instar/replicate). Bars sharing different letters indicate statistically significant treatment differences using Tukey's studentized range test ($\alpha = 0.05$).

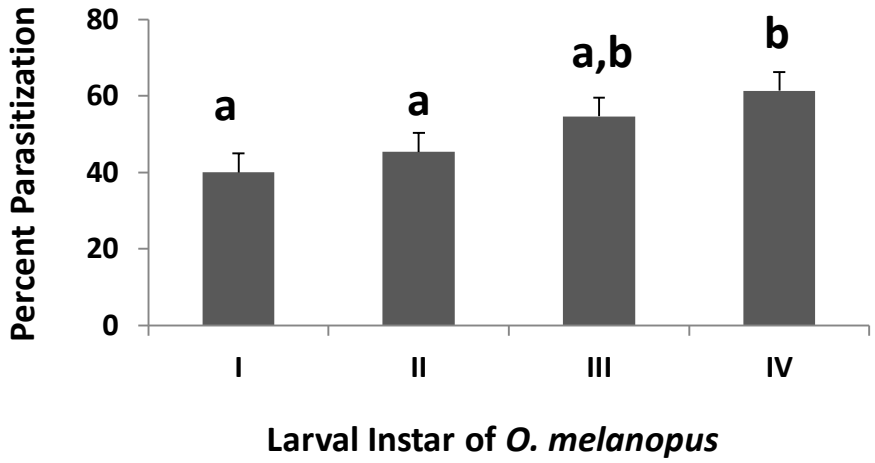

Figure 3.4a. Mean parasitization of different instars of *Oulema melanopus* larvae by *Tetrastichus julis* females in a laboratory no-choice test. Proportions of larvae parasitized in each replicate container are expressed in percentages (n=20 replicates; 5 larvae/replicate). Bars sharing different letters indicate statistically significant treatment differences using Tukey's studentized range test (α=0.05).

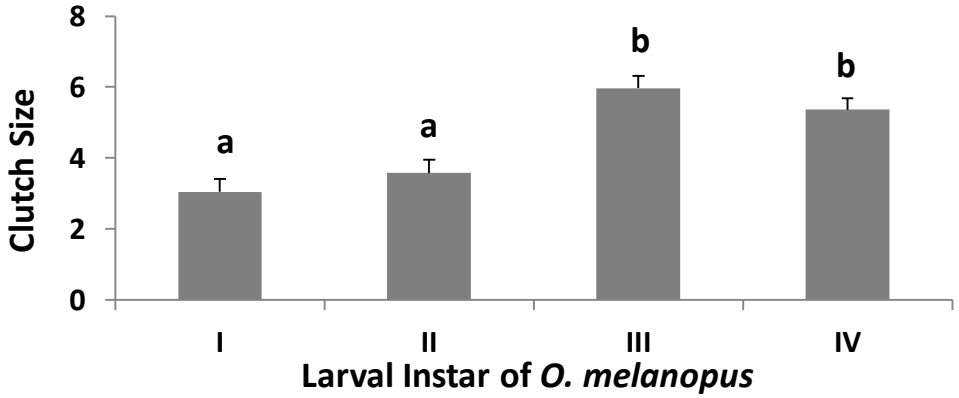

Figure 3.4b. Mean numbers of eggs deposited (clutch size) in different instars of *Oulema melanopus* larvae by *Tetrastichus julis* females in a laboratory no-choice test (n=20 replicates; 5 larvae/replicate). Bars sharing different letters indicate statistically significant treatment differences using Tukey's studentized range test (α=0.05).

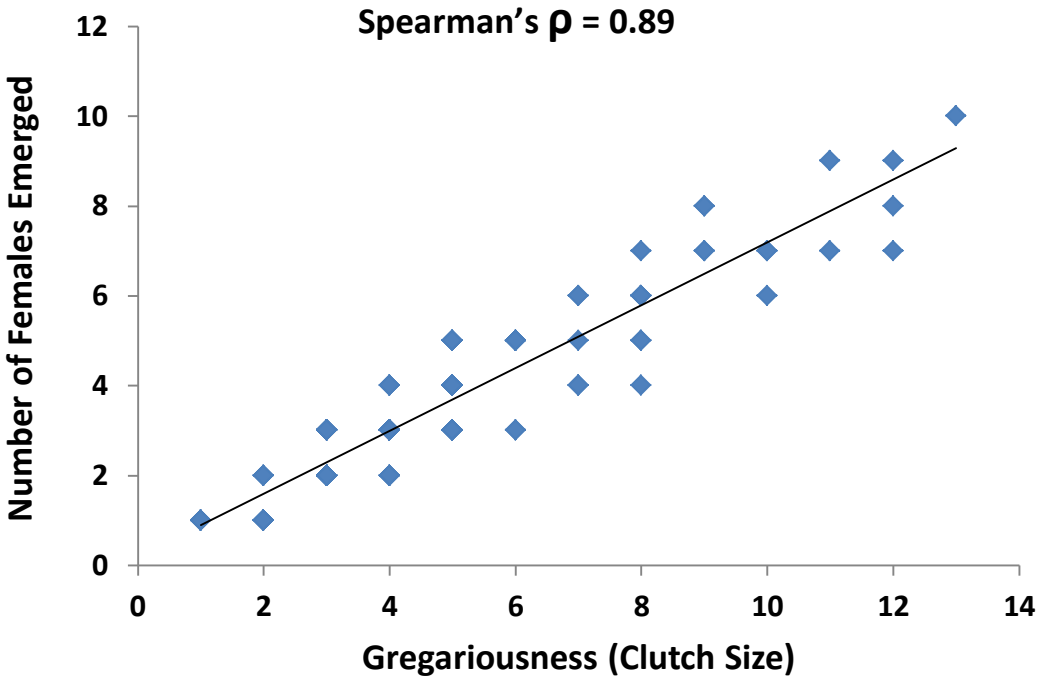

Figure 3.5. The relationship between clutch size and emergence of female *Tetrastichus julis* adults from larvae of *Oulema melanopus* parasitized by *T. julis*.

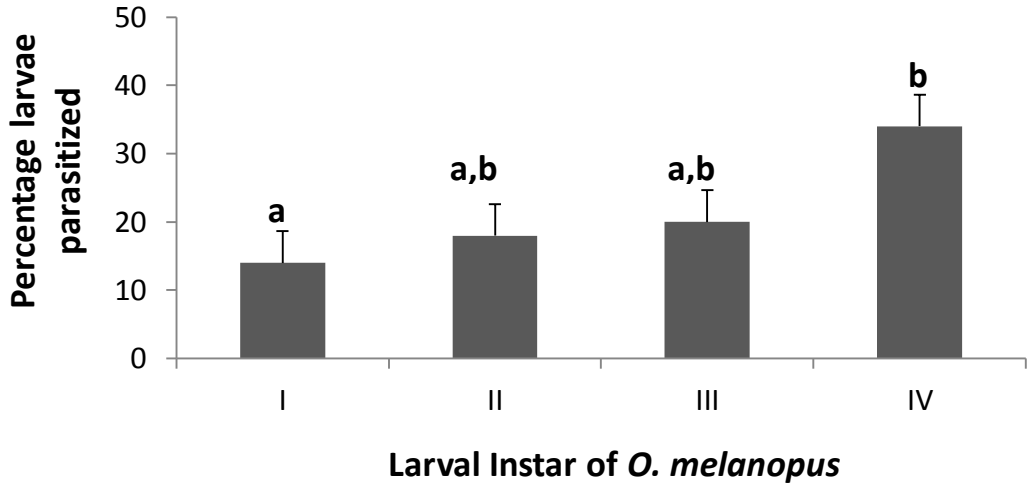

Figure 3.6a. Mean percentages of different larval stages of *Oulema melanopus* parasitized when exposed to field populations of *Tetrastichus julis* in Lethbridge, Alberta, Canada in 2012. Bars with different letters indicate statistical differences in different larval instars using Tukey's studentized range test (α=0.05).

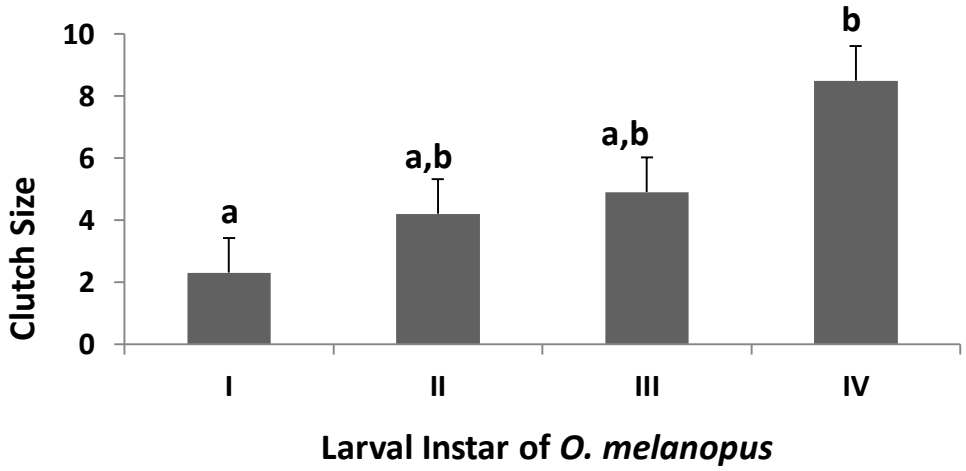

Figure 3.6b. Mean clutch sizes of *Tetrastichus julis* eclosing from different larval instars of *Oulema melanopus* following exposure to field populations of *T. julis* in Lethbridge, Alberta, Canada in 2012. Bars with different letters indicate statistical differences in different larval instars using Tukey's studentized range test (α=0.05).

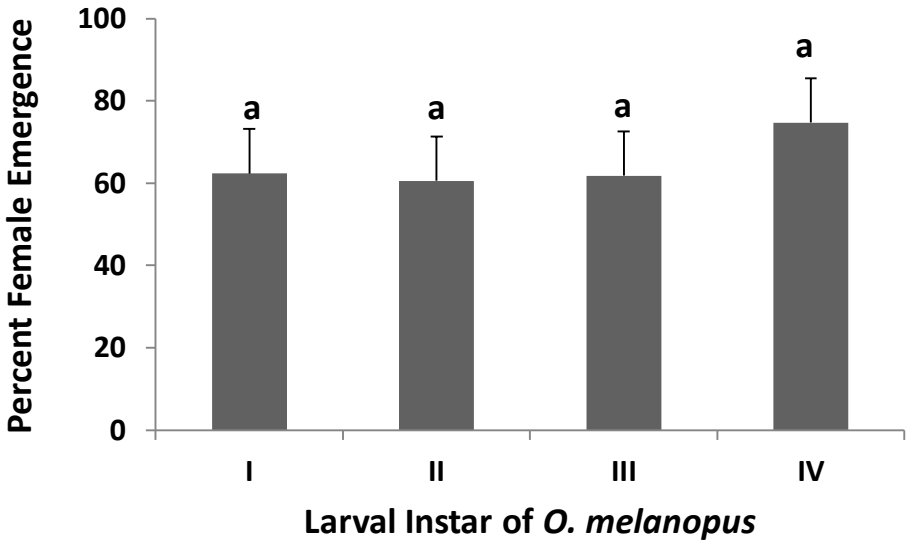

Figure 3.6c. Mean percentages of emergence of *Tetrastichus julis* females from different larval instars of *Oulema melanopus* following exposure to field populations of *T. julis* in Lethbridge, Alberta, Canada in 2012. Bars with different letters indicate statistical differences in different larval instars using Tukey's studentized range test (α=0.05).

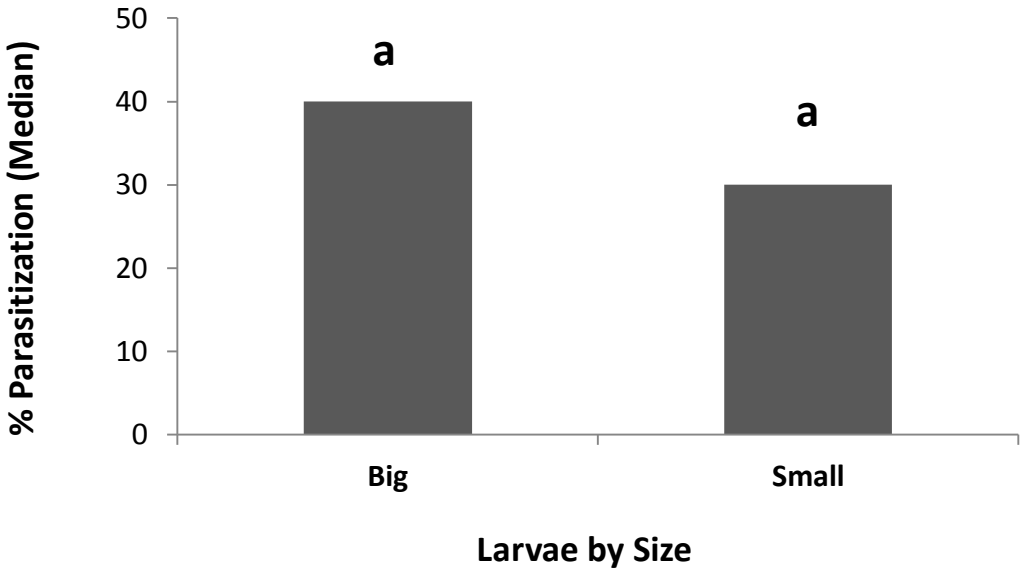

Figure 3.7. Choice of female *Tetrastichus julis* for small (first and second instars) vs. big (third and fourth) instars of *Oulema melanopus* when beetle larvae were exposed to *T. julis* under field conditions. Bars sharing the same letters indicate no statistically significant treatment differences using the Wilcoxon signed rank test.

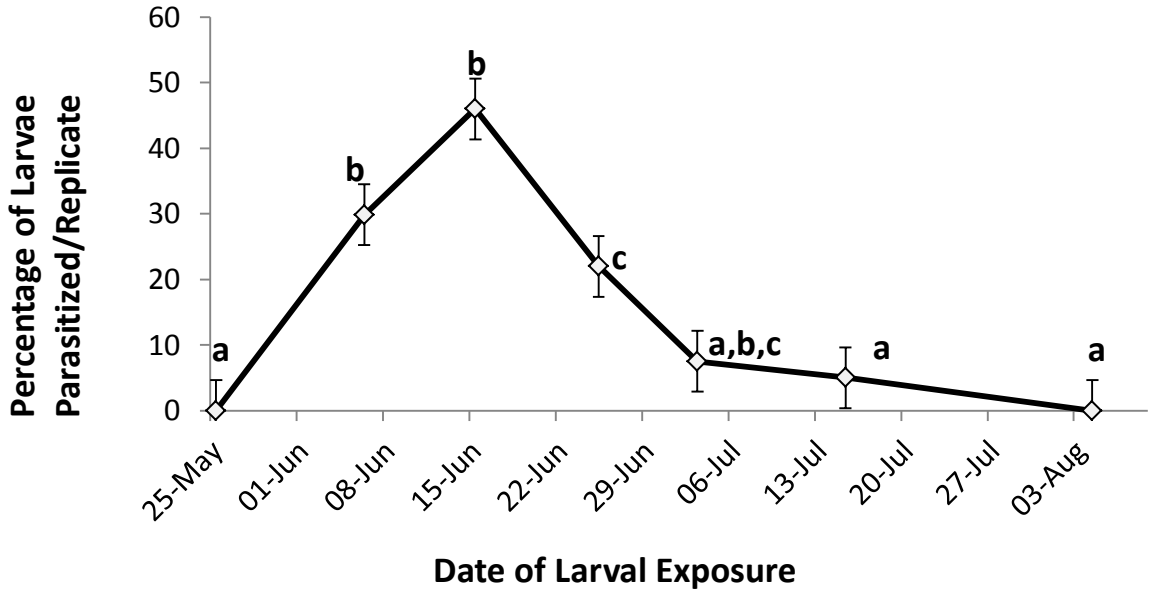

Figure 3.8. The seasonal activity of *Tetrastichus julis* at the cereal leaf beetle nursery, Lethbridge, Canada in 2012. The parasitization activity was calculated by exposing laboratory-reared *Oulema melanopus* larvae to field populations of *T. julis*, and calculating the proportions of larvae parasitized (expressed as percentages) out of the total number of larvae set in the field per replicate. Data points sharing different letters indicate statistically significant differences in parasitization activity between sampling periods.

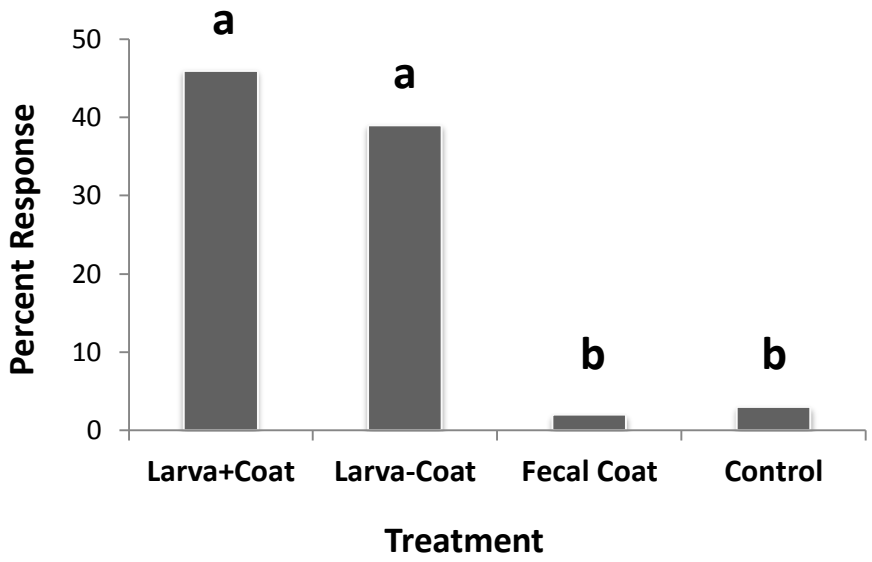

Figure 3.9. Responses of *Tetrastichus julis* females to odours emanating from different treatment sources using a four-choice olfactometer. The treatments comprised a larva of *Oulema melanopus* with its fecal coat, a larva of *O. melanopus* without a fecal coat, a fecal coat of *O. melanopus*, and a blank control. Bars with different letters indicate statistically significant differences in proportions of females attracted to different sources using Tukey's studentized range test.

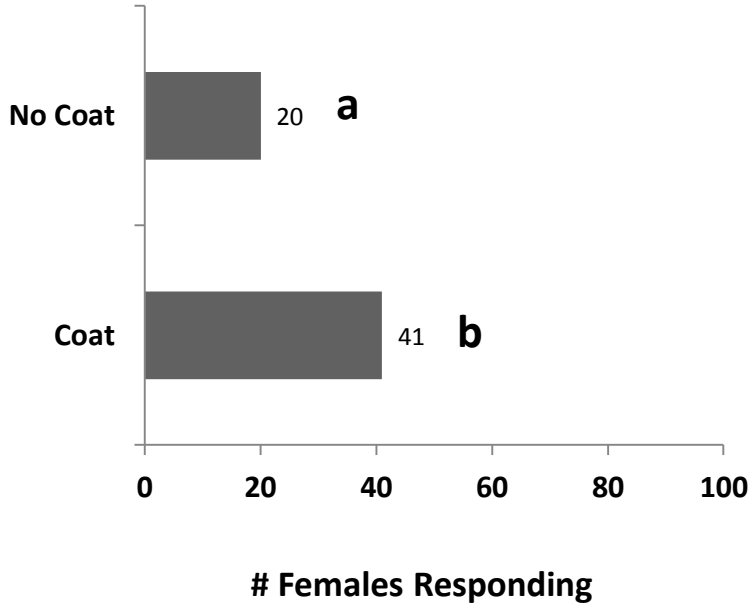

Figure 3.10. Responses of *Tetrastichus julis* females to odours emanating from two different treatment sources, a fecal coat from a larva of *Oulema melanopus*, or a blank control. Bars with different letters indicate statistically significant differences in proportions of females attracted to different sources using two independent samples t-test.

Plate.

Plate 3.1a. A female *Tetrastichus julis* ovipositing in *Oulema melanopus* larva

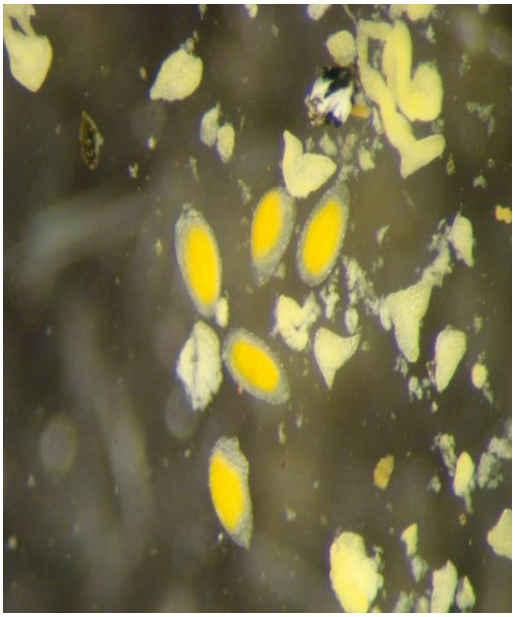

Plate 3.1b. Eggs of *Tetrastichus julis* inside *Oulema melanopus* larva

Plate 3.1c. Larvae of *Tetrastichus julis*

Plate 3.1d. Pupae of *Tetrastichus julis*

Plate 3.1. Life stages of *Tetrastichus julis* observed in parasitized larvae of *Oulema melanopus* in Lethbridge, Alberta, Canada (2010-2012).

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Chapter 4: Spatio-temporal distribution dynamics of the cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), and its larval parasitoid, *Tetrastichus julis* Walker (Hymenoptera: Eulophidae) with reference to host-plant nutrition and plant vigour metrics

4.1. Introduction

The cereal leaf beetle, *Oulema melanopus* L. (Coleoptera: Chrysomelidae), is a relatively recent alien invasive insect pest in western Canada of Eurasian origin that infests agriculturally important cereal crops including wheat, oat and barley (Leibee and Horn 1979; USDA Fact sheet 1995; Kher et al. 2011). The beetle was first discovered in North America in 1962 in Michigan, U.S.A. (Dysart et al. 1973; Evans et al. 2006; LeSage et al. 2007), and has since expanded its range to encompass most regions of cereal production in the U.S.A. (Ihrig et al. 2001; Buntin et al. 2004), eastern Canada (Harcourt et al. 1984), and western Canada (CFIA 2008; Dosdall et al. 2011) including portions of British Columbia, Alberta, southwestern Saskatchewan, and northwestern Manitoba (Dosdall et al. 2011). New disjunct populations were reported in various sites of the three Prairie Provinces in 2013: east of Red Deer in central Alberta, Moosomin in southeastern Saskatchewan and Treherne in southwestern Manitoba (H. Carcamo, personal communication). Adult and larval damage can cause yield losses of 55% in spring wheat, 23% in winter wheat, and 38 to 75% in oat and barley (Webster and Smith 1979; Royce 2000). In Canada, yield losses have not been quantified but the pest is predicted to spread across all cereal-growing

regions (Olfert et al. 2004). Establishment of this pest, therefore, has economic implications for grain production, trade and export.

Biological control of *O. melanopus* using *Tetrastichus julis* Walker (Hymenoptera: Eulophidae), an introduced host-specific larval endoparasitoid from Europe, has been a key strategy in North America (Wellso 1982), and can form a major component of integrated management tactics in western Canada (Kher et al. 2011). *Tetrastichus julis* has dispersed naturally in western Canada and is currently in its early stage of establishment (Kher et al. 2011). Successful implementation of biological control using *T. julis* requires greater understanding of factors underlying field distribution dynamics of *O. melanopus* and the degree of host-parasitoid associations on a spatio-temporal scale (Sarfraz et al. 2010).

Host nutrient availability can be a key determinant of insect pest abundance (Sarfraz et al. 2010). Strong spatio-temporal associations between host plant nutrient availability, insect pest abundance and parasitoid activity have been documented in the region for invasive insect pests such as the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Blake et al. 2010), and diamondback moth, *Plutella xylostella* (L.) (Sarfraz et al. 2010). Host plant nutrients can influence site of oviposition and are indicators of host suitability for optimal population growth of insect herbivores (Thompson 1988). However, spatial associations and the nature of the relationship between host nutrient availability in the field and activity densities of *O. melanopus* and *T. julis* have not been investigated. Positive effects of host plant nitrogen availability on *O. melanopus* colonization are known (McPherson 1983), while there is a negative

influence of a combination of nitrogen and phosphorous on *O. melanopus* infestation due to induced early maturity in host plants by phosphorous (Dimitrijević et al. 1999). Bottom-up effects of host plant nutrients on the distribution of *T. julis* or the role of other host plant nutrients such as potassium and sulphur on *O. melanopus* and *T. julis* associations have not been investigated.

In western Canada, soils are often deficient in nitrogen, sulphur and phosphorous (CCC 2003), and it is important to determine whether such deficiencies may influence *O. melanopus* and *T. julis* distributions. In this context, the present investigation attempts to develop an understanding of the within-field distribution dynamics of *O. melanopus* and *T. julis* with respect to host plant nutrient availability and plant vigour expressed in terms of plant morphological characters such as plant height, basal stem diameter and number of leaves per plant. I hypothesize that *O. melanopus* would colonize areas with high host plant nutrient availability and host plants with high vigour within fields, thus forming population hot spots; the population structure of *T. julis* will therefore be driven by the beetle's larval density (density dependence).

4.2. Materials and Methods

4.2.1. Study area

The study was conducted in commercial fields of winter wheat (*Triticum aestivum* L.) located about 54 km east of Lethbridge, Alberta in the Moist Mixed Grassland Ecoregion (AAFC 2003; Block et al. 2006). The region has a semi-arid climate and warm summers with a mean summer temperature of about 15.5^{o} C

while average annual precipitation ranges from 350 to 400 mm. The soils are mainly sandy loam (CanSIS 1996; AAFC 1999), and the major crops in the region include canola, wheat (winter and spring), barley, sugar beet, and potato.

4.2.2. Study design

A grid design was used to understand spatial associations between *O. melanopus* and *T. julis*. We used three grids, each with 100, 10 m x 10 m cells. Grid 1 (49^o 48N; 111^o 56'W) was established in 2010 and Grids 2 and 3 were in the same commercial field in 2011 and 2012 (49^o 51'N, 111^o 58' W). In all three grids, count data on *O. melanopus* larval populations were collected in each grid cell using sweep net sampling. Sampling was done in mid-July to coincide with the peak activity of life stages of *O. melanopus* (mainly larvae) and *T. julis* (adult, gravid females). Twenty-five 180^o sweep samples were taken in each grid cell by walking in a W-path. The larvae were counted and preserved in 70% alcohol and dissected to estimate the frequency of parasitization by *T. julis*. Data on *T. julis* parasitization comprised counts of numbers of larvae parasitized out of the total number collected in each cell.

Leaf samples were collected from winter wheat plants in each grid cell in 2010, 2011 and 2012 to estimate host plant nutrient availability across grids. The leaf samples were air dried and subjected to chemical analysis (Exova Laboratories, Surrey, BC) to determine the percent composition of nutrients including nitrogen, potassium, phosphorous and sulphur. The combustion method (AOAC-990.03) was followed for determinations of total nitrogen and sulphur

(AOAC International 2007a) while phosphorous and potassium contents were assessed by using the inductively coupled plasma spectroscopic method (AOAC-985.01) (AOAC International 2007b).

In Grids 2 and 3, I collected data on plant vigour metrics in addition to host plant nutrients. Plant vigour was determined in each grid cell by measuring height of plants, basal stem diameter of plants, and number of leaves per plant. For measurements of plant morphological characters, five plants were selected randomly, uprooted from each grid cell and brought back to the laboratory. For each plant, observations were recorded on its height, stem diameter and number of leaves. Height was measured using a measuring tape and averaged for each cell; leaf numbers per plant were counted. Plant diameters were measured using a Marathon^{TM} electronic digital caliper (range 0-150 mm) at the basal portion of the main stem above the root-shoot junction for each plant and average diameters per cell were obtained.

In 2011, I also measured soil-available nutrients. In each alternate grid cell, PRS^{TM} (Plant Root Simulator) probes (Western Ag Innovations, Saskatoon, Canada) were inserted at a soil depth of about 5 cm to determine the soil nutrient availability within the selected grid cells. A PRS probe employs an ion exchange membrane and resembles a plant root surface (Western Ag Innovations 2011). Plant nutrients in ionic form in the soil solution are attracted and adsorbed electrostatically on the probe membrane. Two pairs of probes (two for cations and two for anions) were inserted in each alternate grid cell in a checkerboard fashion and retained for a burial period of four weeks. Upon completion of the exposure

period, which spanned the period of greatest *O. melanopus* larval activity, the probes were removed, washed with distilled water and subjected to soil nutrient analysis. The concentration of nitrogen ions adsorbed in NO_3-N and NH_4-N form was determined using colorimetry through an automated flow injection system, while the concentration of remaining nutrients was measured using inductively-coupled plasma spectrometry (Western Ag Innovations 2011). The concentration of nutrients adsorbed over four weeks was expressed in micrograms (μg) of the nutrient/10 cm^2 of ion-exchange membrane.

4.2.3. Data analysis

4.2.3.1. Spatial analysis

Grid count data of *O. melanopus* and *T. julis* on given sampling date were used along with data on host plant nutrients, plant vigour metrics and soil nutrient availabilities to understand spatial associations among these variables. The degree of clustering of counts of *O. melanopus*, *T. julis*, host plant-available nutrients, and plant morphological characters (vigour metrics) was analyzed using Spatial Analysis by Distance Indices (SADIE) according to the method described by Perry et al. (1999). SADIE uses tests of randomizations to assess the observed arrangements in count data. This helps in identifying areas of a patch (relatively large insect counts close to each other) or gap (relatively few insect counts close to each other) (Perry 1998). We used SADIE to quantify patches and gaps using the distance to regularity (D), which is the minimum distance that individuals must be moved within sampling units to produce a uniform distribution across

each sampling unit (Sarfraz et al. 2010). The flows needed to achieve such distributions are large within patches and gaps based on surrounding values. The sample values at each point are randomized to calculate expected values using a statistical test of significance. The patch index, V_i, indicates a patch (aggregated counts) when $V_i > 1$ while the gap index, V_j, indicates a gap and has the value < 1. Mean values of V_i and V_j indicate the overall spatial pattern in terms of patches and gaps. SADIE provides a value of δ which is the distance between the center of the sampling points and the centroid of sampling values. It is a measure of the degree to which count data are distributed towards the edges of sampling units (Perry and Klukowski 1997).

The spatial association between two sets of counts at a time were then measured to understand associations between *O. melanopus* larvae and its parasitoid, and between the insect species, host plant and soil-available nutrients, and plant vigour metrics (Perry and Dixon 2002). The spatial association between two sets of data is measured by the term X which is equivalent to the simple correlation coefficient between the clustering indices (V_i or V_j) of the two data sets. The degrees of freedom for the correlation are adjusted using the Dutilleul adjustment by removing overall trends in the data to assure stationarity. This method also adjusts for any autocorrelation between two sets of data by using effective sample size. The measure of association, X_k, for each sample location is calculated and used to generate a significance test and associated probability value between X and X_k. For a two-tailed test with the alpha of 0.05, probability values < 0.025 indicate significant association whereas values > 0.975 indicate

significant disassociation. The index of association examines the co-occurrence of patches and gaps in one data set with the patches and gaps in the other data set. The associations between *O. melanopus* larval counts and *T. julis* parasitization frequency, and their associations with host plant nutrients and vigour metrics were assessed to understand spatial relations. Values of the association index (X_k) were interpolated using the spatial analyst extension of ArcGIS (ESRI 2002) to create contour maps of associations.

4.2.3.2. Statistical model to predict host-nutrition and vigour effects

The effects of host plant and soil-available nutrients, and plant vigour on the population dynamics of *O. melanopus* and *T. julis* were estimated by fitting Generalized Estimating Equations (GEEs) to the count data using PROC GENMOD (SAS Institute 2006). I tested several models to explore the effects of the above parameters on the abundance of *T. julis* and *O. melanopus* using different combinations of predictor variables. In general, larval counts for a given grid cell were considered a function of host plant and soil-available nutrients (N, P, K, S) and plant vigour. Similarly, the effects of nutrients and plant vigour on *T. julis* counts were estimated using similar parameters as above. Models with lowest Bayesian Information Criterion (BIC) values were selected for each grid study to estimate the effects of predictors on larval and parasitoid populations (Schwarz 1978).

The deviance parameter estimates for each model selected indicated the presence of over-dispersion in the data, which was corrected by defining the

negative binomial distribution for the data with log as a link function in PROC GENMOD to perform the analysis (SAS Institute 2006).

4.3. Results

4.3.1. Distributions of O. melanopus larvae

Of the three fields examined, Grid 3 had the highest mean larval count per cell (Table 4.1). Significant spatial structures in larval population distributions were observed in all three grids and the patterns of spatial distribution did not vary significantly in terms of their strength among grids. Highly significant patch (V_i) and gap (V_j) index values (Table 4.1) indicated that the observed larval counts among the grid cells were not randomly distributed, and indicated the presence of significant gaps and patches in larval distributions across the grid area (Figs. 4.1-4.3). Higher values of δ (Table 4.1) were observed in Grid 1 compared to Grids 2 and 3; however, the observed beetle and parasitoid distributions were not in the direction of field edges.

4.3.2. Distributions of T. julis

The parasitism level in Grid 1 was 28.7 percent, 31.3 percent in Grid 2, and 37.9 percent in Grid 3. The parasitoid distributions indicated highly significant spatial structures in all three grids. Highly significant patch (V_i) and gap (V_j) index values (Table 4.1) indicated the presence of significant gaps and patches in parasitoid distributions across the grid areas (Figs. 4.1-4.3). The patterns of spatial distribution did not vary significantly in terms of their strength

among grids. Higher values of δ (Table 4.1) were observed in Grid 1 compared to Grids 2 and 3 and this pattern was similar to the one observed for *O. melanopus*. However, specific displacement patterns toward field edges were not observed.

4.3.3. Host plant nutrient availability

Substantial differences were observed among the three grids in the levels of plant nutrients examined. Leaf nutrient compositions indicated high availability of nitrogen in Grid 2 compared to Grids 1 and 3 (Table 4.1). Availability of phosphorous was high in Grid 3 compared to the other two grids. While potassium levels were relatively high in Grid 2, they were low in Grid 3. Sulphur availability was nearly three times greater in Grid 3 compared to Grids 1 and 2 (Table 4.1). Variations in plant-available nutrients resulted in distinct nutrient profiles within and among grids. Spatial patterns in nutrient availability were observed in all grids (Table 4.1). In general, the spatial patterns of variability in distribution of host plant nutrition across grids were similar in all three grids.

4.3.4. Spatial associations among larvae, parasitoid and host plant nutrients

Significant spatial associations were observed between *O. melanopus* and *T. julis* populations with a high index of association (X) in all three grids (Fig. 4.1-4.3). Among the three grids, the level of association between *O. melanopus* and *T. julis* was the highest in Grid 2 ($X = 0.94$, $P < 0.0001$) compared to Grids 1 ($X = 0.84$, $P < 0.0001$), and 3 ($X = 0.88$, $P < 0.0001$). In Grid 1, despite low larval

counts and lower rates of parasitism, the magnitude of association was high (Fig. 4.1).

Significant spatial associations were observed among plant-available nutrients with relationships ranging from strong association to strong disassociation. The association between nutrients in all grids showed complex trends and differences in strengths of relationships. Among the three grids, a greater degree of spatial association between plant-available nutrients was observed in Grid 3 followed by Grid 2. In comparison, the extent of their association was lowest in Grid 1 (Table 4.2). The association among some plant-available nutrients was similar across the three grids. For example, strong positive associations were observed between nitrogen and phosphorous in both Grids 2 and 3, and between sulphur and both nitrogen and phosphorous in all three grids.

In Grid 1, plant-available nitrogen was in high spatial association with sulphur while it showed strong disassociation with potassium (Table 4.2). This indicated that field patches with higher plant-available nitrogen also had higher availability of sulphur but not potassium. High phosphorous availability indicated high potassium but less sulphur availability with no apparent spatial relationship with nitrogen. In Grid 2, however, higher plant nitrogen availability was spatially associated with higher phosphorous and sulphur availability. In Grid 3, all nutrients shared positive spatial associations with each other. Field patches with high nitrogen availability in plants also had high availability of phosphorous, potassium and sulphur. Some contrasting spatial associations across the grid were also observed. While nitrogen and potassium shared a negative association in Grid

1, the association was positive in Grid 3 (Table 4.2). Similarly, sulphur availability in plant tissue had negative association (X = -0.24, P = 0.97) with phosphorous in Grid 1 while it was positive in Grid 3 (X = 0.62, $P < 0.0001$).

The association between *O. melanopus* larval distribution and the distribution of plant-available nutrients in the field indicated variable patterns in all grids (Table 4.3). In Grid 1, no significant spatial associations were found between *O. melanopus* larval counts and plant-available nitrogen (X = 0.11, P = 0.12), phosphorous (X = -0.13, P = 0.12), potassium (X = -0.007, P = 0.52) and sulphur (X = -0.15, P = 0.91). In Grid 2, *O. melanopus* larval counts indicated significant spatial association with plant-available phosphorous (X = 0.24, P = 0.01) and sulphur (X = 0.25, P = 0.01). The probability value for association between larval counts and nitrogen in Grid 2 was slightly greater than the critical significance value of 0.025, and hence no spatial relationship existed between these two (X = 0.17; $P > 0.025$). In Grid 3, a strong spatial disassociation between *O. melanopus* larvae and plant-available potassium was observed (X = -0.23, P = 0.98); however, any such relationship was absent in Grids 1 and 2 (Table 4.3).

No significant spatial associations and disassociations were observed between *T. julis* parasitization levels and host plant nutrient availability of nitrogen and potassium in Grids 1, 2 and 3 (Table 4.3). We observed a significant spatial association between plant-available phosphorous and *T. julis* distribution only in Grid 2 (X = 0.25, P = 0.01). A strong spatial disassociation between *T. julis* and plant sulphur availability in Grid 1 (X = -0.22, P = 0.977) was observed. However, a pattern of spatial association between plant-available sulphur and *T.*

julis counts was observed in Grid 2 with lack of any association or disassociation observed between these two nutrients in Grid 3 (Table 4.3).

4.3.5. Soil-available nutrients and their spatial associations with other parameters

Soil-available nutrients were measured in Grid 2 (2011) only, and the availability of major nutrients including nitrogen, phosphorous, potassium and sulphur in the soil profile varied substantially. Significant spatial structure in the distributions of nutrients in the soil profile was observed. Highly significant patch (V_i) and gap (V_j) index values (Table 4.4) indicated hot-spots of nutrient availability across the grids. Potassium was the most available nutrient followed by nitrogen, sulphur and phosphorous (Table 4.4). Soil-available nitrogen exhibited a significant association with *O. melanopus* distribution (X = 0.20, P = 0.008), but no such association was evident with *T. julis* (X = -0.013, P = 0.47) (Table 4.5). No significant spatial association or disassociation was observed with *O. melanopus* and *T. julis* distributions among soil-available phosphorous, potassium or sulphur (Table 4.5).

4.3.6. Plant vigour metrics and their spatial associations with other parameters

Plant height, basal stem diameter and number of leaves per plant showed significant spatial structures in their distributions across grids in both 2011 and 2012 (Table 4.4). There were significant patches and gaps in the distributions of host plants in terms of height, stem diameter and mean leaves per plant as

indicated by patch and gap indices. Significant spatial associations were found between plant vigour metrics and the distributions of *O. melanopus, T. julis* and soil-available nutrients. Among all spatial relationships, the degree of association between plant vigour metrics and larval and parasitoid distributions was strong (Table 4.6). A significant spatial association was observed between mean numbers of leaves per plant and *O. melanopus* larval counts per grid cell in both 2011 (X = 0.72, P = 0.0001) and 2012 (X = 0.66, P = 0.0001) (Table 4.6, Fig. 4.4), indicating that field patches with plants having greater number of leaves harboured higher number of *O. melanopus* larvae. A similar association was observed between *T. julis* abundance and plant leaves. However, the spatial association between plant leaves and *T. julis* distribution was higher in Grid 3 (X = 0.79; P = 0.001) than in Grid 2 (X = 0.25, P = 0.009). Similarly, plant height was spatially associated significantly with *O. melanopus* larvae in Grids 2 (X = 0.48, P = 0.0001) and 3 (X = 0.66, P = 0.001) (Table 4.6, Fig. 4.4). Field areas indicating higher *O. melanopus* larval activity thus coincided with areas with taller plants and more leaves.

Plant height and *T. julis* abundance did not share any spatial association or disassociation (Table 4.6). Mean basal stem diameters of plants did not associate spatially with larval or parasitoid populations in Grid 2, but significant spatial associations were observed with the abundance of both insect species in Grid 3 (Table 4.6, Fig. 4.4).

Significant spatial associations existed between plant vigour matrices and soil-available nutrients (Fig. 4.5). Soil-available nitrogen showed spatial

associations with plant height ($X = 0.33$, $P = 0.006$) and mean number of leaves per plant ($X = 0.29$, $P = 0.025$). Field patches across the grid with high soil nitrogen availability harbored plants with greater average heights and greater numbers of leaves. Soil phosphorous was spatially associated with basal stem diameter of plants ($X = 0.38$, $P = 0.008$).

Similarly, higher soil potassium availability was spatially associated with all three vigour traits namely, plant height ($X = 0.43$, $P = 0.001$), stem diameter ($X = 0.27$, $P = 0.02$) and number of leaves ($X = 0.37$, $P = 0.006$). Positive associations between soil potassium and the three vigour traits indicated that the patches with high potassium availability harboured plants of greater heights and stem diameters possessing more leaves. Higher availability of soil-available sulphur coincided with plant stands with greater plant heights and greater numbers of leaves (Fig. 4.5).

4.3.7. Estimation of factors influencing O. melanopus and T. julis counts

The statistical model used to estimate the effects of host plant and soil-available nutrients (nitrogen, phosphorous, potassium, sulphur), and plant vigour metrics (plant height, average leaves and stem diameter) on the densities of the beetle and its parasitoid in a given grid cell indicated variable effects of different factors in different grids. For Grid 1, I focused on the effects of host plant nutrition on larval and parasitoid distributions, and hence the model included host plant nutrients as predictors (Table 4.7). For Grids 2 and 3, a variety of factors

were analyzed and hence several regression models were tested with different predictors and compared using BIC values (Table 4.8).

In Grid 1, none of the host plant nutrients except sulphur had a significant impact on the distributions of larvae and parasitoids (Table 4.7). Available sulphur in plant tissue negatively influenced the distributions of larvae and their parasitoids.

In Grid 2, plant-available phosphorous had a negative impact on the abundance of larvae while higher plant potassium availability positively influenced larval colonization of plants (Table 4.7). The model selection based on BIC values deemed all other predictors weak for inclusion in the model (Table 4.8). The results indicated that only larval availability was strong predictor of *T. julis* abundance in Grid 2 (Tables 4.7 and 4.8).

In Grid 3, we obtained data on plant-available nutrients and plant vigour metrics but not on soil nutrient availability. Hence, the models tested to analyze factors affecting larval distribution of *O. melanopus* included different combinations of plant metrics and plant-available nutrients as predictors (Table 4.7). However, the models containing plant-available nutrients and plant vigour metrics together, and the one containing plant-available nutrients only yielded higher BIC values compared to the model containing plant vigour metrics only as predictor variables (Table 4.8). I therefore selected the model containing plant vigour metrics only to estimate *O. melanopus* larval abundance.

The results indicated that plant height and leaf number significantly affected the distribution of *O. melanopus* larvae. Plants with greater average

height and leaf number harboured higher numbers of *O. melanopus* larvae. Along similar lines, the model selected for *T. julis* distribution included the same predictors as for *O. melanopus*. According to this model, the distribution of *T. julis* was positively influenced by the availability of *O. melanopus* larvae, and plant vigour characters such as plant leaves and plant height; the same factors that influenced *O. melanopus* distribution affected the distribution of *T. julis*.

4.4. Discussion

Extensive sampling of *O. melanopus* and *T. julis* from points arranged in a grid pattern in commercial winter wheat fields, and analyses of the spatial associations among the herbivore, its parasitoid and host plant have enhanced understanding of the interactions that exist in this tritrophic system. The current study revealed significant variations in field distribution patterns of all parameters studied. Of these patterns, the patchy distributions of *O. melanopus* and *T. julis* populations were prominent. In all three fields studied, the population structures of the herbivore and its parasitoid indicated significant patches and gaps. This is indicative of population hot-spots for the beetle and its parasitoid in the field as hypothesized. In host-parasitoid dynamics, heterogeneous population patterns continue to arise over time and fixed spatial patterns are not commonly observed (Pearce et al. 2006). Spatial structures of herbivore-host plant populations influence dispersal and assembly dynamics of the natural enemy (French and Travis 2001).

Spatial associations between various life stages of *O. melanopus* populations and patchy distributions of its eggs and larvae based on plant growth stages were reported previously in winter wheat (Reay-Jones 2012). However, how such distributions affect *T. julis* populations has not been investigated, and this is the first report of factors influencing the dynamics of interactions between the beetle and its parasitoid with reference to host plant nutrition and vigour. Such patterns of patchy distribution, however, were identified in field populations of other insects such as diamondback moth, *Plutella xylostella* (L.) (Sarfraz et al. 2010), and the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Dosdall et al. 2006; Blake et al. 2010), in canola in western Canada.

Levels of parasitization showed a gradual increase over years in my study. This increase was especially evident in Grids 2 and 3 which shared same geographic location in consecutive years. The increase in activity of *T. julis* is an indication that the parasitoid is establishing naturally along with its host, and this is encouraging for management of the beetle with a biological control approach. Further, there was a strong spatial association between the activity density of beetle larvae and *T. julis* in all three grids. This indicates high synchronization of *T. julis* with its host both spatially and temporally, and further underlines the density-dependence of the parasitoid. My study shows that *O. melanopus* and *T. julis* represent a tightly coupled host-parasitoid system, and *T. julis* populations are host density-dependent. Density dependence is a key characteristic of host-specific parasitoids (Huu et al. 2008). The success of parasitization depends on the degree to which host populations are supressed by the parasitoid activity and

the extent to which host-parasitoid populations attain stability over time (Hassel and Waage 1984). In this regard, density dependence is a very powerful mechanism that brings about stability in host-parasitoid populations (Hassel and Waage 1984).

Dispersive interactions in host-parasitoid systems give rise to patterns of spatio-temporal heterogeneity and spatial variation in population dynamics (Pearce et al. 2006), as observed in *O. melanopus*-*T. julis* across all three grids. Heterogeneity in insect host populations can affect parasitoid populations and parasitization in several ways. This may alter parasitoid foraging behaviour, selection of patches, encounter rates, and realized fecundity over space and time (Teder and Tammaru 2002; Hastings et al. 2005). With host populations dispersed over a large area, the optimal dispersal strategy for a parasitoid is to colonize patches with high host density (Huu et al. 2008). Parasitoid populations developing in synchrony with its hosts can cause local extinctions in patches (Cronin and Reeve 2005), and maximize reproductive success and progeny sustenance (Matsumoto et al. 2004). One of the major reasons for the success of *T. julis* as a biological control agent is its high degree of synchronization with the life cycle of its host (Haynes and Gage 1981; Evans et al. 2006; Evans et al. 2010), and the current results indicate a similar pattern of synchronization with the host in the newly invaded region. This result was in agreement with my hypothesis that *T. julis* will colonize areas with high *O. melanopus* abundance, and the hot-spots of *T. julis* activity will be in synchrony with beetle activity.

Host plant nutrient availability is a major determinant of host selection and significantly influences fitness and performance of phytophagous insects (Ishihara and Suzue 2011; Blake et al. 2010; Awmack and Leather 2002). Selection of a suitable host for feeding and oviposition can influence the population dynamics of an alien invasive species and its natural enemy (Kim and Lee 2002). Parasitoids that are tightly coupled with their hosts are sensitive to variation in insect host quality (Teder and Tammaru 2002), and understanding bottom-up effects of host plant vigour and nutrition on the pest-parasitoid system is a key to understanding how parasitization success will be affected in a given system. The present investigation revealed a complex pattern of nutrient distribution across grids. Patchy distribution patterns of plant-available nutrients are known to occur in the field (Beckett and Webster 1971; Blake et al. 2010), and could have direct effects on plant morphological characters and leaf tissue quality that are reflected indirectly in insect distribution patterns.

In terms of plant nutrient availability and the distribution of *O. melanopus*, we found substantial variation in spatial associations of host nutrition with beetle abundance. This ranged from no spatial associations in Grid 1 to either variable associations with nitrogen, potassium, phosphorous, or sulphur in other grids. Association of larval activity with greater plant phosphorous and sulphur availability was evident in Grid 2. However, in Grid 3 the larvae correlated spatially with plant potassium availability. These agroecosystems evidently have highly variable nutrient levels, and several more sites and years would be needed

to more accurately determine the roles of the nutrients in *O. melanopus* distributions.

High nitrogen availability increases host colonization by *O. melanopus* adults and larvae (McPherson 1983). However, we did not observe any such association with plant-available nitrogen either spatially or temporally. Despite this, beetle activity indicated spatial association with soil-available nitrogen (Table 4.5). A combination of nitrogen and phosphorous can negatively affect *O. melanopus* infestation and colonization (Dimitrijević et al. 1999), due to induced early maturity of host plants by phosphorous. Regression analysis to predict the effects of plant and soil nutrients and vigour metrics suggested that plant-available phosphorous had a negative impact on larval colonization while potassium had a positive influence on larval colonization. The estimates provided by regression analysis, particularly for Grid 2, corroborate the previous report that phosphorous has a negative influence on larval colonization. However, the results were not consistent in Grid 3. Similar results were obtained for spatial associations of *T. julis* with plant-available and soil-available nutrients. The extent of bottom-up effects of host plant nutrition on the herbivore and its parasitoid varies spatially as a result of site-specific variations in local conditions, regional processes influencing cascade effects and multi-species interactions within local patches (Gripenberg and Roslin 2007). This may have resulted in high spatio-temporal variability in interrelationships between host-available nutrients and dynamics of *O. melanopus* and *T. julis* populations across grids. Despite this, significant positive associations between *O. melanopus* and phosphorous and sulphur, and a

disassociation between *O. melanopus* and potassium were observed in some grid fields. This suggests that such bottom-up effects may play a role in *O. melanopus* field distribution patterns. Potential bottom-up effects of host plant nutrients on distribution dynamics of the *O. melanopus* and *T. julis* require further research.

Plant morphology and vigour traits can significantly influence host-parasitoid interactions (Tscharntke 2000; Hunter 2003; Gingras et al. 2003). Host plant structure has effects on parasitism rates of parasitoids of various lepiodopteran pests (Lill and Marquis 2001). Our results indicate that plant morphological characters expressing host vigour had a strong influence on spatio-temporal distributions of *O. melanopus* and *T. julis*. The mean number of leaves per plant showed strong spatial association with both *O. melanopus* and its parasitoid. Host plants with large numbers of leaves harbored greater larval populations and such densely populated larval patches exhibited more parasitization. Plant height also exhibited high correlation with larval and parasitoid populations. Plant diameter indicated spatial associations with both the beetle and parasitoid in Grid 3 only. However, this highlights the influence of plant architecture and morphological traits on distribution of the beetle and its parasitoid.

Female fecundity and oviposition behaviour of *O. melanopus* are influenced by a variety of factors including plant nutrition, host morphology, and other micro-climatic factors (Wellso 1973). Further, *O. melanopus* life stages were highly spatially correlated with wheat spike counts, and recent research has suggested that wheat stand in the field is a major determinant of the distribution

dynamics of *O. melanopus* (Reay-Jones 2012). This investigation thus corroborates the previous report that plant stand characters play a significant role in field dynamics of the beetle. It is also evident from this study that plant morphology and vigour have significant influences on the population dynamics of *T. julis*. The regression analysis confirmed the influence of plant metrics on the distributions of beetle larvae and the parasitoid. Such bottom-up effects of plant vigour are known to influence not only dispersal but also fitness of parasitoids. For example, taller plants with higher numbers of regenerative shoots of *Typha latifolia* L. have a positive influence on the weight gains of its herbivore, *Nonagria typhae* Thunberg and its ichnumonid parasitoid *Exephanes occupator* Grav. (Teder and Tamaru 2002). On the contrary, negative effects of plant structure and morphological complexity on parasitoid behaviour are also recorded. For example, parasitism of eggs of *Ephestia kuehniella* Zeller by *Trichogramma evanescens* Westwood decreased with increased plant structure complexity as a result of decreased encounter success (Gingras and Boivin 2002). In my study, plant vigour expressed by taller plants, larger basal stem diameters and greater numbers of leaves, and patchy distributions of vigour traits indicated significant variation in canopy characters across the study fields. However, I did not observe any negative influence of these host traits on *T. julis* parasitization. This suggests that the population dynamics of *T. julis* is strongly host-density driven. Hence, positive spatial correlations between plant vigour traits and parasitoid abundance likely resulted from *O. melanopus* colonization of plant patches with high vigour. This, however, suggests that plant canopy characters do

not have a negative influence on host location and colonization by *T. julis*, and are less likely to interfere with *T. julis* dispersal. My results indicate that factors such as plant vigour metrics and morphology may influence beetle distribution under field conditions, with its significant influence on *T. julis* populations.

Besides plant nutrition and plant vigour metrics, several other factors can contribute to the success of a biological control agent. Landscape complexity, matrix diversity and surrounding habitats can influence rates of parasitism (Tscharntke 2000), and hence, research attention needs to be given to the effects of landscape characters on *O. melanopus*-*T. julis* interactions. To understand the extent of parasitism and parasitoid population dynamics, study of parasitoid assemblages at various spatial scales is necessary (Matsumoto et al. 2004). The current investigation underlines the fact that the patterns of beetle-parasitoid associations under field conditions are complex and not restricted by the availability of host plant nutrients only. The limitations of the current investigation include current low population densities of *O. melanopus* and *T. julis*, and limited availability of sites to conduct such studies.

Oulema melanopus is now in an early phase of its invasion in western Canada. Currently, there is no detailed information available on *O. melanopus* host preferences and population dynamics in its new eco-region. However, considering that the infestations in Alberta and Saskatchewan are recent, it provides a unique opportunity to study and understand the initial dispersal characteristics of invading alien species over space and time. This is fundamentally important for understanding community assembly dynamics, and

initial dispersal is the best stage during which to implement management efforts. However, historically this opportunity has occurred very rarely. By directing control efforts early in the invasion phase of the cereal leaf beetle, we can take advantage of the early discovery of this pest to lead to long-term sustainable control. In a management perspective, the natural occurrence of *T. julis* in areas of *O. melanopus* activity is advantageous for designing biological control-based management strategies. Early in the invasion phase of an alien herbivore, parasitoids such as *T. julis* can cease, reverse or slow down the invasion and dispersal (Hastings et al. 2005). Insights developed through my study have application for strengthening biological control efforts. For example, based on density-dependent dispersal of *T. julis* and its strong host-tracking capacity, as evidenced through colonization of high density host patches scattered across grids, targeted relocation of parasitoids in infested areas can be performed. Such releases can facilitate parasitoid establishment in newly infested areas and help keep *O. melanopus* populations well below the economic threshold level.

The role of seeding rate or plant density variations in managing *O. melanopus* populations needs to be given particular attention in future research in view of crop stand and plant vigour effects on the dynamics of *O. melanopus*-*T. julis* interactions. Prior studies indicated that egg and larval densities of *O. melanopus* were low in oat stands seeded at a low seeding rate (Webster et al. 1978). However, with an intermediate or high seeding rate the distribution of eggs and larvae was scattered over a larger area compared to that in a low-seeded crop stand where the number of eggs laid or larvae per tiller per unit area was very

high. Hence, lower than recommended seeding rates in *O. melanopus*-infested areas is not advisable (Webster et al. 1978). In this regard, the recommended seeding rates with judicial use of nutrients can assure uniform plant stands and thus avoid heterogeneous areas with variable vigour that can be severely affected by *O. melanopus* damage. However, field-scale studies are necessary to estimate the effects of seeding rates on the population dynamics of *O. melanopus*. Any cultural management practice, however, should be supplemented with targeted parasitoid releases to mitigate *O. melanopus* damage.

Tables.

Table 4.1. Mean numbers of *Oulema melanopus* larvae, *O. melanopus* larvae parasitized by *Tetrastichus julis* per grid cell, and selected host plant nutrients, and their spatial patterns of distribution as indicated by SADIE patch ($\bar{V}_I$) and gap ($\bar{V}_J$) indices in fields of winter wheat in 2010-2012 near Lethbridge, Alberta, Canada. Figures in the parentheses indicate probability values for SADIE indices at α=0.05.

Grid (Year)	Variable (Counts)	Mean ± SEM	$\bar{V}_I$ (p)	$\bar{V}_J$ (p)	δ
Grid 1 (2010)	*O. melanopus* Larvae	7.97 ± 0.50	2.14 (0.00)	-1.97 (0.00)	9.08
	T. julis abundance	2.29 ± 0.29	2.02 (0.00)	-2.10 (0.00)	14.45
	Nitrogen	3.69 ± 0.59	1.60 (0.00)	-1.67 (0.00)	1.65
	Phosphorous	0.35 ± 0.04	1.62 (0.00)	-1.51 (0.00)	1.29
	Potassium	1.56 ± 0.02	1.31 (0.00)	-1.19 (0.00)	0.30
	Sulphur	0.31 ± 0.00	2.47 (0.00)	-2.49 (0.00)	2.47
Grid 2 (2011)	*O. melanopus* Larvae	14.01 ± 0.91	2.11 (0.00)	-2.13 (0.00)	8.76
	T. julis abundance	4.72 ± 0.47	2.08 (0.00)	-2.06 (0.00)	13.73
	Nitrogen	6.11 ± 0.10	1.76 (0.00)	-1.85 (0.00)	1.97
	Phosphorous	0.28 ± 0.39	2.70 (0.00)	-2.66 (0.00)	-2.55
	Potassium	1.85 ± 0.02	1.42 (0.00)	-1.37 (0.00)	0.63
	Sulphur	0.34 ± 0.53	2.64 (0.00)	-3.39 (0.00)	3.37
Grid 3 (2012)	*O. melanopus* Larvae	17.17 ± 1.71	2.25 (0.00)	-2.17 (0.00)	7.48
	T. julis abundance	6.52 ± 0.65	1.90 (0.00)	-1.98 (0.00)	10.69
	Nitrogen	1.82 ± 0.06	1.87 (0.00)	-1.99 (0.00)	4.82
	Phosphorous	1.07 ± 0.33	1.86 (0.00)	-2.14 (0.00)	4.24
	Potassium	1.27 ± 1.53	1.69 (0.00)	-1.39 (0.00)	0.93
	Sulphur	2.64 ± 0.56	2.24 (0.00)	-2.20 (0.00)	2.87

The indexes $\bar{V}_I$ and $\bar{V}_J$ were calculated using SADIE (see text for details).

Table 4.2. The spatial relationships among the nutrients within leaf tissues indicated by the SADIE point index of association X_k in fields of winter wheat in 2010, 2011, and 2012 near Lethbridge, Alberta, Canada. Values in bold font indicate the probability of significant association or disassociation between the parameters.

Nutrients	Grid 1 (2010)			Grid 2 (2011)			Grid 3 (2012)		
Measured	%Nitrogen	%Phosphorous	%Potassium	%Nitrogen	%Phosphorous	%Potassium	%Nitrogen	%Phosphorous	%Potassium
%Nitrogen	--	0.22	**-0.25****	--	**0.79***	-0.11	--	**0.91***	**0.40***
	--	(0.48)	**(0.99)**	--	**(<0.0001)**	(0.85)	--	**(<0.0001)**	**(<0.0001)**
%Phosphorous	--	--	**0.45***	--	--	0.12	--	--	**0.51***
	--	--	**(<0.0001)**	--	--	(0.15)	--	--	**(<0.0001)**
%Sulphur	**0.50***	**-0.24****	-0.14	**0.65***	**0.77***	-0.14	**0.55***	**0.62***	**0.28***
	(<0.0001)	**(0.98)**	(0.89)	**(<0.0001)**	**(<0.0001)**	(0.87)	**(<0.0001)**	**(<0.0001)**	**(0.007)**

*indicates significant association at $P < 0.025$
**indicates significant disassociation at $P > 0.975$

Table 4.3. The spatial relationships among the percentages of composition of nutrients within leaf tissues and the numbers of *Oulema melanopus* larvae, and the abundance of the larval parasitoid *Tetrastichus julis*, indicated by the SADIE point index of association X_k in fields of winter wheat in 2010 (Grid 1), 2011 (Grid 2), and 2012 (Grid 3) near Lethbridge, Alberta, Canada. Values in bold font indicate the probability of significant association or disassociation between the parameters.

Parameter	**% Nitrogen**			**% Phosphorous**			**% Potassium**			**% Sulphur**		
	Grid 1	**Grid 2**	**Grid 3**	**Grid 1**	**Grid 2**	**Grid 3**	**Grid 1**	**Grid 2**	**Grid 3**	**Grid 1**	**Grid 2**	**Grid 3**
O. melanopus	0.11	0.17	0.24	-0.13	**0.24**	0.01	-0.007	-0.019	**-0.23****	-0.15	**0.25***	0.14
	(0.12)	(0.04)	(0.40)	(0.12)	**(0.01)***	(0.44)	(0.52)	(0.55)	**(0.98)**	(0.91)	**(0.01)**	(0.13)
T. julis	0.16	0.16	0.10	-0.02	**0.25**	0.11	-0.26	-0.047	-0.16	**-0.22****	**0.27***	0.18
	(0.07)	(0.05)	(0.17)	(0.59)	**(0.01)***	(0.15)	(0.59)	(0.65)	(0.93)	**(0.97)**	**(0.007)**	(0.05)

*indicates significant association at $P < 0.025$
**indicates significant disassociation at $P > 0.975$

Table 4.4. The mean compositions of soil-available nutrients, and mean values of plant vigour parameters and their spatial patterns of distribution as indicated by SADIE patch ($\overline{V}_I$) and gap ($\overline{V}_J$) indices for fields of winter wheat in 2011 (Grid 2) and 2012 (Grid 3) near Lethbridge, Alberta, Canada. Nutrient availability was measured using PRS^{TM} probes and is expressed in µg/10 cm^2 over a burial period of four weeks in Grid 2 (2011).

Grid	**Parameters**	**Mean ± SEM**	$\overline{V_I}$	$\overline{V_J}$	**δ**
2(2011)	Soil Nitrogen	112.48 ± 19.87	1.99*	-1.81*	20.48
	Soil Phosphorous	14.98 ± 1.69	2.15*	-2.03*	17.23
	Soil Potassium	192.84 ± 14.01	2.26*	-1.92*	9.38
	Soil Sulphur	120.5 ± 25.0	2.18*	-2.01*	25.97
	Plant Height (cm)	84.48 ± 8.46	1.85*	-1.65*	3.0
	Number of Leaves	23.108 ± 2.30	1.29*	-1.34*	0.98
	Plant Diameter (mm)	2.71 ± 0.27	2.07*	-2.25*	1.87
3 (2012)	Plant Height (cm)	102.66 ± 10.26	1.56*	-1.58*	5.42
	Number of Leaves	24.18 ± 2.41	2.34*	-2.54*	0.49
	Plant Diameter (mm)	2.92 ± 0.29	1.11*	-1.28*	1.51

*the patch ($\overline{V_I}$) and gap ($\overline{V_J}$) indices calculated by SADIE are significant at $P < 0.0001$ indicating presence of hot-spots in the distribution of parameters listed in the table above

Table 4.5. The spatial relationships indicated by the SADIE index of association X_k among soil-available nutrients, the number of *Oulema melanopus* larvae, and the abundance of the larval parasitoid, *Tetrastichus julis* in a field of winter wheat in 2011 near Lethbridge, Alberta, Canada. The values in bold font indicate the probability of significant association or disassociation between the parameters.

Parameter	**Soil Nitrogen (%)**	**Soil Phosphorous (%)**	**Soil Potassium (%)**	**Soil Sulphur (%)**
O. melanopus	**0.20 (0.008)***	0.24 (0.50)	0.26 (0.03)	0.17 (0.11)
T. julis	-0.01 (0.47)	0.14 (0.15)	0.03 (0.40)	0.03 (0.36)

*indicates significant association at $P < 0.025$
**indicates significant disassociation at $P > 0.975$

Table 4.6. The spatial relationships indicated by the SADIE point index of association X_K among selected plant vigour metrics and the number of *Oulema melanopus* larvae, and the abundance of larval parasitoid, *Tetrastichus julis*, in fields of winter wheat in 2011 (Grid 2), and 2012 (Grid 3) near Lethbridge, Alberta, Canada. Values in bold font indicate the probability of significant association or disassociation between the parameters.

Parameter	**Mean plant height**		**Mean leaves per plant**		**Mean plant diameter**	
	Grid 2 (2011)	**Grid 3 (2012)**	**Grid 2 (2011)**	**Grid 3 (2012)**	**Grid 2 (2011)**	**Grid 3 (2012)**
O. melanopus	**0.48***	**0.66***	**0.72***	**0.66***	0.04	**0.32***
	(<0.0001)	**(<0.0001)**	**(<0.0001)**	**(<0.0001)**	(0.32)	**(0.0006)**
T. julis	0.07	0.03	**0.25***	**0.79***	-0.02	**0.30***
	(0.24)	(0.37)	**(0.009)**	**(<0.0001)**	(0.58)	**(<0.001)**

*indicates significant association at $P < 0.025$
**indicates significant disassociation at $P > 0.975$

Table 4.7. Negative binomial regression analysis using generalized estimating equations to analyze factors influencing abundance levels of *Oulema melanopus* and *Tetrastichus julis* in fields of winter wheat in 2010 (Grid 1), 2011 (Grid 2), and 2012 (Grid 3) near Lethbridge, Alberta, Canada. The parameter estimates for each predictor and the associated Wald Chi-square values and probabilities are presented below.

Grid	Model Predictors	Larval Abundance of *O. melanopus* Parameter Estimate (χ2; p)	Parasitoid Abundance Parameter Estimate (χ2; p)
Grid 1	Intercept	**2.14 (12.11; p < 0.001)**	**1.32 (0.00; p = 0.98)**
	Nitrogen	**0.15 (33.90; p > 0.05)**	**0.33 (23.68; p = 0.58)**
	Phosphorous	**0.005 (0.30; p = 0.58)**	**0.01 (2.93; p = 0.08)**
	Potassium	**-0.07 (0.49; p = 0.48)**	**-0.29 (0.02; p = 0.89)**
	Sulphur	**-0.03 (7.24; p = 0.007)**	**-0.07 (6.16; p = 0.01)**
Grid 2	Intercept	**-1.24 (1.12; p = 0.2905)**	**0.66 (12.03; p = 0.0005)**
	Nitrogen	**0.075 (0.24; p = 0.6271)**	***Not included***
	Phosphorous	**-6.30 (1.63; p = 0.02)**	***Not included***
	Potassium	**1.14 (9.61; p = 0.0019)**	***Not included***
	Sulphur	**3.93 (2.74; p = 0.0979)**	***Not included***
	Plant Height	**0.0091 (0.86;p = 0.3540)**	***Not included***
	Plant Leaves	**0.005 (0.00;p = 0.9617)**	***Not included***
	Plant diameter	**0.17 (1.13; p = 0.2876)**	***Not included***
	Larval abundance	***Not included***	**0.05 (52.89; p <.0001)**
	Soil Nitrogen	**-0.008 (0.98; p = 0.3217)**	**-0.001 (1.13; p= 0.2880)**
	Soil Phosphorous	**-0.0082 (0.67; p=0.4133)**	**0.001 (1.18; p = 0.2769)**
	Soil Potassium	**0.0023 (5.83; p =0.0157)**	**-0.009 (0.75 ; p = 0.3858)**
	Soil Sulphur	**0.0002 (0.07; p= 0.7885)**	**0.0004 (0.24;p = 0.6275)**
Grid 3	Intercept	**-1.56 (3.62; p=0.057)**	**-3.53 (4.69; p = 0.03)**
	Larval availability	***Not included***	**0.033 (13.71; p = 0.0002)**
	Plant Leaves	**0.047 (133,41; p<0.001)**	**0.02 (4.78; p = 0.02)**
	Plant diameter	**-0.12 (0.86; p= 0.35)**	**-0.21 (0.82; p= 0.36)**
	Plant Height	**0.03 (14.99, p=0.0001)**	**0.04 (6.90; p=0.0086)**

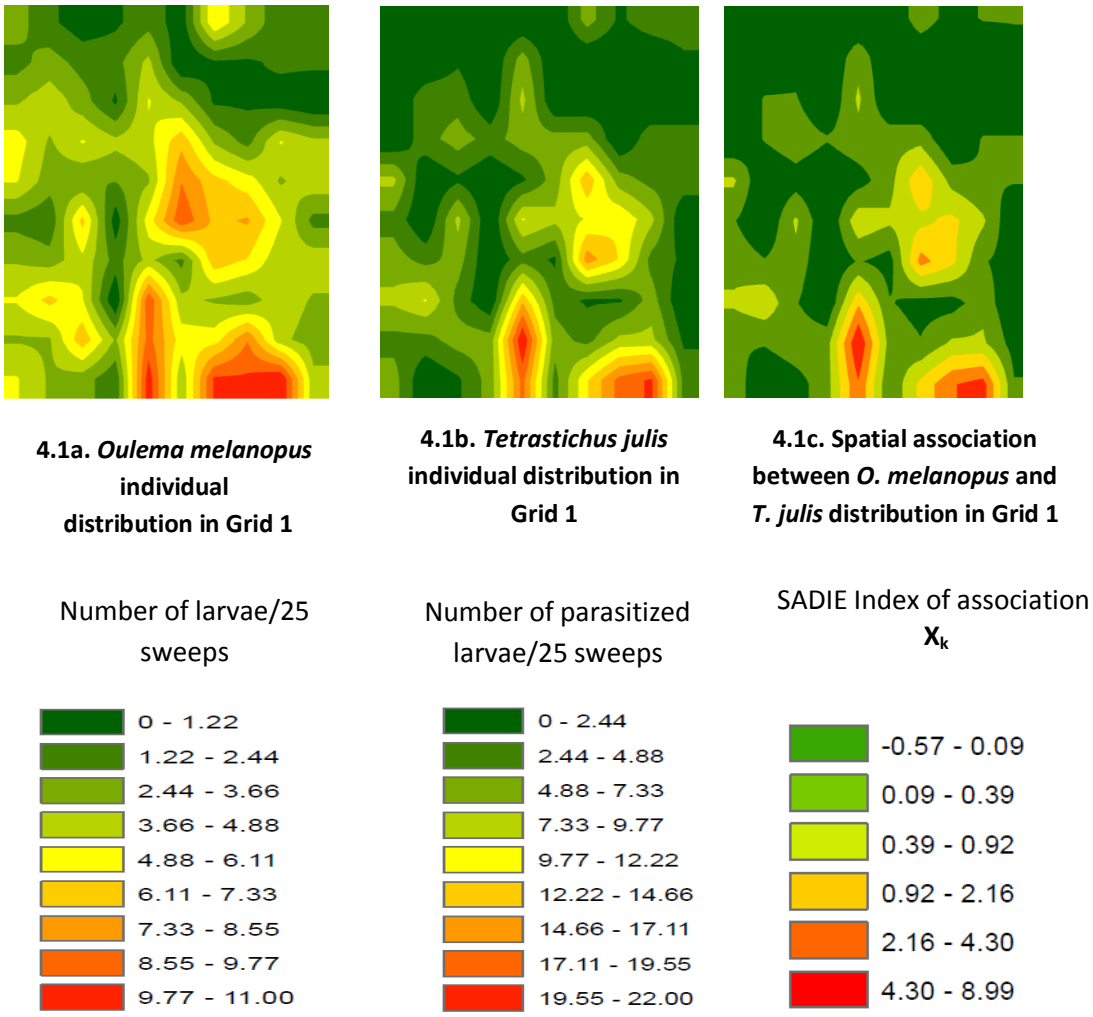
Table 4.8. Regression model selection using Baysian Information Criterion (BIC) to predict factors influencing abundance of *Oulema melanopus* and *Tetrastichus julis* under field conditions of winter wheat in Alberta, Canada. For each grid study year, several models with a dependent variable and predictors have been presented below with their respective BIC values. The model with the lowest BIC value is declared selected.

Model tested with predictors	Baysian Information Criterion (BIC) Value
Grid 2- 2011:	
Larval abundance = N+P+K+S+Soil N +Soil P+Soil K+Soil S+Plant leaves+Plant stem diameter+Plant height	337.84 (Selected Model)
Larval abundance = N+P+K+S	682.63
Larval abundance =Height+Leaves+Diameter	692.64
Larval abundance = N+P+K+S+ Height+Leaves+Diameter	584.82
Parasitoid abundance= Larva+N+P+K+S+Plant height+Plant leaves+Plant stem diameter	460.00
Parasitoid abundance= Larva+Plant height+Plant leaves+Plant stem diameter	445.00
Parasitoid abundance= Larva+ N+P+K+S	454.00
Parasitoid abundance= Larva+ Soil N+ Soil P+ Soil K+ Soil S	232.48 (Selected Model)
Grid 3- 2012:	
Larval abundance = N+P+K+S+Plant leaves+Plant stem diameter+Plant height	597.74
Larval abundance = N+P+K+S	729.23
Larval abundance = Plant leaves+Plant stem diameter+Plant height	579.66 (Selected Model)
Parasitoid abundance= Larva+N+P+K+S+Plant height+Plant leaves+plant stem diameter	511.49
Parasitoid abundance= Larva+N+P+K+S	509.57
Parasitoid abundance= Larva+ Plant height+Plant leaves+Plant stem diameter	495.24 (Selected Model)

Note: The following abbreviations denote respective plant and soil nutrients:

N=Nitrogen, P=Phosphorus, K=Potassium, S= Sulphur

Figures.



4.1a. *Oulema melanopus* individual distribution in Grid 1

4.1b. *Tetrastichus julis* individual distribution in Grid 1

4.1c. Spatial association between *O. melanopus* and *T. julis* distribution in Grid 1

Figure 4.1. Distributions of larvae of *Oulema melanopus* and *Tetrastichus julis* during their peak activity periods at the flag leaf stage in Grid 1 (2010), and the spatial association between the beetle and the parasitoid. The counts of larvae and parasitized larvae per grid cell were used to obtain interpolated maps of individual distribution and spatial association between the two species.

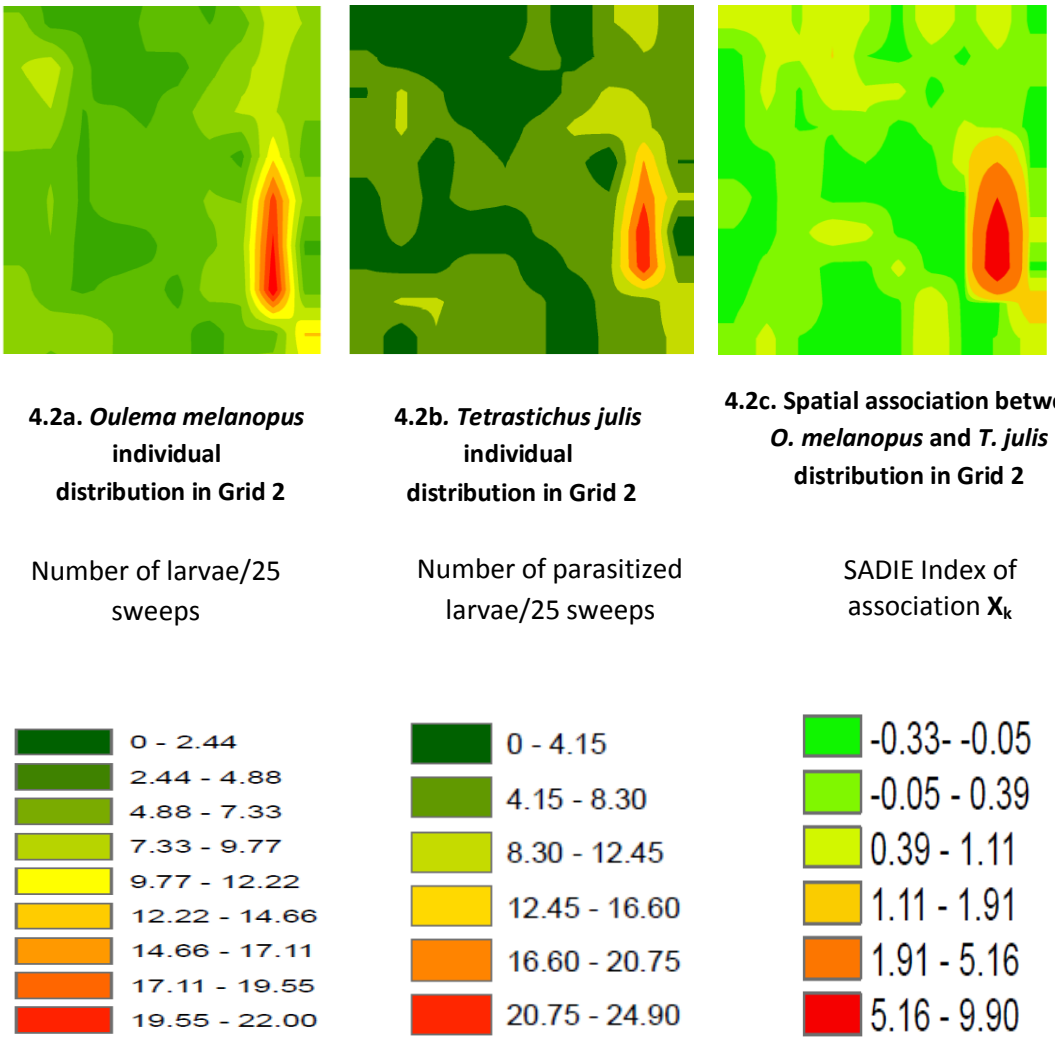


Figure 4.2. Distributions of larvae of *Oulema melanopus* and *Tetrastichus julis* during their peak activity periods at the flag leaf stage in Grid 2 (2011), and the spatial association between the beetle and the parasitoid. The counts of larvae and parasitized larvae per grid cell were used to obtain interpolated maps of individual distribution and spatial association between the two species.

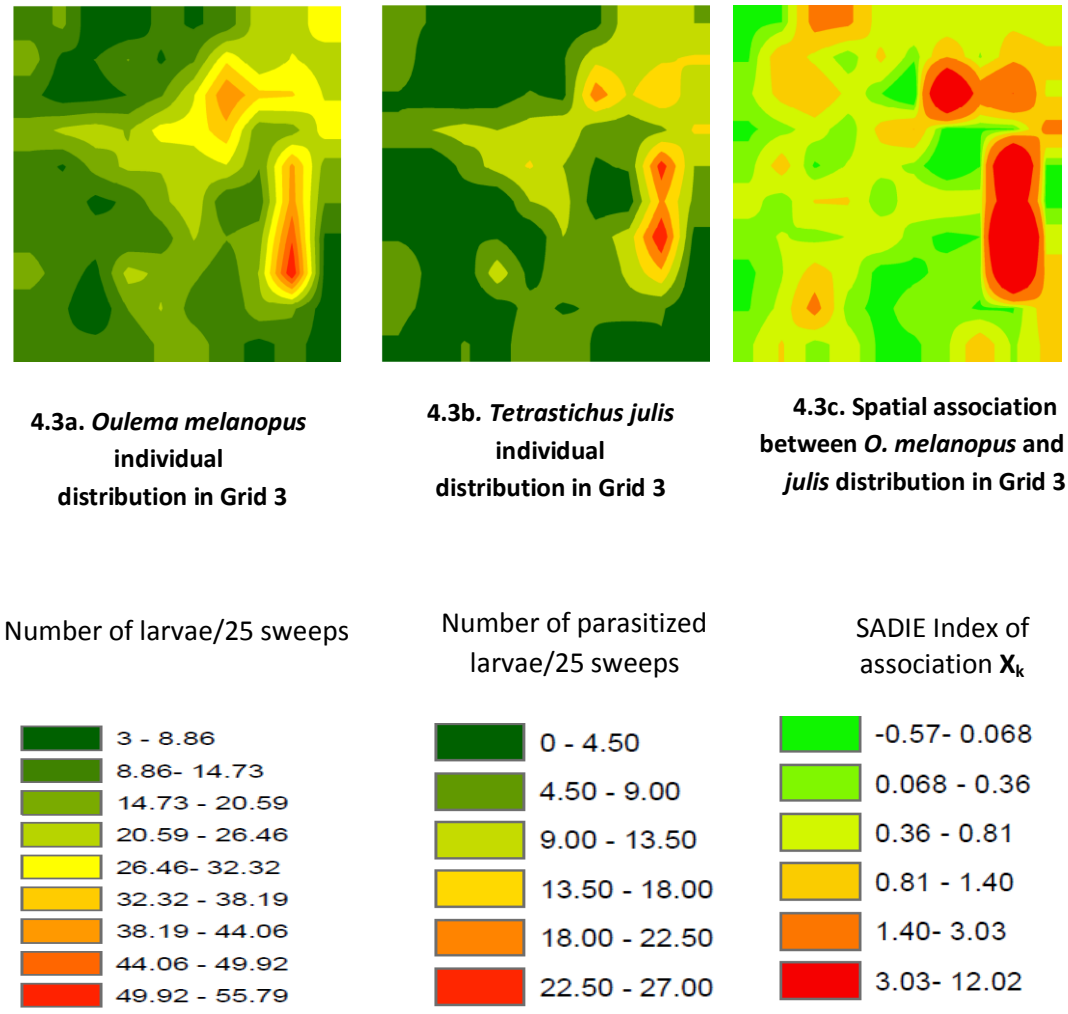


Figure 4.3. Distributions of larvae of *Oulema melanopus* and *Tetrastichus julis* during their peak activity periods at the flag leaf stage in Grid 3 (2012), and the spatial association between the beetle and the parasitoid. The counts of larvae and parasitized larvae per grid cell were used to obtain interpolated maps of individual distribution and spatial association between the two species.

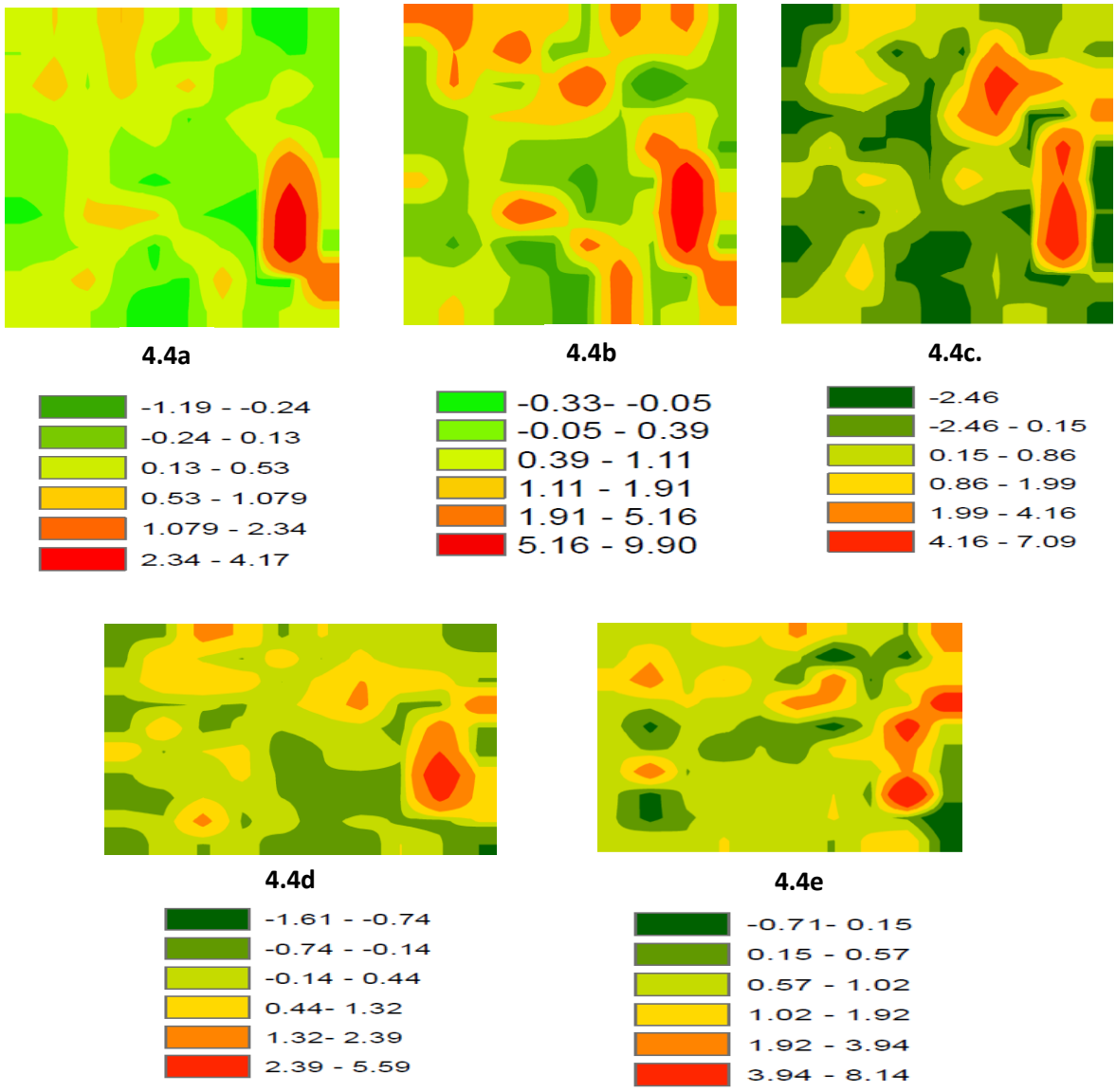
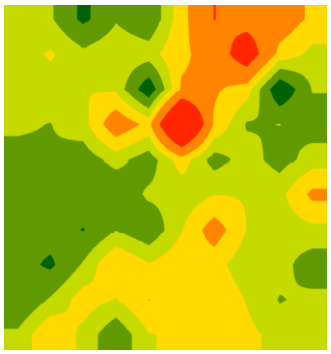
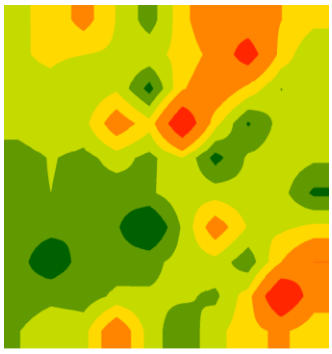


Figure 4.4. Contour maps interpolated from SADIE individual point index of association X_k showing the distributions of areas of association or disassociation between *Oulema melanopus* and a) plant height (2011); b) number of leaves per plant (2011); c) plant height (2012); d) number of plant leaves (2012); and 4e) stem diameter (2012).



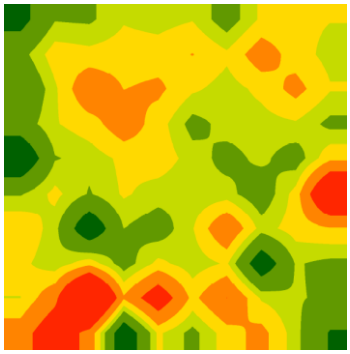
4.5a. Nitrogen and plant height

-0.62 - -0.20
-0.20- 0.08
0.08 - 0.38
0.38 - 0.69
0.69 - 1.21
1.21 - 1.86



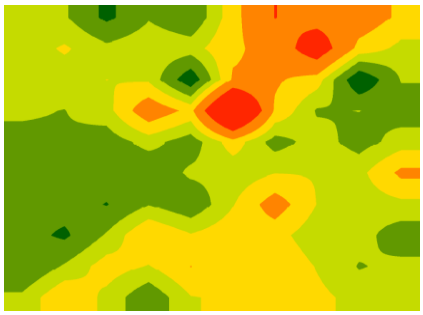
4.5b. Potassium and plant height

-0.80 - -0.28
-0.28 - 0.26
0.26 - 0.70
0.70 - 1.23
1.23 - 2.06
2.067 - 4.14



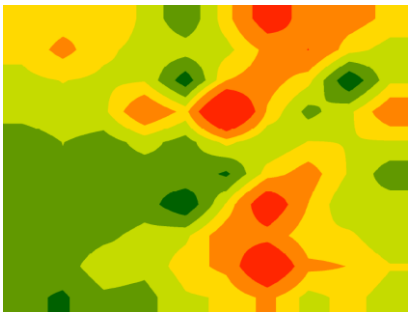
4.5c. Potassium and plant leaves

-1.48 - -0.63
-0.63 - 0.12
0.12 - 0.49
0.49 - 0.95
0.95 - 1.69
1.69 - 4.39



4.5d) Potassium and Plant Height

-0.80 - -0.28
-0.28 - 0.26
0.26 - 0.70
0.70 - 1.23
1.23 - 2.06
2.067 - 4.14



4.5e) Phosphorous and plant height

-0.69 - -0.27
-0.27 - 0.17
0.17 - 0.56
0.56 - 0.93
0.93 - 1.53
1.53- 3.12

Figure 4.5. Contour maps interpolated from SADIE individual point index of association X_k showing the distributions of areas of association or disassociation between soil nutrients and plant vigour metrics (Grid 2: 2011).

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Chapter 5: Antixenosis resistance to cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), in exotic wheat germplasm

A version of this chapter has been accepted for publication:

Kher, S. V., L. M. Dosdall, H. A. Cárcamo, and M. El-Bouhssini. Antixenosis resistance to cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), in exotic wheat germplasm. Journal of Economic Entomology.

5.1. Introduction

The cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), is a relatively recent alien invasive insect pest of Eurasian origin in western Canada that infests agriculturally important cereal crops including wheat, oat and barley (Leibee and Horn 1979; Dosdall et al. 2011; Kher et al. 2011). The beetle was initially discovered in North America in 1962 in Michigan, U.S.A. (Dysart et al. 1973; Evans et al. 2006; LeSage et al. 2007), and has since expanded its range to encompass most regions of cereal production in the U.S.A. (Ihrig et al. 2001; Buntin et al. 2004), eastern Canada (Harcourt et al. 1984), and western Canada (CFIA 2008; Dosdall et al. 2011) including portions of British Columbia, Alberta, south-western Saskatchewan, and north-western Manitoba (Dosdall et al. 2011). In Alberta, the beetle was first discovered near Lethbridge (49^{o} 41’ 39”N, 112^{o} 49’ 85”W) and Taber (49° 47′ 5” N, 112° 09′ 03” W) in 2005, and annual surveys have indicated significant population hot-spots across southern Alberta (Dosdall et al. 2011; Kher et al. 2011). Adult and larval damage

can cause yield losses as high as 55% in spring wheat, 23% in winter wheat and 38-75% in oat and barley (Webster and Smith 1979; Royce 2000). In Canada, the pest is predicted to spread across all cereal growing regions (Olfert and Weiss 2006). Establishment of this pest thus has several economic implications for grain production, trade and export.

Oulema melanopus is univoltine and active in the field from May to August with the peak oviposition period being late May to mid-June in western Canada (Kher et al. 2011). Eggs are laid on the upper surfaces of leaves along the margins or close to the leaf mid-rib either singly or in multiple clusters (McPherson 1983; Piesik and Piesik 1998). Female fecundity ranges from 50 to 275 eggs in its lifetime (Schmitt 1988), and factors such as leaf surface texture, leaf width, and orientation of leaves influence oviposition (Wilson and Shade 1966). Incubation period is four to six days (Barton and Stehr 1970), followed by a larval period consisting of four instars that feed on adaxial leaf surfaces (Smith et al. 1971). Larvae are more damaging than adults and have been reported to consume plant biomass one to 10 times their body weight (Livia 2006). Larval feeding leads to significant losses in crop quantity and quality due to reduced photosynthetic activity (Haynes and Gage 1981; Grant and Patrick 1993; Kostov 2001), and the flag leaf stage is the most susceptible stage to the damage (Wilson et al. 1969). *Oulema melanopus* pupates in the soil forming earthen cocoons (Dysart et al. 1973); first-generation adults emerge in about three weeks and feed on various monocotyledonous plants before overwintering until late April of the following spring (Grant and Patrick 1993; Kher et al. 2011).

The importance of host-plant resistance as an alternative management tool for *O. melanopus* is well recognized, given the economic and ecological consequences of chemical control (Papp and Masterhazy 1996). Attempts to explore sources and mechanisms of resistance in cereals were initiated immediately after the discovery of *O. melanopus* in Michigan (Everson et al. 1966; Gallun et al. 1966; Ringlund and Everson 1968; Smith et al. 1971; Wallace et al. 1974). Wheat demonstrates strong resistance compared to oat and barley (Hahn 1968). The major mechanism of resistance in wheat genotypes is non-preference (antixenosis) by *O. melanopus* (Gallun et al. 1966; Wellso 1973; Hoxie et al. 1975). Leaf pubescence is a major non-preference mechanism (Wallace et al. 1974; Ringlund and Everson 1968), and it can deter oviposition, and affect hatchability, larval survival and adult feeding on resistant wheat varieties (Gallun et al. 1966; Wellso 1973; Hoxie et al. 1975; Papp et al. 1992). Wheat varieties with shorter and fewer trichomes are more preferred as oviposition hosts for *O. melanopus* (Hoxie et al. 1975).

Host-plant resistance for *O. melanopus* control was successful with the development of the moderately resistant wheat variety, "Downy", in the United States (Wellso 1982). Papp and Masterhazy (1996) also reported 34% reduction in feeding damage on resistant wheat genotypes when compared to susceptible ones. No particular reports on exploration of resistant genotypes are available from Canada.

Oulema melanopus is currently in its early stage of invasion in western Canada (Dosdall et al. 2011) with scattered local populations and patchy

distributions. This provides a unique opportunity to design integrated management strategies that incorporate sustainable approaches such as host-plant resistance. Reports of the existence of certain genotypes of wheat of central Asian origin with putative resistance to this insect form the basis of our study (El-Bouhssini, unpublished data). This region of the world has an extended history of wheat infestation by *O. melanopus* (Haynes and Gage 1981; LeSage et al. 2007), hence such genotypes could be invaluable for eventually developing locally adapted yet resistant genotypes of bread wheat (*Triticum aestivum* L.). Here we present results of investigations of feeding and oviposition preferences by the beetle to these genotypes, and I investigated the role of antixenosis as a resistance mechanism.

5.2. Materials and Methods

5.2.1. Wheat germplasm

Six genotypes used in our experiment (Table 5.1) were obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (referred to hereafter as ICARDA genotypes). The seventh genotype (CDC GO) was a local cultivar grown widely in Canada selected as a vulnerable control. Six promising genotypes of central Asian origin (Kyrgyzstan) with putative resistance traits for *O. melanopus* were selected based on the information available from ICARDA. These genotypes were observed to suffer less damage from *O. melanopus* under local field infestation conditions in Uzbekistan, Kyrgyzstan and Tajikistan (El Bouhssini, unpublished data).

However, the mechanism(s) of resistance in these genotypes has not been explored. Plants were propagated in a greenhouse potting mixture in Terracotta pots placed in greenhouse plastic trays (53 cm X 26 cm) with individual cups for seedlings and maintained at 16L: 8D and 60% relative humidity.

5.2.2. Insect culture

Overwintered *O. melanopus* adults were collected from a winter wheat field (49^{o} 41' 49" N, 112^{o} 46' 59" W) at the experimental farm of the Lethbridge Research Centre of Agriculture and Agri-Food Canada, and other commercial winter wheat fields near Lethbridge using sweep nets. The adult colonies were maintained under standard laboratory conditions at 21^{o} C and 16: 8 L:D regime and starved for 24 h before releasing them in the antixenosis test arena.

5.2.3. Experimental design and procedures

Antixenosis assays under choice scenarios were conducted using arenas consisting of plastic plug trays (53 cm X 26 cm) with 35 individual square cells each with a depth of 6 cm. The experiment was a randomized complete block design with seven genotypes randomized within a column, and five such columns within a tray. Thus, each tray served as a block representing seven treatments (genotypes) randomized across columns within the block. Six ICARDA genotypes and CDC GO were planted in a column with each cell representing a particular genotype. There were three such blocks. Upon emergence, seedlings were thinned to maintain one seedling per cell of a given genotype.

Approximately six weeks after planting, the entire antixenosis arena was confined within an insect rearing cage (47.5 X 47.5 X 93 cm, BugDorm^{TM}, MegaView, Taiwan). Five mating pairs of *O. melanopus* adults were released for an exposure period of 96 h to allow them to mate, feed and oviposit on the genotypes of their choice. At the end of the exposure period, adults were removed from the cage and the number of eggs laid on each genotype was counted across different blocks.

The choice antixenosis assays were also conducted with teneral adults emerging from laboratory-reared *O. melanopus* larvae in late July-early August. The genotypes were exposed to five adult pairs for 96 h to allow the adults to feed. The experiment was replicated three times simultaneously. At the end of the exposure period, the percentage feeding on each genotype across different blocks was estimated using image analysis.

Antixenosis assays under no choice scenarios were conducted using a plastic pot (15 cm diameter) to grow five seedlings of each host genotype and replicated in five such pots. Each pot was caged (BugDorm^{TM}) and exposed to five pairs of overwintered *O. melanopus* for 96 h and the number of eggs laid was then counted.

Seedlings of each genotype were inspected visually for adult feeding damage. If the damage was apparent, such leaves were cut at the base and scanned (Epson Perfection 4990 Photo scanner) at a resolution of 600 dpi. Analysis was performed using image analysis software, Image Pro Plus, v 4.1 (Media Cybernetics, Silver Springs, Maryland). Threshold values for blue, green and red

channels were set, such that the damaged portions would be segmented out from the background. A particle count was then executed measuring leaf area, damaged area and the ratio of damaged area to the entire leaf area. The damage caused to a particular genotype was expressed in terms of percentage leaf area fed upon by *O. melanopus* adults over the exposure period. Leaves with folds, curls and other abnormalities were excluded from the analysis. For each replicate plant, observations were taken from a minimum of three leaves.

5.2.4. Statistical analysis

The differences in oviposition for overwintered adults on test genotypes under choice scenario were analyzed by using PROC GLIMMIX (SAS Institute 2010). Initial exploratory analysis suggested that the data on oviposition counts followed a negative binomial distribution. Hence, a generalized linear mixed model with negative binomial distribution defined using "log" as a link-function was fitted to these data. Model parameters were estimated using pseudo-likelihood technique (SAS Institute 2010). Genotype (treatment) was considered as a fixed effect in the model while "tray" (block), and the column nested within the tray were considered as random effects. The differences in the means of oviposition counts were compared using "DIFF" statement in PROC GLIMMIX. The genotype, NN-100, consistently yielded zero egg counts across all blocks and hence it was not included in the analysis.

Differences in feeding for overwintered and teneral adults on genotypes were analyzed using analysis of variance (PROC MIXED, SAS Institute 2008).

Each tray with columns representing seven genotypes in the antixenosis arena was considered a block and treated as a random factor. Similarly, columns nested within the tray were treated as random while genotypes were treated as fixed factors. Assumptions of normality and variance homogeneity were tested using Shapiro-Wilk and Leven's tests, respectively, prior to performing ANOVA. The proportions of feeding damage for both overwintered and teneral adults were arcsine transformed to achieve a normal distribution. Differences in least square means were compared using the PDIFF statement in PROC MIXED (SAS Institute 2008).

Differences in oviposition for overwintered adults under the no-choice scenario were analyzed using analysis of variance. The differences in means were compared using a Tukey test performed using the LSMEANS statement with the PDIFF option in PROC MIXED. At the end of the trials, the damage values for different genotypes from trials involving overwintered adults and teneral adults were pooled and analyzed to understand whether genotypes differed in their susceptibility to teneral and overwintered adults, and whether new and overwintered adults differed significantly in terms of their mean feeding on various genotypes. Genotype, adult type (overwintered or teneral adult) and the interactions between them (genotype X adult type) were tested and the treatment differences were compared with the PDIFF statement as explained before.

5.3. Results

Genotypes differed significantly in the mean number of eggs laid per plant across all blocks ($F = 3.55$; df = 5, 82; $P < 0.05$) in the choice arena. *O. melanopus* laid more eggs on plants of the susceptible cultivar, CDC GO, than on the ICARDA genotypes (Table 5.2). No eggs were laid on NN-100 plants. Similarly, fewer eggs were laid on plants belonging to genotypes NN-105, NN-41, NN-45 and NN-78 than on CDC GO. Among exotic genotypes, plants of NN-27 and NN-41 harboured slightly higher numbers of eggs than the remaining genotypes.

In the no choice study, the results were similar to those observed in the choice assay. Mean oviposition was highest on the susceptible genotype, CDC GO (Table 5.2). Furthermore, CDC GO plants differed significantly ($P < 0.001$) in terms of mean oviposition and harbored more eggs when compared to other genotypes such as NN-100, NN-105, NN-27, and NN-78. Among the ICARDA genotypes, the lowest number of eggs was laid on plants of genotype NN-100. NN-100 had significantly lower mean oviposition compared to genotypes NN-41 and NN-45. Mean oviposition was highest on NN-41 plants, which was followed by NN-45 plants (Table 5.2); both of these genotypes appear as suitable as CDC GO.

Feeding damage caused by overwintered adults to different seedlings over 96 h differed significantly among genotypes ($F = 2.60$, df = 6, 84; $P < 0.05$, Fig. 5.1). Mean feeding values for plants of the susceptible cultivar, CDC GO, were

higher than for plants from genotypes NN-100 and NN-105 ($P < 0.05$), but similar to the damage observed in plants of all other genotypes tested ($P > 0.05$).

When feeding damage was expressed as mean percent leaf area consumed per plant, plants from NN-45 had greater feeding, which was comparable to feeding observed on plants of CDC GO (Table 5.3). Feeding on plants of NN-45 differed significantly from those of genotypes such as NN-100 ($P < 0.01$), and NN-78 ($P < 0.05$). Arcsine transformed values for mean feeding were comparatively similar for plants of genotypes NN-100 and NN-105, and also for those of NN-27 and NN-45 (Table 5.3), and no significant differences were found among plants of ICARDA genotypes.

Feeding damage by teneral adults was similar among all genotypes evaluated (Fig. 5.1, $F = 0.95$, df = 6, 84; $P > 0.05$). Although plants of the susceptible cultivar, CDC GO had numerically higher feeding damage than plants of putatively resistant ICARDA genotypes, the differences were not significant statistically ($P > 0.05$).

The seven test genotypes differed significantly in terms of their susceptibilities to feeding by overwintered and teneral adults ($F = 2.46$, df = 6, 196; $P < 0.05$). However, the extent of feeding on various genotypes did not differ significantly between overwintered and teneral adults ($F = 2.21$, df = 6, 196; $P > 0.05$). The values of feeding damage caused were slightly higher for teneral adults on various genotypes. The interaction between genotypes and adult type was statistically insignificant ($F = 0.61$, df = 6, 196; $P > 0.05$). In terms of particular genotypes susceptibility to feeding by overwintered and teneral adults, CDC GO

was the most susceptible genotype ($P < 0.001$). CDC GO differed significantly ($P < 0.05$) from NN-100, NN-105, NN-41, and NN-78 in terms of damage. Among ICARDA genotypes, no statistically significant differences in terms of mean feeding were noted ($P > 0.05$).

5.4. Discussion

Results indicate that some of the ICARDA wheat genotypes selected for their putative resistance to *O. melanopus* have mechanisms of non-preference (antixenosis) in terms of oviposition and feeding by the beetle. Lower oviposition and feeding on plants of certain genotypes such as NN-100, NN-105 and NN-78 are indicative of antixenosis, and consistently lower oviposition rates on some ICARDA genotypes were observed in both choice and no-choice trials. Although the genotypes involved in the trials were known to possess certain mechanisms of resistance, such mechanisms were not explored before and the current investigation helps to provide insights into possible causes of their resistance. This information is needed for more detailed studies that may result in development of commercial resistant cultivars.

Mechanisms of resistance in wheat for *O. melanopus* have been widely explored (Everson et al. 1966; Gallun et al. 1966,; Ringlund and Everson 1968; Smith et al. 1971; Wallace et al. 1974), and antixenosis for oviposition and feeding is regarded as the principal mechanism of resistance against the beetle in most wheat genotypes (Wellso 1973; Hoxie et al. 1975). Our results concur with these studies.

The term antixenosis mainly implies behavioural response of an insect to a plant (Kogan and Ortman 1978), and is a response to plant properties that impart unsuitability to the plant in terms of feeding and/or oviposition. Most studies have indicated leaf pubescence as the main component of antixenosis in wheat for *O. melanopus* (Gallun et al. 1966; Papp et al. 1992); however, the phenomenon may not necessarily be restricted to pubescence, and other plant morphological characters have been reported to induce non-preference for oviposition and feeding. Wellso et al. (1973) pinpointed the influence of leaf width on oviposition and feeding by *O. melanopus* females, and reported an inverse relationship between leaf width and rate of oviposition. They found that plants with narrow leaves were less preferred for oviposition and suggested that leaf size characters can be considered important traits to screen for resistant germplasm. Similarly, silicaceous trichomes on leaves were noted to impart indigestibility of leaves causing antixenosis for larvae (Wellso 1973). Narrow leaf margins between veins were deterrent for larval feeding due to the inability of larvae to accommodate their mouthparts to hold and skeletonize the leaves of certain genotypes (Shade and Wilson 1967). Hence, antixenosis in most wheat genotypes results from a variety of plant morphological and physiological characters. We have not explored what mechanisms confer antixenosis in the genotypes we studied and this warrants further research.

We observed higher preference for plants of the locally popular commercial cultivar of spring wheat, CDC GO, for both oviposition and feeding by *O. melanopus*. This variety has been consistently seen as susceptible to *O.*

melanopus infestation in other related studies (Kher, unpublished data). This trend of greater oviposition on a susceptible host when exposed to a choice of resistant and susceptible hosts is consistent with the reports by Hoxie et al. (1975) and Wellso (1979).

Although the genotypes selected for the antixenosis tests were chosen based on their putative resistance to *O. melanopus* from field screenings in their native range, we noted relatively high variability in antixenosis. *Oulema melanopus* did not feed actively on plants of the genotype NN-100. Adults feed actively when they are reproductively active and cause substantial damage to host plants (Haynes and Gage 1981; Kher et al. 2011); therefore, it was unusual that there was only slight feeding on NN-100 plants. This needs further exploration to identify plant morphological characters that may be involved in hindering feeding and oviposition by adults. Other genotypes that appeared to exhibit greater antixenosis included NN-105 and NN-78. Overall oviposition and feeding were lower on both of these genotypes when compared not only with susceptible CDC GO but also with other genotypes such as NN-27, NN-41 and NN-45, which did not exhibit stronger resistance to *O. melanopus* in terms of hindering its feeding and oviposition. Given that strong antixenosis is absent in NN-41, NN-45 and NN-27, it is important to test whether any of these genotypes possess antibiotic characters that may hinder *O melanopus* physiology resulting in cessation of continued feeding or death.

The current investigation was conducted through a series of laboratory experiments and it is important to test the performance of these exotic genotypes

under field conditions to validate the results. Webster et al. (1978) observed that the results of trials involving pubescent wheat genotypes differed between field and laboratory tests as a result of the dynamic nature of insect-plant interactions under field conditions, and effects of environment on growth stages, maturity and expression of resistant traits. In genotypes with pubescence as a major resistance mechanism, leaf pubescence may decrease with plant maturity, and larval cohorts feeding on older resistant plants can effectively complete their life cycle (Webster et al. 1978). However, most field studies have shown the efficacy of the host-plant resistance approach in managing *O. melanopus* populations.

The current investigation has implications for strengthening the sustainable management framework for *O. melanopus* in a region experiencing a recent invasion by this pest. Identifying key sources and mechanisms of resistance is an important first step in implementation of host plant resistance as a component of an integrated program of pest management. Resistance traits may be associated with yield reduction (Philips et al. 2011), and host plant resistance may not prove efficient as a standalone pest management strategy. However, integration of host plant resistance with other pest management strategies such as biological control with natural enemies can efficiently improve the success of the pest management programme (Papp et al. 1992; Philips et al. 2011). Further, antixenosis for oviposition and feeding can help to mitigate *O. melanopus* damage at an early stage by reducing initial infestation levels. Feeding on resistant genotypes reduces adult and larval fitness of *O. melanopus* (Smith et al. 1971), and this can negatively impact pest performance, helping to reduce potential yield

losses. Thus, host plant resistance strategies can help to minimize insecticidal applications, and so mitigate negative impacts on ecosystem biodiversity.

Tables.

Table 5.1. Genetic background information on the wheat genotypes evaluated.

Genotype	Parental Germplasm	Source
NN27	Ferrugineum 205/ Frunsenskaya 60	ICARDA, Syria
NN41	Lutescencs 42/ Odesskaya Krasnokolosaya	ICARDA, Syria
NN45	Intensivnaya/Norin38 / Krasnovodopadsk	ICARDA, Syria
NN78	Odesskaya	ICARDA, Syria
NN100	Erythrospermum 13 / Obriy	ICARDA, Syria
NN105	Frunsenskaya60/Tardo/Intensivnaya/ Eryt.	ICARDA, Syria
CDC GO (control)	Grandin/SD3055	AAFC, Lethbridge

Table 5.2. Oviposition by overwintered *Oulema melanopus* adults on seedlings of test genotypes with putative resistance and the check cultivar in two laboratory assays.

Genotype	**Mean eggs/plant ± S.E.**	
	Antixenosis arena: Oviposition under choice scenario	Cage study: Oviposition on plants in a no-choice scenario
NN-100	0.00 *	2.20 ± 1.26 [b]
CDC GO	1.13 ± 0.22 [a]	16.60 ± 1.26 [a]
NN-27	0.73 ± 0.22 [a, b]	7.20 ± 1.26 [b]
NN-41	0.26 ± 0.22 [b, c]	15.80 ± 1.26 [a]
NN-45	0.13 ± 0.22 [b, c]	14.00 ± 1.26 [a, c]
NN-78	0.06 ± 0.22 [c]	5.40 ± 1.26 [b]
NN-105	0.06 ± 0.22 [c]	6.00 ± 1.26 [b]

A different letter denotes significant differences in means for oviposition under choice scenario detected using the DIFF statement in ANOVA using PROC GLIMMIX.
Means not sharing letters were significantly different according to the Tukey-adjusted test after ANOVA under no-choice scenario.
Exposure period was 96 h.
*NN-100 was not included in the analysis pertaining to zero egg counts across all blocks.

Table 5.3. Genotype susceptibility to feeding expressed as pooled means of feeding damage and compared between overwintered and teneral adults of *Oulema melanopus*.

Genotype	**Percentage feeding/Plant/Genotype (Mean ± S.E)**
CDC GO	2.06 ± 0.27 **a**
NN-100	0.71 ± 0.27 **b**
NN-105	0.95 ± 0.27 **b**
NN-27	1.31 ± 0.27 **a, b**
NN-41	1.00 ± 0.27 **b**
NN-45	1.39 ± 0.27 **a, b**
NN-78	1.13 ± 0.27 **b**

Adult type	**Percent feeding over the exposure period/Genotype (Mean ± S.E)**
Overwintered adults	1.07 **a** ± 0.12
Teneral adults	1.38 **a** ± 0.12

The differences in oviposition are tested using analysis of variance. A different letter denotes significant differences in means detected using the PDIFF statement in ANOVA.

Figures.

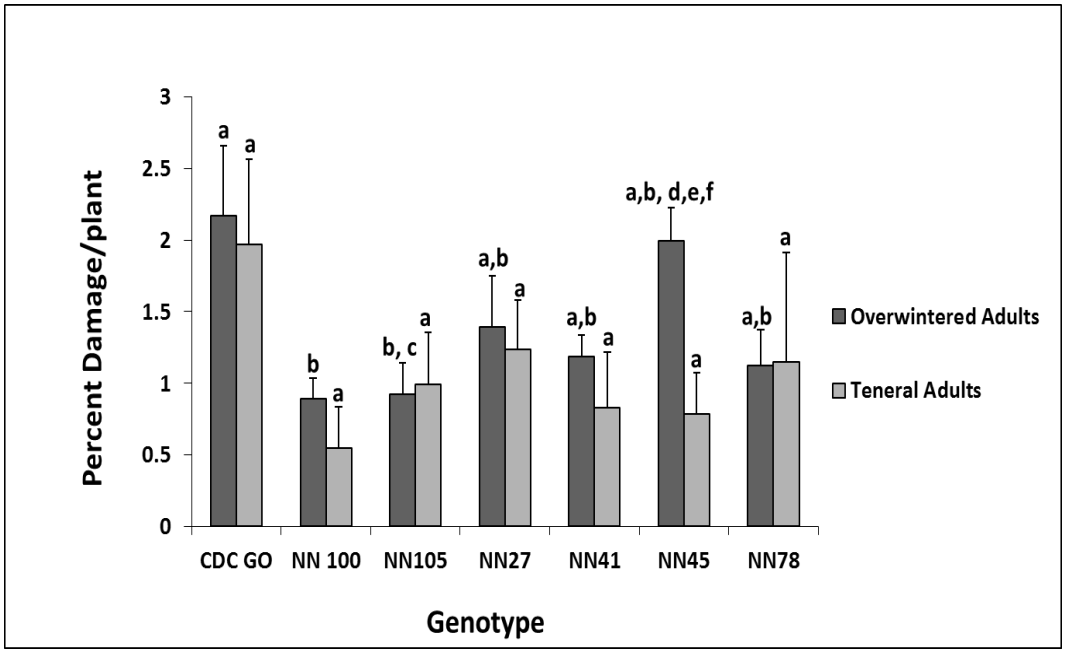

Figure 5.1. Feeding damage caused by overwintered and teneral adults of *Oulema melanopus* to plants of selected genotypes determined using image analysis. The proportions of damage caused to plants of each genotype have been arcsine transformed. Bars with different letters indicate significant treatment differences.

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Chapter 6: Antibiosis resistance to cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), in central Asian wheat germplasm

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6.1. Introduction

The cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), is an emerging pest of Eurasian origin of commercially important cereals including wheat, oats and barley in western Canada (Leibee and Horn 1979; Dosdall et al. 2011; Kher et al. 2011). Since its discovery in North America in 1962 in Michigan, U.S.A. (Dysart et al. 1973; Evans et al. 2006; Lesage et al. 2007), the beetle has expanded its geographic range significantly, encompassing most regions of cereal production in the U.S.A. (Ihrig et al. 2001; Buntin et al. 2004). In Canada, the beetle was first discovered in Ontario (Harcourt et al. 1984), and has recently invaded western Canadian provinces including portions of British Columbia, Alberta, southwestern Saskatchewan, and northwestern Manitoba (CFIA 2008; Dosdall et al. 2011). In Alberta, the beetle was first discovered near Lethbridge (49^{o} 41’ 39” N, 112^{o} 49’ 85” W) and Taber

(49° 47′ 5” N, 112° 09′ 03” W) in 2005, and annual surveys have indicated significant population hot-spots across southern Alberta (Dosdall et al. 2011; Kher et al. 2011).

Adult and larval damage of this univoltine pest can cause yield losses of 55% in spring wheat, 23% in winter wheat, and 38-75% in oat and barley (Webster and Smith 1979; Royce 2000). In Canada, the pest is predicted to spread across all cereal-growing regions (Olfert and Weiss 2006). Establishment of *O. melanopus* has several economic implications for grain production, trade and export. This warrants research to develop sustainable management practices that reduce reliance on chemical insecticides.

Host plant resistance in *O. melanopus* management has been researched extensively (Everson et al. 1966; Gallun et al. 1966; Ringlund and Everson 1968; Smith et al. 1971; Wallace et al. 1974; Papp and Masterhazy 1996; Konyspaevna 2012), and is considered a potential integrated management component in regions experiencing recent invasions (Haynes and Gage 1981; Kher et al. 2011; Philips et al. 2011). Host plant resistance can be manifested as antixenosis (non-preference), antibiosis, tolerance, or combinations of these (Painter 1968; Renwick 1983; Kogan and Paxton 1983). Two potential mechanisms of resistance reported against *O. melanopus* infestation in cereals are antixenosis and antibiosis (Gallun et al. 1966; Schillinger 1966; Wellso 1973; Hoxie et al. 1975; Wellso 1979). In terms of availability of potential genotypes with resistance, wheat has greater potential than other cereal hosts (Hahn 1968; Steidl et al. 1979).

Antibiosis entails negative effects of resistant host plants on pest physiology (Painter 1958; Renwick 1983; Smith 2005). Antibiosis mechanisms for *O. melanopus* are known in wheat (Schillinger 1966; Ringlund and Everson 1968; Wellso 1979), oat (Steidl et al. 1979), barley (Hahn 1968), and corn (Wellso 1978). Glandular trichomes in certain wheat genotypes exert antibiotic effects on *O. melanopus* eggs and larvae; the effects on eggs result in non-viability and failure to hatch (Wellso 1979). The antibiotic effects associated with trichomes may be exerted by both physical and chemical means (Schillinger 1966). Reduced feeding and fitness of *O. melanopus* larvae on plants with very low trichome density are indicative of the presence of associated mechanisms of antibiosis (Ringlund and Everson 1968). The genes controlling leaf pubescence in wheat may be linked with genes responsible for chemical antibiosis (Ringlund and Everson 1968); however, such associations have not been explored. Varieties with greater trichome density may also exert antibiotic effects on *O. melanopus*. For example, lower fitness and feeding of the beetle larvae on some pubescent wheat varieties have been reported in the U.S.A. and were attributed to antibiosis rather than to antixenosis (Smith and Webster 1974). Further, production of volatile compounds by some host species also has antibiotic effects on the beetle. For example, a secondary volatile chemical in corn, 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), negatively affects longevity of overwintered *O. melanopus* adults (Wellso 1978). Strong biochemical antibiosis resulting in low larval weight gains and reduced fitness was noted in oat genotypes (*Avena sterilis*

L.) (Steidl et al. 1979). There are no studies elucidating biochemical antibiosis in wheat.

Although both antixenosis and antibiosis are known to be components of wheat resistance to *O. melanopus*, much more research has focused on antixenosis (Haynes and Gage 1981; Battenfield et al. 1982; Papp and Masterhazy 1996; Konyspaevna 2012) than on antibiosis. Greater density of longer trichomes on leaves is a desirable trait invoking non-preference (Gallun et al. 1966; Hahn 1968). However, non-preference may be associated with or augmented by antibiotic mechanisms (Wellso 1979; Smith and Webster 1974), and may indicate antibiosis exerted by physical or chemical means (Schillinger 1966).

It is argued that the mechanisms of antixenosis and antibiosis can overlap in host plants making the distinction between these categories difficult (Renwick 1983; Smith 2005; Hesler and Dashiell 2011). Understanding of antibiosis effects thus requires detailed studies of host plant effects on insect pest biology and physiology (Renwick 1983). Understanding the underlying antibiotic mechanisms can help strengthen the resistance development efforts in commercial crop varieties.

Here I present the results of laboratory assessments of egg viability, developmental success, survivorship, and fitness of *O. melanopus* on genotypes of wheat (*Triticum aestivum* L.) of central Asian origin with putative resistance to *O. melanopus*. The aim of the current investigation was to determine whether antibiosis constitutes innate resistance in any of the test genotypes. I considered any negative effects of test genotypes on *O. melanopus* biology such as reduced

egg viability, failure to develop, early mortality and low adult fitness to indicate antibiotic response.

6.2. Materials and methods

6.2.1. Germplasm

Seed of six genotypes used in our experiment, with code letters NN (Table 6.1), was obtained from the International Center for Agricultural Research in the Dry Areas (ICARDA), Aleppo, Syria (referred to hereafter as ICARDA genotypes). Asia Minor is one of the major centers of availability of resistant genotypes for *O. melanopus* (Ringlund and Everson 1968; Hahn 1968), and the genotypes included in this study originated from central Asia (Kyrgyzstan). Six promising genotypes with putative resistant traits to *O. melanopus* were selected based on the information available from ICARDA. These genotypes are known to incur less damage in regions with established *O. melanopus* populations such as Uzbekistan, Kyrgyzstan and Tajikistan (El Bouhssini, unpublished data); however, the underlying resistance mechanisms remain undetermined. One genotype, CDC GO, is a commercial wheat variety commonly grown in western Canada and seed was obtained from Agriculture and Agri-Food Canada.

6.2.2. Insect culture

Overwintered *O. melanopus* adults were collected using insect sweep nets from a winter wheat field (49^{o} 41’ 49” N, 112^{o} 46’ 59” W) designated as the cereal leaf beetle nursery at the experimental farm of the Lethbridge Research

Centre of Agriculture and Agri-Food Canada, and other commercial winter wheat fields near Lethbridge (49^{o} 41' 39"N, 112^{o} 49' 85" W). The adult colonies were maintained under standard laboratory conditions at 21^{o} C and 16L: 8D (L:D) regime on wheat plants and starved for 24 h before conducting antibiosis tests.

To avoid handling damage, the sexes of adult beetles were determined by picking mating pairs of newly emerged overwintered beetles kept together in plastic containers (2 L capacity). Males were characterized based on a clearly visible, curved aedeagus protruding from distal abdominal sternites. Mating pairs were carefully placed in individual plastic containers and released in experimental cages.

6.2.3. Effects on egg hatchability and viability

Oulema melanopus preference for oviposition on various test genotypes was studied on live potted plants maintained in cages. For each genotype, a plastic greenhouse potting container (15 cm diameter) was used to grow seedlings. In each container, five seedlings of a given genotype were maintained. Plants in pots were allowed to grow for about eight weeks until they reached the five-leaf stage and were caged in BugDorm^{TM} insect rearing cages. For each genotype tested, a minimum of five such cages with several live plants set inside as food material were maintained in a completely randomized design. The plants were maintained at standard laboratory conditions (16L: 8D and 60% relative humidity).

Plants of each genotype in a given cage were exposed to five pairs of *O. melanopus* overwintered beetles for 96 h for oviposition. *Oulema melanopus*

females prefer to oviposit singly on individual host plants, so maintaining several host plants in a cage was designed to avoid oviposition on the same plant. If multiple eggs were laid on the same host plant, such plants were removed to avoid competition among the larvae sharing the same host. The position of leaves on which eggs were laid was marked at the base of the stem of the seedling using a coloured tape to facilitate daily observations of the relatively sessile larvae.

The eggs laid were monitored further for hatchability to test whether any genotype had negative effects on the normal development and incubation of eggs. Numbers of non-viable eggs were counted for each genotype. Eggs that showed symptoms such as desiccation, crumpling, or blackening that finally resulted in a failure to hatch were considered non-viable.

6.2.4. Development and survivorship studies

The eggs deposited on plants of each genotype in cages in the oviposition experiment were maintained intact and allowed to hatch. Handling of larvae and transfer to new hosts can result in mortality of neonate instars thus confounding the results of antibiosis expression (Ringlund and Everson 1968). Therefore, the larvae developing from such eggs were allowed to develop on the same plants without changing the experimental conditions. We studied biological parameters of *O. melanopus* on test genotypes from hatching to adult emergence.

Observations were recorded daily to calculate larval period, pupal period and total developmental period on each test genotype. Due to the small size of early instars, it was difficult to record the observations on the developmental

periods of individual instar stages. Hence, we measured larval period as a whole. Observations were recorded for any observed larval mortality in early instar stages. Larvae in their first instar stage were mainly considered "early" and if their death occurred within a few hours of hatching or before reaching second instar it was considered as early mortality. The number of larvae dying in early stages was thus counted and compared among hosts.

Fitness of *O. melanopus* emerging from each genotype was determined by weighing individual adults. Survivorship of *O. melanopus* was calculated as the percentage of adults emerging from the total number of eggs laid per test genotype in each cage.

6.2.5. Statistical analyses

Differences in oviposition, developmental parameters, survivorship and fitness of *O. melanopus* on various test genotypes were compared using analysis of variance (PROC MIXED, SAS Institute 2010). Assumptions of normality and variance homogeneity were tested using Shapiro-Wilk test and Leven's test, respectively, prior to performing ANOVA. Differences among mean oviposition, and time taken for larval, pupal and total development were compared among genotypes using a Tukey test and the LSMEANS statement with the PDIFF option in PROC MIXED. Along similar lines, *O. melanopus* fitness on various host genotypes was determined by comparing adult weight gain between genotypes using analysis of variance. Means were compared using a Tukey's studentized range test following ANOVA as described before.

6.3. Results

6.3.1. Effects on egg hatchability and viability

The test genotypes did not negatively affect the development of eggs and hatching. The number of non-viable eggs was not significantly different among the test genotypes ($F = 1.02$, df = 6, 28, $P > 0.05$), and we did not observe symptoms such as desiccation of eggs, blackening and failure for the embryonic development. However, genotypes differed in terms of oviposition ($F = 16.94$, df = 6, 28, $P < 0.0001$). *Oulema melanopus* adults laid significantly more eggs on plants of the susceptible cultivar, CDC GO, included as the check genotype, compared to other ICARDA genotypes except NN-41 and NN-45 ($P < 0.0001$, Table 2). Fewest eggs were laid on genotypes NN-78, NN-100, NN-105, and NN-27. Among ICARDA genotypes, significantly more eggs were laid on plants of genotypes NN-41 and NN-45 than genotypes NN-27, NN-78, NN-100 and NN-105. Both genotypes differed significantly from other ICARDA genotypes ($P < 0.0001$ for all comparisons, Table 6.2).

6.3.2. Development and survivorship

The mean incubation time for eggs did not differ significantly among the genotypes ($F = 1.89$, df = 6, 249, $P > 0.05$) (Fig. 6.1). The average development time of *O. melanopus* larvae differed significantly among test genotypes ($F = 32.14$, df = 6, 249, $P < 0.0001$). Larvae developed faster on plants of cultivar CDC GO (20 days) when compared to ICARDA genotypes ($P < 0.0001$ for all

comparisons, Fig. 6.1). The shortest larval period occurred on plants of genotypes NN-45 and NN-41, respectively, relative to all other ICARDA genotypes ($P <$ 0.0001).

Developmental time required for pupation differed significantly among the test genotypes ($F = 80.22$, df = 6, 241, $P < 0.0001$). The shortest pupal period occurred on plants of CDC GO (24 days) while the longest was on genotype NN-27 (32 days). CDC GO differed significantly in terms of pupal development from ICARDA genotypes ($P < 0.0001$ for all comparisons, Fig. 6.1). Among ICARDA genotypes, pupal period was shortest on plants of NN-41 followed by that on NN-45; both of these genotypes differed significantly from each other ($P < 0.0001$). Whilst the pupal developmental times on NN-100 and NN-105 were comparable, both genotypes differed from NN-78 in terms of mean pupal period ($P < 0.0001$ for both comparisons, Fig. 6.1). NN-27 required the longest time for pupal development, and differed from all other ICARDA genotypes ($P <0.0001$).

Finally, differences were observed in the total developmental time required by *O. melanopus* ($F= 94.14$, df = 6, 241, $P < 0.0001$). The beetle completed its development in the shortest time on CDC GO (51 days), while the developmental time was the longest on the plants of NN-27 (64 days). CDC GO thus differed significantly from all other test genotypes ($P < 0.0001$). Among ICARDA genotypes, the total developmental time was shortest on plants of NN-41 and NN-45, respectively. These two genotypes differed significantly from other genotypes namely, NN-27, NN-78, NN-100 and NN-105 ($P < 0.0001$). Although NN-27 resulted in delayed *O. melanopus* development, it did not differ

significantly from genotypes such as NN-78, NN-100 and NN-105 ($P > 0.05$, Fig. 6.1).

The survivorship and success of development of *O. melanopus* differed significantly among test genotypes ($F = 14.97$, df = 6, 28, $P < 0.0001$). The rate of survival and successful life cycle completion was highest on the susceptible cultivar CDC GO, and lowest on genotype NN-100. CDC GO differed from other ICARDA genotypes such as NN-27, NN-78, NN-100 and NN-105 ($P < 0.0001$). Among ICARDA genotypes tested, the highest survivorship was recorded on plants of the genotype NN-41 followed by NN-45. NN-41 differed significantly ($P < 0.05$) in terms of survivorship from other genotypes such as NN-100, NN-105 and NN-78, and NN-27. Similar differences were observed between NN-45 and other ICARDA genotypes. The survivorship pattern on NN-41 and NN-45 was comparable to CDC GO with no significant differences observed (Table 6.1).

With respect to physiology of *O. melanopus* larvae, no particular abnormalities were observed on any of the test genotypes or significant differences in early larval mortality ($P > 0.05$, Table 6.1).

6.3.3. Adult fitness

Host genotype significantly affected adult weights ($F = 154.61$, df = 6, 241, $P < 0.0001$). *Oulema melanopus* developing on the susceptible cultivar CDC GO had significantly higher body weights compared to all other genotypes ($P < 0.0001$ for all comparisons, Fig. 6.2). Adults reared on plants of genotype NN-100 had the lowest weight gain. Among ICARDA genotypes, adults reared on plants

of genotypes NN-41 and NN-45 differed significantly from all other genotypes in terms of adult weights ($P < 0.0001$ for all comparisons, Fig. 6.2). Those reared on genotype NN-105 had moderate weights that differed significantly from all other ICARDA genotypes (Fig. 6.2).

6.4. Discussion

Results indicated that some of the ICARDA wheat genotypes exerted antibiotic effects on performance and fitness of *O. melanopus*. Extended developmental period, reduced adult weights, and low survivorship on genotypes including NN-100, NN-78, NN-105 and NN-27 are indicative of antibiosis. The observed effects negatively affect normal development of *O. melanopus*, influencing its physiology, and thus conform to the definition of antibiosis (Painter 1958; Renwick 1983; Dent 2000; Smith 2005). Identification of expression of antibiosis in ICARDA genotypes thus confirms that the resistance in certain ICARDA genotypes is not putative but falls into classical categories of resistance. Antibiosis may be manifested through physical and/or biochemical mechanisms (Kogan and Paxton 1983; Smith 2005) but I did not explore the *modus operandi* of antibiosis expression.

Previous research on *O. melanopus* resistance has focused mainly on antixenosis and very few studies have explored potential antibiotic effects in resistant genotypes (Wellso 1979; Haynes and Gage 1981). In this regard, it is important to note that the mechanisms of antibiosis and antixenosis may overlap (Kogan and Paxton 1983). For example, the death of test insects on resistant

genotypes may be due to antibiotic effects or a result of extreme non-preference leading to starvation (Renwick 1983). On the contrary, the non-preference in host plants may also be linked with chemical antibiosis as noted in certain wheat genotypes resistant to *O. melanopus* (Ringlund and Everson 1968). To distinguish antibiosis from antixenosis, detailed studies on pest performance on resistant cultivars are necessary. Cage studies on live plants under no-choice conditions allow measurements of biological parameters to detect the presence of antibiotic effects in test cultivars (Smith 2005), and this formed the basis of our laboratory assessments.

Among the ICARDA genotypes tested, expression of antibiosis was prominent in NN-100, NN-78, NN-105 and NN-27. The major antibiotic effects observed included prolonged larval and pupal periods, lower adult weights, and lower survivorship on plants of these genotypes.

Antibiotic effects on pest biology can be observed as soon as oviposition occurs. Host plants may exert negative effects on arthropod pests by potentially hampering the development of eggs by mechanisms such as hypersensitive response, formation of neoplasms, or by biochemical defenses (Hilker and Meiners 2002). Most studies on antibiosis have focused on the negative effects of resistant hosts on larval development upon feeding, rather than the effects on egg development (Walling 2000). However, oviposition-induced defenses of plants are of interest in understanding antibiosis. Such potential negative effects of host plant physiology on the survival and hatchability of eggs of insect pests are known (Smith 2005). Reduced hatchability and death of eggs on resistant host

genotypes have been reported for pest species such as Colorado potato beetle (Balbyshev and Lorenzen 1997), and bean pod weevil (Garza et al. 2001), and the early mortality resulting from negative effects on eggs is a desirable trait for enhancing early control of pest populations.

In my studies, however, the test genotypes did not exert any negative effects on egg hatchability and viability. The incubation patterns on all hosts were comparable and death of eggs was not observed. Hence, I conclude that the antibiosis associated with the test genotypes was expressed only upon feeding by larvae and was not exerted on the egg stage.

However, I observed that ovipositing *O. melanopus* exhibited preferences among the test genotypes. The beetle preferred NN27>NN-100>NN-105>NN-78 for oviposition. Because some test genotypes are preferred over others for oviposition, it may point to involvement of non-preference for certain hosts for oviposition (Wellso 1979; Steidl et al. 1979) over antibiotic effects on egg survivorship.

The nature of antibiotic effects as a result of continued feeding on resistant plants can vary (Painter 1958), and the effects may be acute or chronic (Smith 2005). Antibiotic effects on early life stages can cause death, and those in later stages reduce fitness expressed in terms of failure or extended time for pupation, reduced adult weights and body size, and prolonged developmental periods (Smith 2005). Previous studies on antibiosis in wheat genotypes for *O. melanopus* have shown adverse effects on larval weight gains, reduction in egg hatch, and lower survivorship on resistant genotypes (Hoxie et al. 1975; Schillinger and

Gallun 1968; Webster et al. 1975). Feeding on oats with antibiosis expression affected larval fitness and survivorship of *O. melanopus* adults (Steidl et al. 1979). In our studies, the extended larval period of *O. melanopus* on ICARDA genotypes was associated with lower adult fitness and lower survivorship, as observed on plants of genotypes NN-100, NN-105, NN-78 and NN-27. This indicates chronic rather than acute effects of antibiosis on development.

Not all of the ICARDA genotypes possessed antibiotic properties as hypothesized. The developmental parameters and fitness on plants of genotypes NN-45 and NN-41 followed a normal growth pattern, comparable to the susceptible genotype, CDC GO. The plants of these genotypes were not only attractive for oviposition but the feeding on these plants also resulted in higher survivorship and greater adult fitness of *O. melanopus*. No negative effects on hatchability of eggs, larval survivorship, or developmental times were observed (Kher et al. 2011).

In a pest management context, oviposition deterrence and unsuitability for feeding are both desirable traits in a resistant genotype (Kogan and Ortman 1978; Dent 2005). In this regard, plants of genotypes NN-100, NN-105, NN-78 and NN-27 performed well as they harbored lower egg loads and resulted in higher *O. melanopus* mortality. Based on lower oviposition rates observed, it may be concluded that these test genotypes express both antixenosis and antibiosis as reported for other insects (Smith 2005). For example, in Canada both resistance mechanisms were reported in field pea for pea aphid (Soroka and Mackay 1991), in *Brassica napus* L. for cabbage seedpod weevil (Dosdall and Kott 2006; Tansey

et al. 2010), and for diamondback moth in some canola cultivars (Sarfraz et al. 2007). However, none of the test genotypes in our experiment appeared highly non-preferred for oviposition, and antibiosis effects on larval growth and development were more prominent than antixenosis.

Our assessment of expression of antibiosis in test genotypes was laboratory-based. Field assessment of antibiosis can be complicated by environmental factors that affect the expression of resistance (Wellso and Hoxie 1982), difficulty in observing individual life stages and fitness parameters due to insect movements (Smith 2005). The laboratory studies thus provide a foundation for behavioural and biological assessment. Field studies conducted in Tajikistan showed lower damage ratings on the genotypes NN-27 and NN-78 (Safarzoda et al. 2011). Our finding that these genotypes are unsuitable for continued feeding by *O. melanopus* thus concurs with the field study.

Given that antibiosis is expressed through larval feeding on the test genotypes, further research is needed to explore the mechanism of this response in resistant genotypes. Antibiosis may be influenced by the production of volatile organic compounds (VOCs), and certain plant volatiles (the green leaf volatiles) may influence feeding by insect pests on resistant hosts (Piesik et al. 2009, 2010). The green leaf volatiles in wheat and barley determine attraction of *O. melanopus* for oviposition (Delany et al. 2013). However, the release of VOCs as a plant defense response may be invoked by mechanical injury caused by larval or adult feeding (Piesik et al. 2011). Continued feeding by larvae can induce release of cis-jasmone derivatives (indoles and terpenes) that can deter continued feeding on

such hosts (Delany et al. 2013; Piesik et al. 2011, 2013), and thus would negatively influence the performance on such hosts. Hence, further research attention should be given to the biochemical defenses that may determine antibiosis in resistant hosts.

On a worldwide basis, *O. melanopus* occurs over a vast geographical range extending from central and eastern Europe (Haynes and Gage 1981) to Asia (Kher et al. 2011) and North America. In North America, the species is transcontinental, and occurs from the Maritime Provinces of Canada (LeSage et al. 2007) and eastern U.S.A. (Haynes and Gage 1981; Philips et al. 2011) to western U.S.A. and Canada (Buntin et al. 2004; Evans et al. 2006; Dosdall et al. 2011). Studies reported here utilized field-collected specimens from southern Alberta, but the possibility exists that different molecular biotypes of *O. melanopus* exist with genetic differences that may affect their antibiotic responses, and hence results on antibiosis may be somewhat influenced by the beetle population used in these studies.

The importance of host-plant resistance tactics in *O. melanopus* management is well recognized (Everson et al. 1966; Gallun et al. 1966; Papp et al. 1992), and resistance can be readily integrated with other measures such as biological control (Haynes and Gage 1981; Philips et al. 2011; Kher et al. 2011). Antibiosis resistance in particular may be beneficial to improve biological control efforts. For example, among indirect effects of antibiosis, affected insect pests may be prone to greater exposure and higher susceptibility to natural enemies (Singh 1986). Various acute and chronic antibiotic effects on *O. melanopus*

populations result in lower fitness (Smith et al. 1971), and thus help to reduce economic yield losses. Host plant resistance can thus help in developing an economically viable and environmentally sustainable pest management framework for *O. melanopus* control.

Tables.

Table 6.1. Background information on the exotic wheat genotypes selected for the antibiosis trial reported in this study

Name of the Genotype	Parental Germplasm	Source
NN-27	Ferrugineum 205/ Frunsenskaya 60	ICARDA, Syria
NN-41	Lutescencs 42/ Odesskaya krasnokolosaya	ICARDA, Syria
NN-45	Intensivnaya/Norin38 / Krasnovodopadsk	ICARDA, Syria
NN-78	Odesskaya	ICARDA, Syria
NN-100	Erythrospermum 13 / Obriy	ICARDA, Syria
NN-105	Frunsenskaya60/Tardo/Intensivnaya/ Eryt.	ICARDA, Syria
CDC GO (check)	Grandin/SD3055	AAFC, Lethbridge

Table 6.2. Comparison of developmental parameters of *Oulema melanopus* on various test genotypes: (a) mean oviposition over an exposure period of 96 h on plants of genotypes included in the antibiosis study, (b) survival calculated as the number of *O. melanopus* completing the life cycle and expressed as percentages for each genotype, (c) the average number of non-viable eggs per replicate per genotype, and (d) the average number of larvae that died in early instar stages per replicate per genotype

Plant genotype and number of replicate beetles in parentheses	(a) Mean oviposition (Mean ± SE)	(b) Survival rate (%) (Mean ± SE)	(c) Average number of non-viable eggs/replicate (Mean ± SE)	(d) Average number of larvae that died in early instar stage/replicate (Mean ± SE)
CDC GO (Check) (N=70)	15 ± 1.1 [a]	97.32 ± 4.08 [a]	0.00 ± 0.49[a]	0.40 ± 0.51[a]
NN-100 (N=32)	6.48 ± 1.1 [b]	58.05 ± 4.08 [b]	1.40 ± 0.49 [a]	1.40 ± 0.51[a]
NN-105 (N=30)	6.2 ± 1.1 [b,c]	60.83 ± 4.08 [b,c]	1.00 ± 0.49 [a]	1.20 ± 0.51[a]
NN-27 (N=36)	7.5 ± 1.1 [b,c,d]	66.77 ± 4.08 [b,c,d]	1.20 ± 0.49 [a]	2.20 ± 0.51[a]
NN-41 (N=79)	15.8 ± 1.1 [a,e]	88.88 ± 4.08 [a,e]	0.60 ± 0.49 [a]	1.20 ± 0.51[a]
NN-45 (N=70)	14.7 ± 1.1 [a,f,e]	83.36 ± 4.08 [a,d,e,f]	1.40 ± 0.49 [a]	1.00 ± 0.51[a]
NN-78 (N=27)	5.4 ± 1.1 [b,c,d,g]	61.2 ± 4.08 [b,c,d,g]	1.00 ± 0.49 [a]	1.20 ± 0.51[a]

Means not sharing letters were significantly different according to the Tukey-adjusted test after ANOVA.

Figures.

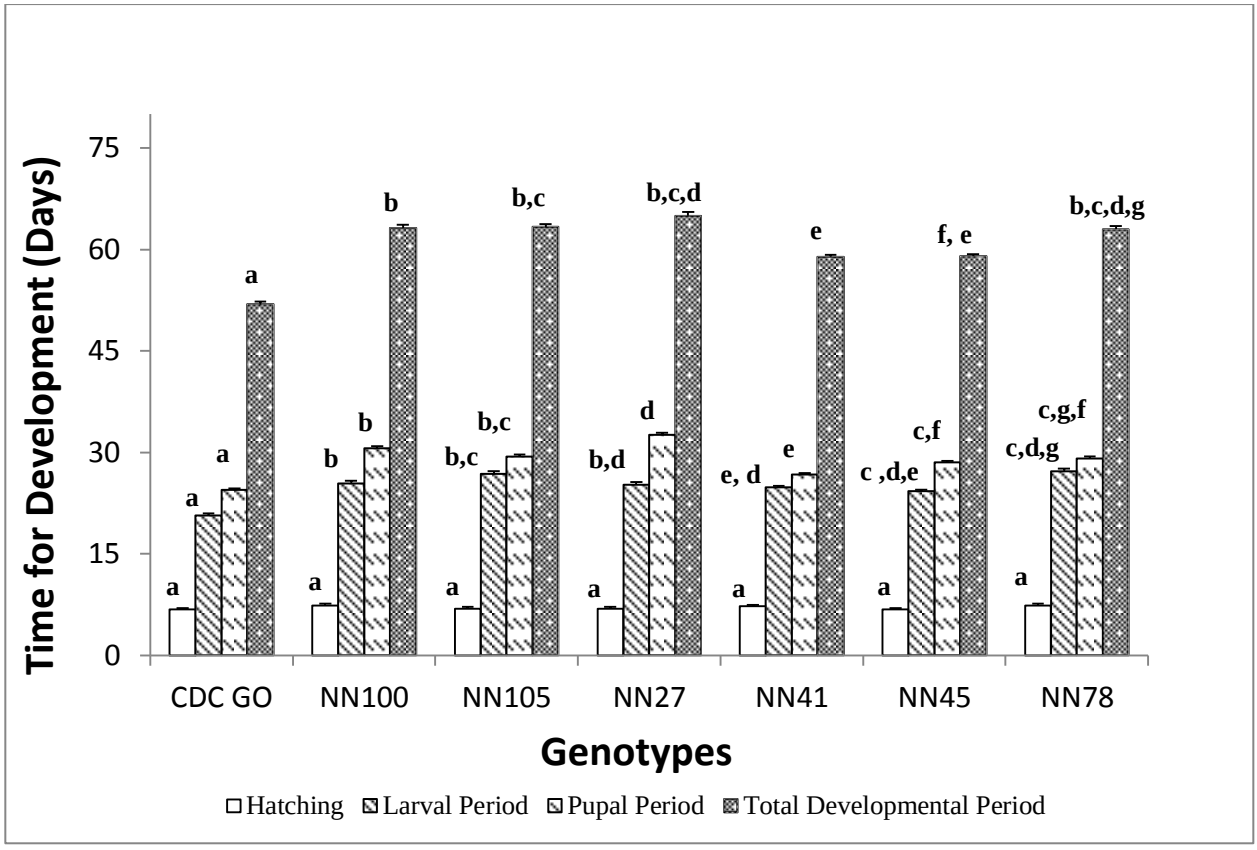

Figure 6.1. Mean developmental time in days (+ S.E.) for the completion of the life stages of *Oulema melanopus* on plants of various wheat genotypes investigated. Bars with different letters indicate significant treatment differences among genotypes for each beetle developmental stage.

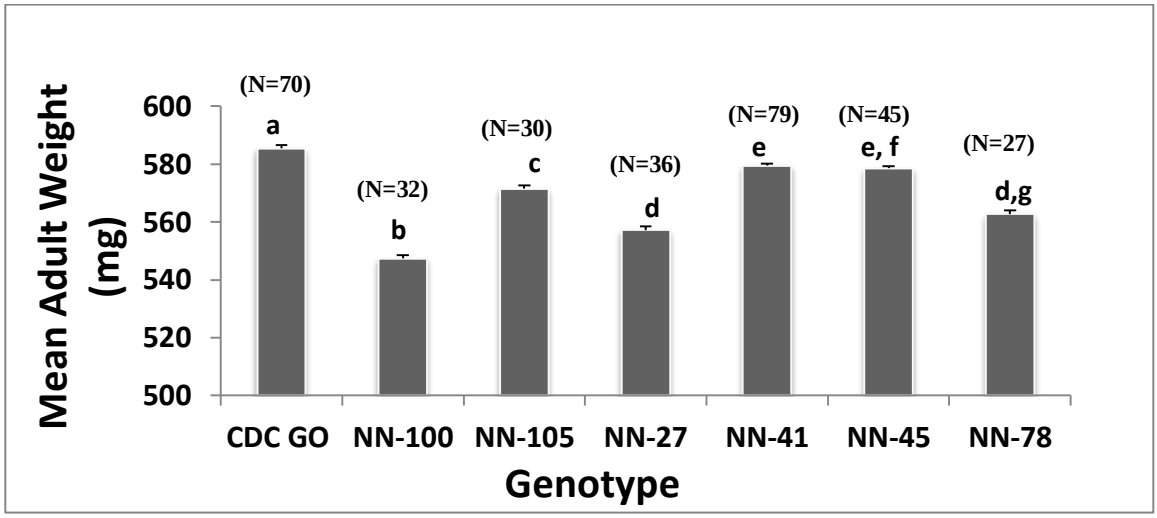

Figure 6.2. Mean adult weight of *Oulema melanopus* (+ S.E.) measured upon successful completion of the life cycle on plants of various genotypes investigated. Bars with different letters indicate significant treatment differences.

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Chapter 7: General Discussion

This thesis elaborates on results of my investigations of various bioecological aspects of the invasion of the cereal leaf beetle, *Oulema melanopus* (L.) (Coleoptera: Chrysomelidae), in western Canada, and consequent implications for sustainable management of the beetle in this new ecoregion. Through this investigation, I have attempted to explore important interrelations defining tritrophic interactions between the cereal host plants, *O. melanopus* and *Tetrastichus julis* (Walker) (Hymenoptera: Eulophidae), the principal parasitoid of the beetle. I investigated life histories and host preferences of *O. melanopus* and *T. julis*, their spatio-temporal distribution dynamics, and explored host-plant resistance mechanisms in exotic wheat genotypes to discern interrelations between these species.

It is more than five decades since *O. melanopus* was first discovered in North America in Michigan, U.S.A. (Philips et al. 2011). The pest has continued to spread throughout North America despite considerable attention given to various aspects of its management, and successful implementation of a classical biological control programme using introduced natural enemies from Europe, relying especially upon the larval endoparasitoid, *T. julis*. The recent invasion by *O. melanopus* of western Canadian cereal-growing regions and its continued range expansion throughout the region is indicative of its invasive capability. Invasion of western Canada was, however, somewhat unique in that the beetle invasion was soon followed by its principal parasitoid, *T. julis*, as the eulophid expanded its geographic range in western Canada thus requiring no intentional

introduction. This presents an historical opportunity to bolster natural suppression of beetle populations by augmenting the activity of *T. julis* at an early stage of beetle invasion, before populations reach economically damaging levels. Understanding the assembly dynamics and dispersal characteristics of the beetle and its principal natural enemy can facilitate effective implementation of biological control and other pest management strategies. Further, life history studies are a key to understanding critical processes underlying host-parasitoid interactions (Hassell 2000). The invasions of western Canada by *O. melanopus* and *T. julis* therefore provided an opportunity to investigate key aspects of their life histories, especially pre-imaginal development on various host plants, to strengthen understanding of how best to implement some key management practices.

It is argued that the gap between laboratory behavioural studies and field dynamics studies has limited the applications of ecological theory to the practice of pest management (Hochberg et al. 1996). Bridging this gap has potential implications for sustainable management particularly when the insect invasion is recent and the insect is in its initial stage of dispersal. My investigation attempts to couple components of field population ecology with life history traits to present a broader picture of interactions between the beetle and its parasitoid.

To design a sustainable management framework applicable to western Canada, a detailed understanding of the bioecology of the pest and its interactions with host plants in its new environment was important. Hence, I studied life history traits of *O. melanopus* (Chapter 2) and its parasitoid, *T. julis* (Chapter 3),

to understand their biologies, adaptive strategies, and fitness characters. I expanded the study of their associations to develop an understanding of how such interactions function under dynamic field conditions at a spatial scale (Chapter 4). Detailed studies on field dynamics of *O. melanopus* and *T. julis* with reference to plant vigour metrics and nutrition at a spatio-temporal scale thus facilitated improved understanding of tritrophic interactions (Chapter 4). I further focused on exploring mechanisms of host plant resistance in the form of antixenosis (Chapter 5) and antibiosis (Chapter 6) in wheat genotypes of central Asian origin.

Results of my study on developmental patterns of *O. melanopus* on potential cereal hosts in western Canada (oat, wheat, barley, corn, rye and triticale) indicate that the preferences for these hosts and their utilization differed within the fundamental host range of *O. melanopus* (Chapter 2). Significant differences were noted in the developmental times for different life stages, survivorship, fitness gains, female fecundity and the duration of active oviposition on different hosts. These findings confirmed that the host range of *O. melanopus* can vary geographically as it expands its range (Kher et al. 2011). In my studies, wheat (winter and spring), oat (cv. Morgan) and barley were the best hosts in terms of prompting rapid development, fitness gains, and fecundity. Prolonged development with low fitness gains was noted on corn, rye and triticale but survivorship was high on the latter two hosts. Hence, rye and triticale can act as secondary hosts of *O. melanopus* in western Canada. Recent surveys in southern Alberta indicated high population densities of the beetle in winter triticale fields (Cárcamo, personal communication). This observation corroborates results of my

study. Development and survivorship on a local cultivar of oat, Waldern, indicated prolonged developmental times and low survivorship. Developmental trends showed differences among and within species. Given that the studies on biology and fitness were laboratory-based, care must be taken in estimating the impact of these host plants on beetle populations under field conditions. This is particularly important when interpreting negative consequences of continued feeding of *O. melanopus* on Waldern oat. In my studies, Waldern oat has proven to be an attractive host for oviposition. However, I observed a negative influence on larvae following their feeding on this cultivar. Field trials are necessary to validate such effects on field populations of *O. melanopus* to further explore the potential of this cultivar as a candidate for a trap crop.

As the oat cultivar, Waldern, seems to exert antibiosis resistance upon larval feeding, no additional strategies (for example, spraying with chemical insecticide) to regulate *O. melanopus* populations may be needed. Thus, this result from studies on the biology of *O. melanopus* provides a potential avenue for investigation of an important pest management strategy.

Despite lower fitness gains on certain hosts such as rye and triticale, oviposition by *O. melanopus* was not significantly different among the hosts, and high numbers of eggs were laid on these plants. Such variable utilization of hosts can be an adaptive strategy of the beetle for continued dispersal and range expansion in this new eco-region (Mayhew 1997). This strategy can enable *O. melanopus* to sustain itself on suboptimal hosts in years or seasons when more preferred hosts are unavailable.

Survival of *O. melanopus* and its ability to complete its life history on suboptimal plants can also enhance its ability to disperse. *Oulema melanopus* is predicted to establish in all regions of cereal crop production in western Canada, including agricultural fields of the Peace River region of northern Alberta (Olfert et al. 2004). However, the Peace River region is separated geographically from agricultural fields in west-central Alberta by coniferous forest that encompasses a width of at least 150 km^2. Nevertheless, this should not present an appreciable barrier to dispersal of *O. melanopus* because roadways connecting northern and central Alberta harbour many different gramineous plant species, and along with some nonfavored crop species like rye and triticale, many species of wild plants like wild oats can support adult feeding and larval development (Government of Alberta 2010).

A considerable volume of research literature has been published on *O. melanopus* host affinities in different regions. However, after five decades of research on *O. melanopus* management with emphasis on classical biological control, a number of knowledge gaps remain on the biological parameters of its principal larval endoparasitoid, *T. julis*. To implement biological control strategies successfully it is important to understand the ecology of both the natural enemy and its host (Memmott et al. 1998). This formed the basis of my investigation on life history traits and host preferences of *T. julis* (Chapter 3). My study reports for the first time aspects of the biology of *T. julis* through a comprehensive investigation of its larval stage host preferences, clutch size characters and host-finding behaviour using a series of laboratory and field experiments. My studies

thus provide a detailed picture of the strategies that *T. julis* employs to synchronize with its host.

It was previously reported that *T. julis* generally accepts all the larval stages of its host and does not have specific preferences (Stehr 1970), but my investigation indicates that although all host stages can be parasitized, *T. julis* females prefer advanced larval instars; such a selection may lead to greater clutch size, and improved fitness. The sex ratio of *T. julis* was consistently female-biased and a strong positive correlation between female emergence and clutch size was observed. Activity of *T. julis* females was highly synchronized with that of *O. melanopus*. Greater survival of female *T. julis* occurred if no potential hosts were encountered, and this indicates a fitness attribute to promote parasitization. For a parasitoid species that has invaded a new ecoregion while tracking its host, the suite of all the beneficial characters such as a female-biased sex ratio, sibling mating, and adjustment of clutch size based on instar size represent high adaptability to its host. Further, density dependence of *T. julis* on its host (Chapter 4) explains how population structure of the parasitoid is shaped under field conditions and exploiting patches of high host density can assure the best utilization of available resources for the parasitoid. Parasitoid behaviour and field population dynamics are interconnected (Ives 1995). Strong adaptive characteristics of parasitoids for host exploitation have been reported. At low densities, selective behaviour with preference for oviposition in larger instars is observed, particularly when the host is available at low densities (Fidgen et al. 2000). For example, *Elachertus cacoeciae* (Howard) (Hymenoptera: Braconidae),

prefers advanced larval instars of spruce budworm, *Choristoneura fumiferana* (Clemens) (Lepidoptera: Tortricidae), and the female-biased sex ratio is proportional to the instar size (Fidgen et al. 2000). Host-related preferences may be indicative of decisions based on handling time, encounter rates and may influence sex ratio and within-instar preferences (Price 1986; Fidgen et al. 2000).

In early invasion phases of an alien insect pest, two characters determine the insect distribution and success of biological control: environmental stochasticity and density dependent distribution of the natural enemy (Fagan et al. 2002). Field dynamics and dispersal characteristics of *O. melanopus* are influenced by micro- and macro-climate, nutrient availability, inter-field variations in crop maturity, wind patterns and initial population source (McPherson 1983; Grant and Patrick 1993; Sawyer and Haynes 1985). Dispersal plays a crucial role in life history and population dynamics (Casagrande et al. 1977). To predict seasonal variation in population dynamics it is important to consider the distribution and movement of the beetle in space and time (Sawyer and Haynes 1985) in relation to agro-ecosystem characteristics.

Studies on dispersal characteristics of *O. melanopus* have shown that the random diffusion model predicting random and unidirectional movement of the beetle to crop fields provides relatively accurate predictions on a wide regional scale but not on an individual field scale. Simulation models based on the hypothesis of attraction to host crops and movement away from non-hosts fit better than random diffusion for individual field scales (Sawyer and Haynes 1985). Reay-Jones (2010) studied spatial distribution patterns of *O. melanopus* in

wheat fields and suggested that considerable population variability in *O. melanopus* occurs on a spatial scale. However, this research focused mainly on the aspects of localized management of *O. melanopus* in wheat fields and reducing the beetle migration to neighbouring fields. Further studies on *O. melanopus* distribution in relation to crop developmental stages indicated that wheat stand characters and growth stages influenced field dynamics of the beetle (Reay-Jones 2012). The research also indicated spatio-temporal synchrony among eggs and larval stages of the beetle. However, none of the studies conducted on field dynamics of the beetle have explored interrelations between beetle and parasitoid dynamics, and how the beetle distribution patterns and host plant characters may influence *T. julis* distributions. To bridge this gap in knowledge, my investigation examined bottom-up effects of host plant nutrient availability, plant vigour metrics and soil-available nutrients on field distribution dynamics of both *O. melanopus* and *T. julis* on a spatio-temporal scale.

Under field conditions, the relationship of *O. melanopus* and *T. julis* indicated a tightly coupled host-natural enemy system (Chapter 4). The distribution patterns of the beetle and the parasitoid revealed the presence of significant gaps and patches across grid plots. Such patchy distributions are reported for *O. melanopus* in winter wheat (Reay-Jones 2012). *Oulema melanopus* and *T. julis* exhibited strong spatial association indicating density dependence of the parasitoid on its host as hypothesized. The relative dynamics of parasitoid density dependence may vary over space and time and may greatly influence host dynamics (Hochberg et al. 1996). Such patchy distributions of host

and parasitoids, and strong density dependence have been reported for other pests such as the cabbage seedpod weevil, *Ceutorhynchus obstrictus* (Marsham) (Coleoptera: Curculionidae), in canola (Blake et al. 2010), and contribute to the success of natural enemies in pest suppression.

Patchy host distributions have consequences for natural enemy population dispersal as evidenced in my studies. When the host is distributed over a wide area and in discrete patches, a parasitoid gains a strong advantage by colonizing areas with high host abundance. Parasitoid behaviour can also be modified, resulting in a high dispersal rate by parasitoid females to areas with high host density once successful host encounter occurs (Hassell and May 1973). The ability of parasitoids to search and exploit areas with high host abundance is critical for success of the parasitoid to stabilize its populations and prevent extinction (Hassel and May 1974). Parasitoids with limited dispersal ability benefit from colonizing patches with high host density (Hassell and May 1988): once a host patch is discovered, comparatively little energy is needed to parasitize the hosts. *Tetrastichus julis* has high reproductive potential while its dispersal rates are low (Haynes and Gage 1981). Density dependence on its host populations as seen in my studies is thus advantageous to *T. julis*.

Further, parasitization under field conditions is not a random phenomenon but is driven by spatial processes (Hassell 2000). Spatial density dependence of parasitoid distribution as a form of spatial heterogeneity is considered to stabilize natural enemy populations over an area particularly with patchy host distributions (Hochberg et al. 1996). Under field conditions, spatial patterns of parasitism may

vary primarily from direct density dependence to inverse density dependence to no relationship at all. Both direct and inverse density dependence of natural enemies on their host bring about stability in host-parasitoid distribution dynamics (Hassell and May 1988). Direct density dependence is more important in regulating host populations.

In Chapter 4, I reported that host plant characteristics influence field dynamics of *O. melanopus* which in turn influence *T. julis* distribution. Plant vigour expressed in terms of basal stem diameter, number of leaves per plant, and plant height indicated a high degree of spatial association with beetle activity, and thus were major determinants of the distribution dynamics of the beetle. Plant- and soil-available nutrients and vigour indices significantly influenced the distribution dynamics of both species. The occurrence of abundant natural enemy populations in areas with high plant density was recorded in soybean (*Glycine max* (L.) Merr.) (Fabaceae), and was attributed to changes in microclimatic conditions that favor herbivore natural enemy abundance in high density plantings (Price 1986). Effects of host plant vigour on herbivore fitness are known (Sarfraz et al. 2010), and the results of my study indicate how herbivore adaptation to plant vigour brings about respective changes in population structures of the parasitoid. Host habitat and local landscape characters can also influence spatio-temporal interactions between host and parasitoid. A poor habitat and unsuitable landscape traits may reduce parasitoid activity (Hirzel et al. 2007). Thus, research is needed to understand how habitat and landscape characters can influence population dynamics and interactions between *O. melanopus* and *T. julis*.

The importance of resistance breeding against *O. melanopus*, in view of the economic and ecological consequences of chemical control, is well documented (Papp and Masterhazy 1996). Sources of resistant germplasm for *O. melanopus* are concentrated mainly in Asia Minor and southeastern Europe (Ringlund and Everson 1968; Hahn 1968). Host plant resistance is a major component of *O. melanopus* management particularly in North America (Haynes and Gage 1981; Philips et al. 2011), and has garnered significant success. The genotypes included in my study originated from Uzbekistan, Kyrgyzstan and Tajikistan of central Asia and were described as possessing putative resistance to *O. melanopus* infestation (M. El-Bouhssini, personal communication). This suggests that the resistant germplasm for *O. melanopus* is not only concentrated in Asia Minor but further explorations in central Asia can provide valuable genetic resources for future breeding programmes. Non-preference for oviposition and feeding is the major resistance mechanism in wheat genotypes against *O. melanopus* (Everson et al. 1966; Gallun et al. 1966; Wallace et al. 1974; Ringlund and Everson 1968; Smith et al. 1971). However, antibiosis has not been given due consideration and may contribute to resistance in novel genotypes. I tested both modalities of resistance (antixenosis and antibiosis) in central Asian genotypes. Three of the six genotypes tested (NN-100, NN-78 and NN-27) were less attractive for *O. melanopus* oviposition and feeding (Chapter 5), and further trials on biology and fitness suggested prolonged beetle development and low fitness on the genotypes listed above (Chapter 6), which indicated the presence of both antixenosis and antibiosis mechanisms.

The presence of both resistance mechanisms in the same genotype has a dual advantage. Such resistant genotypes may not be selected for oviposition or may harbour fewer eggs, and if oviposition occurs, the plants would exert negative effects on larval development and feeding; consequently adult fitness would be low. Antibiotic effects resulting in prolonged larval periods are desirable for integrating host plant resistance and biological control for *O. melanopus* management. For example, antibiosis resistance can increase the exposure period of larvae to *T. julis* through prolonged larval growth. However, the timing of larval death as a consequence of feeding on resistant host plants is also an important factor as far as success of natural enemies is concerned (Price 1986). This is an important issue for a gregarious, koinobiont parasitoid like *T. julis* as any form of early mortality of host larvae may endanger survival of the parasitoid progeny. I observed that although antibiotic effects in genotypes such as NN-100, NN-27 and NN-78 brought about negative effects on *O. melanopus* physiology and adult emergence, death did not occur in larval stages. I found that the effects of antibiosis were chronic in nature and the mortality in pupal stages was higher than for larvae. This can be beneficial for *T. julis* development as its progeny could complete their growth before the beetle pupae have died. I did not expand my investigation to test the mechanisms underlying antixenosis and antibiosis effects in test genotypes and this warrants further research to understand the factor(s) driving these mechanisms.

Another concern in utilizing host plant resistance in combination with biological control is the consequences of negative effects of resistant hosts on pest

physiology for parasitoid development. If the mechanism of resistance is chemical antibiosis, there may be direct or indirect effects of host plant chemicals sequestered in the herbivore body on the third trophic level (Price 1986). However, this aspect of bottom-up effects of antibiosis mechanisms in host plants has received very limited attention, and it is not documented if any such effects may negatively influence the fitness of *T. julis*. To address this concern, detailed studies of parasitoid biology using larvae fed on resistant host genotypes will be needed. Such studies should be coupled with investigations of the chemical compounds that occur in antibiotic-resistant plants in comparison with those from susceptible plants.

In general, investigations have shown that the effects of resistant host plants on herbivore pests do not influence growth rates of natural enemies. For example, Krips et al. (1999) found that the population growth rates of the predatory mite, *Phytoseiulus persimilis* Athias-Henriot (Acarina: Phytoseiidae), were not influenced by feeding of its spider mite host, *Tetranychus urticae* Koch (Acari: Tetranychidae), on resistant gerbera plants.

Prior studies on host plant resistance for *O. melanopus* have shown that resistant wheat cultivars with glandular trichomes did not influence parasitism and activity of egg and larval parasitoids of the beetle, including *T. julis* (Lampert et al. 1983). Large-scale cultivation of hairy resistant wheat varieties did not interfere with biological control efforts in the U.S.A. (Lampert et al. 1983). Extensive field studies have reported that the use of resistant wheat genotypes did not influence parasitization activity of *T. julis* or activity of the other larval

parasitoids (Casagrande and Haynes 1976). These studies, devoted to understanding interactions between host plant resistance and biological control strategies for *O. melanopus*, have helped to strengthen biological control programmes for *O. melanopus* in the U.S.A. (Casagrande and Haynes 1976). This underlines the fact that biological control and host plant resistance are compatible strategies, and can provide a sustainable, integrated option for pest management in newly invaded eco-regions such as western Canada.

Webster (1977) argued that antixenosis resistance may be better compared to antibiosis resistance as the development of pest biotypes resistant to non-preference traits is less likely to occur compared to that for antibiosis traits. However, population shifts to other crops are hypothesized to be due to non-preference for resistant wheat cultivars (Webster 1977). On the contrary, field studies in the U.S.A. showed that the rates of migration to fields surrounding resistant wheat cultivars were low (Casagrande and Haynes 1976). Hence, although the approach has some shortcomings, it can certainly be installed as an important component of an integrated pest management programme. Furthermore, results of my study have implications not only for western Canada but also for regions in central Asia experiencing *O. melanopus* infestations. My studies have established a platform for further studies to investigate the genetic basis of resistance for future introgression breeding programmes. The genotypes identified as potential resistant lines in my study can act as effective genotypes for breeding explorations in North America and can be locally used in central Asia as resistant cultivars for mitigating losses associated with *O. melanopus* infestations. In North

America, a key consideration for introgression of *O. melanopus* resistance to elite wheat cultivars will be to ensure that resistance traits are not associated with yield reductions, and that grain quality (including bread-making characteristics) is not diminished in the resistant germplasm.

Assuming that *O. melanopus* in western Canada caused only 10% of yield losses, this amounts to substantial economic losses. Consequently there is an urgent need to develop strategies for sustainable management of the beetle. My detailed studies on *O. melanopus* local biology, spatio-temporal associations, and exploration of host plant resistance provide a foundation for designing such strategies for western Canada where the beetle invasion is recent.

From a management perspective, it is important to combine the results from pest monitoring and field dynamics studies. The management priority is to monitor the current range expansion of *O. melanopus* and identify new areas of infestation. Most invasions occur through multiple invasion foci or through continued dispersal and reestablishment from the origin of the invasion over a period of time (Moody and Mack 1988). However, the persistence of populations and continued dispersal depend on availability of suitable habitats in areas adjacent to points of invasion, and randomness in population dynamics of small invading populations (Moody and Mack 1988). In managing invading populations, large foci are easily detected due to visible population increase and ease in sampling, and are generally a target of management strategies. Such targeted management can help to manage populations of invasive pest species; however, small foci of invasion with low population densities are often neglected,

and these can develop future avenues for dispersal and continued spread for invasive species (Moody and Mack 1988; Ives and Settle 1996). Such nascent or satellite foci can contribute significantly to rapid establishment and range expansion of an invading species (Moody and Mack 1988; Memmott et al. 1998). Targeted efforts to identify nascent foci can help to reduce the rate of establishment of the invading species (Moody and Mack 1988). In the case of *O. melanopus*, the current population structures indicate sporadic populations scattered over a vast geographic area and may be indicative of the presence of several satellite populations currently acting as sources of invasions for previously uninfested areas. For example, although the major population hot-spots and spread have been observed mainly in the southwestern part of the Canadian Prairies, particularly in the province of Alberta, recent collection records in central Alberta near Edmonton, east of Red Deer, Moosomin in south-eastern Saskatchewan, and Treherne in southwest Manitoba are indicative of the presence of smaller, distinct populations that can act as sources for infestation across the cereal-growing areas in central Alberta (Carcamo, personal communication). From a management perspective, it is important that such foci be identified and targeted to avoid potential future establishment of the beetle. With the populations of *T. julis* establishing in beetle-infested areas, it provides opportunities for targeted releases of parasitoids in the areas where the beetle populations exist but have escaped parasitism.

The optimal release strategy for biological control agents depends upon the probability of establishment in reference to release sizes (Shea and

Possingham 2000). Insights from the *O. melanopus-T. julis* spatial association study are particularly helpful in this case (Chapter 4). Large releases of parasitoids in areas with high as well as low beetle densities, and small releases in areas with newly detected infestations may yield high success rates owing to the strong host-tracking capacities of the parasitoid. Given that *T. julis* chooses advanced larval stages on which to oviposit more eggs, releases in the field between mid-June to early July to synchronize the parasitoid activity with the peak larval activity of the beetle can help parasitoid females to optimize their fitness under field conditions when exposed to different larval stages.

In view of the time and economic resources required for mass rearing the parasitoid, it is important that *T. julis* releases are made at several different sites with known beetle infestation levels, with equal emphasis placed on the newly discovered sites with low beetle populations. The best strategy for parasitoid establishment would be to engage in many small releases at all known locations of low-density *O. melanopus* infestations. Monitoring of populations at release sites will help to track the establishment rates of the parasitoid and will help to understand the beetle-parasitoid population dynamics in newly infested regions. Hence, for the newly infested provinces of western Canada, mixed strategies involving both releases of small and large numbers at different infested localities can be done. The releases can be based on the pest population density and parasitoid availability. Such strategies can help to mitigate negative impacts of *O. melanopus* infestation and to maintain the beetle population below an economic injury level. At each release location, the pest population can be reduced locally

and the parasitoid can have a chance to expand its populations in nearby areas where the pest populations have spread (Fagan et al. 2002). Releases at multiple localities can thus allow natural enemies to keep pace with the host population and avoid lag times between pest invasion and parasitoid activity (Ehler 1998).

Such targeted releases of *T. julis* following laboratory-based mass rearing of the parasitoid were performed in Manitoba, Canada in 2010. The beetle was discovered in the Swan River Valley but there was no evidence of the presence of *T. julis* (Dosdall et al. 2011). Laboratory-reared and field-collected larvae were exposed to the laboratory colony of *T. julis*. Parasitized larvae were relocated in Manitoba at 24 sites in 2010 to supplement smaller releases in 2009. In 2011, adult *T. julis* were recovered from approximately 22% of *O. melanopus* larvae collected (n = 103) from release sites (H. Cárcamo 2012, unpublished results). This confirms that *T. julis* has established in the Swan River Valley, Manitoba (Kher et al. 2013). A similar strategy can be adopted in the future to augment the activity of *T. julis*.

Based on the results of my investigation, and combining the results from prior research on *O. melanopus* in North America, I have presented below some recommendations for *O. melanopus* surveillance and management in western Canada. I have also presented the rationale behind the tactics suggested and identified areas for focusing future research attention. The management approach discussed below focuses on following major components: monitoring and surveillance, appropriate agronomic practices, relocation of *T. julis*, and timing

and judicial use of chemical insecticides. I have discussed each approach and related tactics below.

Monitoring and surveillance

Early detection of *O. melanopus* activity in winter and spring cereals will help to track the beetle activity and help alert and educate farmers regarding presence of the beetle, and sustainable management options. Hence, annual surveys in different cereal crops across western Canada are necessary to monitor beetle populations at established sites and to detect new foci of infestation.

In the U.S.A., degree-day models have been developed to determine the time of scouting and enable targeting management operations for *O. melanopus* in some areas (Philips et al. 2012). However, such models are not currently available for western Canada. However, there is a strong positive correlation between temperature and development of *O. melanopus* (Guppy and Harcourt 1978), and hence, the onset of the beetle activity coincides with warmer spring temperatures (Philips et al. 2012). Further, if the daytime high temperatures in spring exceed 14^{o}C, the beetle adults emerge from overwintering sites and migrate to cereal fields (Gutierrez et al. 1974). Given the influence of temperature, scouting can be started for winter cereals with the onset of warm spring days. For spring cereals, scouting in early to mid-June coincides with adult feeding and oviposition activity in early spring. The surveys for *O. melanopus* can be performed using sweeping for adult and larval stages, visual monitoring for eggs, and surveillance for adult and larval damage (WCLBWG 2001). A minimum of 120 sweeps at 180^{o} are

recommended; however, the number of sweeps can be higher in larger fields (WCLBWG 2001).

Agronomic practices

Plant stand management

Results of my study indicate high spatial variability in soil- and plant-available nutrients, and plant stand characters. Such site-specific variability in resources can significantly influence insect herbivore and natural enemy populations. My investigation suggests that plant stand characters influence population dynamics of *O. melanopus* and *T. julis*. A heterogeneous plant stand with variable vigour can result in colonization of areas with high plant vigour that may result in beetle-induced yield reductions. Hence, an optimum plant stand with uniform vigour can help to mitigate losses caused by adult and larval feeding of *O. melanopus*.

For winter wheat, time of seeding determines emergence success, spring establishment and yield (AWC 2013). Seeding in early September is recommended for southern Alberta to ensure establishment of the plant crown before first freezing to assure spring establishment (AWC 2013). However, optimum seeding dates differ throughout western Canada and recommended sowing times by provincial agriculture authorities should be followed. A plant density of 250 plants/m^2 (AWC 2013; Government of Alberta 2011; Government of Manitoba 2013) is desirable for optimum productivity and the seed rates can be adjusted based on this (AWC 2013). An optimum seeding rate of 135 kg/ha is

recommended (Government of Alberta 2007). However, a major factor affecting the development of a uniform plant stand across a winter wheat field is winter-kill. Low temperatures can damage the crown of winter wheat plants resulting in lower survival and sparse plant stands in spring (University of Saskatchewan 2013). Hence, maintaining stand uniformity can be a greater challenge in winter wheat than in spring wheat. However, early application of nitrogen to encourage tillering, and early weed management in spring to reduce resource competition can help to maintain desired plant stands (Government of Manitoba 2013). Fertilizers should be applied judiciousy and overuse of nitrogenous fertilizers should be avoided to maintain uniform vigour. Depending on soil nutrient testing and moisture availability, the rates of nutrient application may vary. Applications of nitrogen and phosphorous are advised for southern Alberta and it is recommended that other nutrients be supplied only if they are deficient (AWC 2013). Nitrogen and phosphorus applied at rates of 65-75 and 50 kg/ha, respectively, can help to maintain optimum plant stands. A banded application of 75 kg/ha of nitrogen (ammonium nitrate) early in the spring can facilitate tillering (Government of Alberta 2000). Less than recommended seeding rates are not recommended in cereals, particularly for oat (Webster et al. 1978) as they result in higher population densities of *O. melanopus* per tiller. Hence, seeding should be done at recommended rates. The use of recommended agronomic practices for spring wheat will ensure desired and uniform plant stands.

Effect of tillage

Conventional tillage can kill up to 89% of *T. julis* populations as the beetle cocoons inside the soil containing overwintering parasitoid larvae are killed (Leibee and Horn 1979). To augment the activity of *T. julis* and enhance biological control of *O. melanopus* it is thus important to implement conservation tillage options. In western Canada, direct seeding is the recommended method for winter wheat and has advantages in terms of maintaining snow cover and for moisture management for optimum growth (AWC 2013). This method will also help conserving *T. julis* populations where parasitism is established by avoiding injury to the overwintering parasitoids. Direct seeding in spring and winter wheat crops has proven effectiveness against insect pests like wheat stem sawfly, *Cephus cinctus* Norton (Hymenoptera: Cephidae) (Beres et al. 2011), and thus can serve multiple, complementary purposes.

Parasitoid relocation

As discussed earlier, an approach for enhancing biological control of *O. melanopus* in western Canada would be to release *T. julis* at the newly infested sites. In the U.S.A., parasitoids including *T. julis* were reared through development of field insectaries and by relocating parasitized larvae to other regions with beetle infestations (Dysart et al. 1973; Vail et al. 2001). Due to the limited success of releases of adult *T. julis* and cost considerations, field insectaries are a popular approach over laboratory parasitoid rearing in the U.S.A. (Dysart et al. 1973). However, as *O. melanopus* is in its initial phase of establishment and has patchy distributions in western Canada, both approaches

(laboratory rearing and field nurseries) should be given consideration for effective parasitoid relocation.

In my experiments, parasitization of *O. melanopus* larvae by *T. julis* could be achieved with 48 h exposure to gravid parasitoid females. Parasitization levels as high as 82% could be achieved with 96 h exposure. Hence, exposure of laboratory-reared and field-collected larvae to *T. julis* females for up to 96 h can be performed to achieve high parasitization. *Tetrastichus julis* has a preferred affinity for attacking third and fourth instars, and larger instars represent greater ease of handling, so these larval stages are recommended for exposure to parasitoids. Parasitization levels can be determined by dissecting a subset of exposed larvae to check for the presence of parasitoids in the host body (WSU Extension 2001); alternatively, the parasitoids can be reared to emergence. Parasitized larvae or cocoons formed by such larvae can then be transported to relocation sites and released. For winter wheat, parasitoid releases in mid-June can coincide with peak larval activity, while releases in late June to early July are ideal for spring cereals.

Timing and judicious use of chemical insecticides

Avoiding unnecessary use of chemical insecticides in cereal crops upon detection of *O. melanopus* infestations is recommended for avoiding negative effects on *T. julis* activity. Currently, chemical insecticides are not used extensively for *O. melanopus* management in western Canada. However, recent surveys have indicated increases in population hot-spots of *O. melanopus* (Kher et

al. 2013), and insecticidal applications may be necessary in the future. The only product registered in Canada for *O. melanopus* control is the organophosphate compound, malathion (WCCP 2013), although it should be noted that other products like the carbamate, carbaryl, can also control the beetle when applied against other crop pests (Government of British Columbia 2011). However, negative effects of applications of insecticides on survival and establishment of *T. julis* are known (Coats et al. 1979), and it is therefore important to monitor field activities of *O. melanopus* and *T. julis* through scouting to determine whether the insecticidal spraying is necessary. Economic threshold levels commonly used in most parts of the U.S.A. of three eggs or larvae per plant at the boot stage and one larva per flag leaf at the flag leaf stage (Webster and Smith 1979), and can be applied to western Canada. To achieve sustainable management with biological control as the main tool, it is recommended that the insecticides be used only when necessary (Philips et al. 2011). This will also require effective communication with farmers through extension activities to educate them about the effectiveness of *T. julis* in naturally suppressing *O. melanopus* populations.

The management tactics suggested here will depend on levels of *O. melanopus* infestation at a given location. Current trends of spatial variability observed in my research fit the criteria for site-specific management (SSM) (Plant 2001). If spatial variability is observed in factors that can influence crop yield (such as nutrient availability), and if the underlying mechanisms causing such variability can be discerned, the variability can be addressed using SSM (Miller et al. 1999). The SSM approach is technology-intensive and utilizes inputs from

global positioning systems, yield monitors, and variable rate chemical application systems to develop management tactics at a spatial scale that is below the whole-field scale (Plant 2001). This approach can help to identify field areas where targeted strategies in terms of nutrient management, insecticidal spraying, and weed management need to be applied. It can also help in avoiding unnecessary use of agricultural inputs and precision in their application, thus improving the efficiency of crop production (Plant 2001).

Applications of SSM for *O. melanopus* management have not been reported. However, if the beetle attains pest status, SSM strategies can be used based on understanding developed from field dynamics of the beetle and parasitoid. For example, identifying areas with nutrient deficiency and targeting fertilizer applications to address such deficiencies can be a part of a SSM strategy to maintain homogeneous plant stands with uniform vigour to avoid patchy distributions. The use of SSM tactics has generated interest among producers and is expected to be widely used in future (Alberta Agriculture and Rural Development 2001), and has prospects for strengthening sustainable management of *O. melanopus* with emphasis on augmentation of *T. julis* activity.

Future research

Future research attention needs to be directed to developing predictive models based on temperature requirements of the beetle and the parasitoid for western Canada to forecast peak activity periods. Such models will be particularly useful for cereal farmers in monitoring beetle populations and making

management decisions. Research should also focus on agronomic practices to determine optimum seeding rates and times, optimum fertilizer rates and their effects on plant stand characters. Field research needs to be conducted to quantify *O. melanopus* damage to various crops, and to establish economic threshold levels. It is necessary to validate the threshold levels calculated for North America for the local conditions in western Canada.

The role of bottom-up effects of plant nutrients also needs to be explored further to accurately estimate the effects of specific plant-available nutrients on the distribution dynamics and fitness of *O. melanopus* and *T. julis*. This will facilitate decisions regarding nutrient applications for winter and spring cereal crops to mitigate losses caused by *O. melanopus*. Climate change is another concern that can significantly influence population spread and dispersal of the beetle (Olfert et al. 2004). Previous research has predicted effects of incremental temperatures on the spread and distribution of *O. melanopus* in western Canada (Olfert et al. 2004; Olfert and Weiss 2006). However, effects of incremental temperatures and climate change on *T. julis* range expansion with reference to *O. melanopus* have not been investigated. Predicting dispersal patterns of *T. julis* with reference to incremental temperatures will help to identify areas in this eco-region that are favourable for parasitoid establishment and the areas that can restrict dispersal and establishment. This will help to identify suitable tactics for augmenting the activity of *T. julis*.

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