

**ECOLOGICAL INTERACTIONS BETWEEN *LYGUS* (HEMIPTERA: MIRIDAE)
AND THEIR NYMPHAL PARASITOIDS *PERISTENUS* (HYMENOPTERA:
BRACONIDAE) IN SOUTHERN ALBERTA**

DIANA CATALINA FERNÁNDEZ
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DIANA CATALINA FERNÁNDEZ

Date of Defence: August 9, 2016

Dr. Robert A. Laird Co-Supervisor	Associate Professor	Ph.D.
Dr. Héctor Cárcamo Co-Supervisor	Research Scientist	Ph.D.
Dr. Alice Hontela Thesis Examination Committee Member	Professor	Ph.D.
Dr. Robert Bouchier Thesis Examination Committee Member	Research Scientist	Ph.D.
Dr. Robert Longair External Examiner University of Calgary Calgary, Alberta	Senior Instructor	Ph.D.
Dr. Theresa Burg Chair, Thesis Examination Committee	Associate Professor	Ph.D.

Abstract

Lygus bugs cause significant losses in commercial crops. Parasitoids (*Peristenus*) attack *Lygus* nymphs and have been considered to manage *Lygus* populations. The thesis provides information on the seasonal activity of three *Peristenus* species in southern Alberta, and a possible outcome of the European *P. digoneutis* in terms of *Lygus* control in canola, if present in the Prairies. Three native *Peristenus* species (*P. braunae*, *P. carcamoi* and *P. broadbenti*) occur sequentially during the season in southern Alberta. Overall parasitism did not exceed 16% during the season. In greenhouse trials, *P. digoneutis* parasitized *Lygus* nymphs on alfalfa and canola. Parasitism was on average up to 19% in alfalfa, and from 10-15% in canola. Nymphs' mortality was higher in canola than alfalfa, and it increased with the presence of *P. digoneutis*. The potential of *P. digoneutis* to attack *Lygus* in canola can improve the latter's management in the Prairies.

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List of Abbreviations

°C	degree Celsius
cm	centimeters
Inc	incorporated
L	liters
LRDC	Lethbridge Research and Developmental Centre
mm	millimeters
mL	milliliters
ADD	accumulated degree-days
g	grams
m ³	cubic meters
L-150	Liberty-150

Chapter 1 - General introduction and literature review

Goals of Thesis

This thesis provides baseline information on parasitoids in the genus *Peristenus* that attack *Lygus* spp., and their potential role as biological control agents of this pest in southern Alberta. The goals are: (1) Describe the seasonal occurrence of native *Peristenus* spp. in southern Alberta; (2) Determine the potential of the exotic *Peristenus digoneutis* to attack *Lygus* bugs on canola plants, through choice and no-choice greenhouse trials, and (3) Investigate and develop the methodology to assess the potential negative indirect effects of an exotic species, *P. digoneutis*, on native *Peristenus* species, through larval competition studies.

Literature Review

Parasitoid-host-plant interactions

A parasitoid is an insect that feeds on its host organism's body to survive and reproduce, killing the host in the process (Godfray, 1994). Parasitoids usually lay their eggs on or inside their host and, eventually, the larva stage will hatch and start feeding on the host, causing the host's death. Depending on the oviposition site, parasitoids are classified as *endoparasitoids* or *ectoparasitoids* (Godfray, 1994). The larvae of endoparasitoid individuals develop within the host, while those of ectoparasitoids develop on the host's surface. If, after ovipositing in/on the host the parasitoid larva restrains its host's on movement and development, the parasitoid is an *idiobiont*, whereas if the host continues developing, the parasitoid is a *koinobiont* (Godfray, 1994; Hochberg & Ives, 2000). Parasitoids also can be solitary or gregarious. Based on their reproductive biology,

some parasitoids are classified as *proovigenic*, which means that female parasitoids emerge with a complete set of eggs, and no more eggs are produced during their lifetime. On the other hand, when females continue to produce eggs after emergence, they are considered as *synovigenic* (Flanders, 1950). Insects within the orders Hymenoptera and Diptera have the most species displaying this life-style (Waage & Hassell, 1982).

Parasitoids and their hosts are intimately related, and much of the knowledge of these interactions has been built on the use of parasitoids as biological control agents in managed ecosystems (Waage & Hassell, 1982; Rosen & De Bach, 1991; Godfray, 1994). To survive, a parasitoid must select an appropriate host for its offspring but first: (1) it needs to find the host habitat, and immediately after, (2) locate a suitable host (Salt, 1935; Vinson, 1976). These processes are mediated by the food source of its insect host. The temporal and spatial distribution of host plants at local and landscape scales, plant architecture, plant diversity, chemical, and visual cues are among the factors involved in these processes (Barbosa, 1998; Carrillo et al., 2008).

Successful parasitism requires an efficient foraging strategy (Godfray, 1994). Moreover, the level of parasitism of insect herbivores depends on their host species (Streams et al., 1968; Firake et al., 2012). For example, complex leaf surfaces decrease parasitism relative to simple surfaces (Andow & Prokrym, 1990). Understanding these complex trophic interactions is important to manage insect pests effectively. The manipulation of plant species, either as barriers, reservoirs, or lures for parasitoids, can optimize the suppression of insect pests (Cortesero et al., 2000)

Parasitoid competition

Competition in nature occurs when individuals within the same species (*intraspecific*) or in different species (*interspecific*) interact negatively to obtain resources. In parasitoid communities, free-living adults searching for hosts compete *extrinsically*, while immature stages developing within the host compete *intrinsically* (Godfray, 1994). Intrinsic competition occurs when (1) a parasitoid female lays a number of eggs that exceed the capacity of the host (*superparasitism*) or (2) when a female parasitizes an already-parasitized host (*multiparasitism*).

Frequently, parasitoid species overlap in space and time exploiting the same host stage, and, as a result of overlap, competition may occur (DeBach & Sundby, 1963; Van Nouhuys & Punju, 2010; Teder et al., 2013). Eventually, this interaction may lead to the displacement of the less-competitive species (DeBach & Sundby, 1963). However, cases of coexistence between parasitoid species have been reported (De Moraes et al., 1999; Van Nouhuys & Punju, 2010; Teder et al., 2013). Additionally, the presence of multiple parasitoid species can increase the level of host suppression (Xu et al., 2013).

Introductions of foreign parasitoids can disrupt resident species in the new ecosystem (Bennett, 1993). DeBach and Sundby (1963) reported the displacement of the ectoparasitoid wasp *Aphytis chrysomphali* Mercet by its congener *A. lingnanensis* Compère in southern California. A subsequent introduction of a third species in the system, *A. melinus* DeBach, displaced *A. lingnanensis* from part of its range. In eastern North America, the introduced *Cotesia rubecula* L. has become the dominant biological control agent of the cabbageworm *Pieris rapae* L. This parasitoid has displaced the former dominant species *C. glomerata* L. from northeastern US, and also adjacent Canadian provinces (Van Driesche, 2008; Herlihy et al., 2012). *Aphidius ervi* Haliday

was introduced in North America in 1961 by the United States Department of Agriculture from Europe (Mackauer & Finlayson, 1967) to control the pea aphid *Acyrtosiphon pisum* Harris. Prior to this introduction, the native *Praon pequodorum* Viereck was a common parasitoid of the pea aphid (Halfhill et al., 1972). In a period of 21 years after its release, the abundance of *P. pequodorum* has been decreasing steadily (Danyk, 1992). Schellhorn et al. (2002) reported that the decline of the aphid parasitoid *P. pequodorum* in alfalfa fields is the result of competition between this native species and the exotic *A. ervi*.

Before releasing foreign parasitoids, screening assessments need to be carried out to avoid undesirable outcomes. Usually, attention is given to non-target hosts that can be affected, and not much information is available regarding the effects within the existing parasitoid community. The lack of surveys describing the state of the native parasitoid community before introduction makes it difficult to identify changes caused by the new species. Therefore, it is essential to determine not only an exotic parasitoid's host range but also the native natural enemies associated with the target host to consider potential negative impacts on native parasitoid species.

Study species

The study system used in this work is based on the relationships among parasitoid wasps (*Peristenus* spp.), their hosts (*Lygus* spp.), and the hosts' food sources, canola (*Brassica napus* L.) and alfalfa (*Medicago sativa* L.) plants.

Lygus plant bugs

The genus *Lygus* Hahn (Heteroptera: Miridae) comprises a group of plant-feeding insects associated with cultivated and non-cultivated plant species (Kelton, 1975; Fye, 1982; Young, 1986; Wheeler Jr, 2000). Due to their feeding preferences and sucking mouthparts, they are responsible for significant damage to their host plants and can lead to fruit abscission, necrosis, and deformation of fruits and seeds (Allen & Gaede, 1963; Tingey & Pillimer, 1977). *Lygus* overwinter as adults in tree shelters or field margins under the leaf litter (Cárcamo and Otani, unpublished data). When the temperature increases, overwintered adults emerge from their shelters, mate, and the females start laying eggs in the plant tissue of their host plants (Kelton, 1975). *Lygus* nymphs hatch after 10-14 days, and molt five times until reaching the adult stage in about 30-45 days.

In North America, four species of *Lygus* are recorded as crop pests: *L. lineolaris* Palisot, *L. elisus* Van Duzee, *L. borealis* Kelton, and *L. hesperus* Knight. The first species, the tarnished plant bug, is widespread in the humid regions of Canada and USA (Schwartz & Footitt, 1998). In an extensive survey of *Lygus* spp. throughout the Canadian provinces, Schwartz and Footitt (1992) reported that *L. elisus* and *L. lineolaris* were the dominant species in oilseed and mustard. These species can cause significant losses in canola crops (Butts & Lamb, 1991a). Also, *L. borealis* was common in alfalfa fields (Butts & Lamb, 1991b). In addition, later studies also reported *L. keltoni* affecting canola fields in Alberta (Cárcamo et al., 2002; Otani & Cárcamo, 2011), and even dominating the *Lygus* species assemblage in southern areas of the province (Cárcamo et al., 2002).

Nymphal parasitoids of Lygus bugs

Efforts to identify natural enemies to control *Lygus* populations have focused on nymphal parasitoids. These include solitary endoparasitoid wasps in the genera *Leiophron* Nees and *Peristenus* Foerster (Braconidae: Euphorinae) (Carignan et al., 1995; Goulet & Mason, 2006). After emergence from a cocoon, adult wasps in these genera parasitize early instars of *Lygus* (Loan, 1974a). Depending on their biology, geographical range, and host timing, these species can have one, two, or multiple generations per year (Loan, 1974b; Goulet & Mason, 2006). The number of generations is mediated by diapause requirements and developmental rates (Day, 2005b). Geographical overlap, similarities in morphology, and constraints in obtaining adults have hampered species identification in *Leiophron* and *Peristenus* (Loan, 1974a), and gaps in taxonomic information still remain (Goulet & Mason, 2006). Although larval morphology of different stages of these parasitoids has been described to distinguish species, identification is not reliable (Lim & Stewart, 1976b; Carignan et al., 1995). Taxonomic keys are based on adults' description, and morphological similarities among species are a constraint in identification, especially for sympatric species (Bickford et al., 2007). Geographical range, number of generations, phenology, principal host, and developmental time are some of the characteristics that have been used to distinguish cryptic species (Goulet & Mason, 2006).

Recently, the development of specific molecular primers for some *Peristenus* and *Leiophron* species has reduced time and improved accuracy in identifying cryptic species that share geographical and host ranges (Tilmon et al., 2000; Ashfaq et al., 2004; Mowry & Barbour, 2004; Zhu et al., 2004; Gariepy et al., 2005; Gariepy et al., 2007; Bon et al., 2008; Gariepy et al., 2008a). Moreover, Erlandson et al. (2003) developed molecular

markers to distinguish *Peristenus* species present in Saskatchewan, only *P. pallipes* Loan (syn. *P. mellipes*) was found associated with *Lygus* bugs in this location.

Native parasitoid species in North America

Leiophron. There are nineteen *Leiophron* species in North America, although only four (*L. lygivorus* Loan, *L. uniformis* Gahan, *L. australis* Goulet and *L. simoni* Goulet) parasitize *Lygus* spp. (Goulet & Mason, 2006). *Leiophron lygivorus* and *L. uniformis* are bivoltine. The former occurs along the cold-temperate regions of eastern North America, whereas the latter is found in warm areas from Ontario to Mexico (Goulet & Mason, 2006). Both species attack *L. lineolaris* but *Leiophron uniformis* has a preference for *Halticus bractatus* Say (Goulet & Mason, 2006). *Leiophron australis* and *L. simoni* have only been reared from *L. lineolaris*, and recently described by Goulet and Mason (2006). *Leiophron asutralis* is bivoltine and found along the Atlantic coast of the US, whereas *L. simoni* has been recorded from the provinces of Ontario and Quebec (Goulet & Mason, 2006).

Peristenus. Cresson (1872) was the first to describe the species *P. mellipes* in North America under the genus *Euphorus*. Since then, several authors have contributed to the taxonomy of the Nearctic species. Extensive revision based on morphological characters and other aspects of their basic biology has increased knowledge and the number of species recognized.

Currently, 29 *Peristenus* species occur in North America, although only twelve are associated with *Lygus* (Goulet & Mason, 2006). Out of these, nine species are native to North America and the remaining three (*P. digoneutis* Loan, *P. relictus* Ruthe and *P. rubricollis* Thomson) were introduced from Europe. They differ in geographical range,

voltinism, and main host species. *Peristenus mellipes* Cresson, *P. pseudopallipes* Loan, and *P. dayi* Goulet are univoltine species occurring mostly in eastern North America (Goulet & Mason, 2006). *Peristenus gillespiei* Goulet has also one generation per year and attacks *Lygus* spp. on the lowlands of British Columbia to California. *Peristenus howardi* Shaw is a bivoltine species reared from *Lygus hesperus* in the western United States (Day et al., 1999; Mowry & Barbour, 2004; Seymour et al., 2005; Goulet & Mason, 2006). The remaining species, *P. otaniae*, *P. braunae*, *P. carcamoi*, and *P. broadbenti* were recently described in Goulet and Mason (2006). They are all univoltine parasitoids usually found in the Canadian Prairies.

The time of appearance in the field is critical for the survival of an organism. For parasitoids, the time required for emergence determines the possibility to find a suitable host for their offspring. Phenological events between parasitoid and host are expected to be synchronized by adaptation, allowing a stable interaction (Godfray et al., 1994). Conversely, hosts may avoid synchronization, reducing encounters with the parasitoids (Hochberg & Ives, 2000). In a study carried out by Day (1999), three *Peristenus* species emerged from different mirid hosts. Results were based on percentage of individuals per species that emerged from a determined host. Each parasitoid species corresponded to their principal or preferred host. In another study, Day (2005b) found differences in the number of days required for adult emergence of six *Peristenus* spp. from field-collected mirids. Averages ranged between 8 to 60 days after cocoons were moved out of cold storage. In a field study in Southern Ontario, the native *P. pallipes* and *P. pseudopallipes* species had distinct abundance peaks during the season. The first species appears early in the summer, and the second about 15 days later (Broadbent et al., 2006).

Studies on *Peristenus* phenology, species composition, and parasitism levels on *Lygus* spp. have been carried out in North America on multiple species. Lim and Stewart (1976b) described the seasonal occurrence of *P. pallipes* and *P. pseudopallipes* in alfalfa crops and weeds in Quebec. In a subsequent study, Carignan et al. (2007) found that the percentage of parasitism attributed to these species in cultivated and wild plants ranged between 0-27% before the establishment of a European parasitoid in the area. In southern Ontario, also prior to the invasion by *P. digoneutis*, Broadbent et al. (2006) reported overall mean parasitism levels below 11% in alfalfa, and in weedy sites, for three different regions. In western Canada, field surveys in Saskatchewan reported peak values on a particular date and site of up to 70% parasitism of *Lygus* spp. in alfalfa fields, although overall seasonal parasitism did not exceed 28%. Parasitism in canola fields was less than 1% (Braun et al., 2001). In the pacific coast of the United States, single values of parasitism during the peak of occurrence of *Lygus* nymphs were 45% and 80% in the first and second generation, respectively, by the native *P. howardi*. This native species has two generations per year and it is principally thelytokous (i.e., unfertilized eggs produce female offspring), which may explain its success (Day et al., 1999; Seymour et al., 2005). Despite single parasitism values/date greater than 50% reported in previous studies (Braun et al., 2001; Broadbent et al., 2006), overall parasitism attributed to native *Peristenus* in Canada is not sufficient to keep *Lygus* numbers below the economic threshold level in some crops (Craig & Loan, 1969; Loan & Craig, 1976). As a result there has been significant interest in supplementing parasitism using European congeners in biological control programs.

Biological control of Lygus bugs in North America

Biological control is the use of natural enemies to maintain the population of an organism under pest levels (Waage et al., 1988; Howarth, 1991). There are different types of biological control, depending on the technique, the origin of the target organism, and the natural enemy used. One of the most common is the *classical* approach, which consists of introducing an exotic agent to control an exotic pest. However, an unusual form of biological control has increased interest among practitioners. *Neo-classical* biological control considers new associations with the target pest and the natural enemy (Lockwood, 1993). This technique proposes the introduction of an exotic agent to control a native pest.

The *Lygus* species considered crop pests in Canada are native to North America (Schwartz & Foothit, 1998), and these species serve as hosts for the native *Peristenus* and *Leiophron* wasps (Cresson, 1872; Clancy & Pierce, 1966; Lachance et al., 2001; Goulet & Mason, 2006; Carignan et al., 2007). However, attempts to find biological control agents to effectively reduce Miridae populations in North America have resulted in the identification of promising European parasitoid species. In Europe, the most common *Lygus* species considered as pests are *L. rugulipennis* Poppius and *L. pratensis* L. (Coutinot & Hoelmer, 1999). Although *L. rugulipennis* is associated with more than 450 host plant species (Holopainen & Varis, 1991), usually is not considered as a serious pest. These *Lygus* species are hosts of the European parasitoids *P. digoneutis*, *P. stygicus*, and *P. rubricollis* (Loan, 1974a).

Initial field surveys carried out in Pakistan, Iran, India, Indonesia, Turkey, and parts of Africa and Europe (Kuhlmann et al., 1999) led to the release of *Peristenus digoneutis* in New Jersey to diminish *L. lineolaris* populations in alfalfa fields. Within 10

years of its initial release, *P. digoneutis* had successfully established (Day et al., 1990) and spread northward (Day, 1996; Day et al., 1998; Day et al., 2000; Day et al., 2004), including to the eastern provinces of Canada (Broadbent et al., 1999; Day et al., 2008).

European Peristenus in North America

In addition to *P. digoneutis*, two other European *Peristenus* parasitoids released in North America are *P. relictus* (syn. *P. stygicus*) and *P. rubricollis*. *Peristenus relictus* was first released in California along with *P. digoneutis* to control the western tarnished plant bug *L. hesperus* in 1998 (Pickett et al., 2007; Pickett et al., 2009). *Peristenus relictus* is multivoltine and has a broader host range than *P. digoneutis* (Haye et al., 2006; Mason et al., 2011). Subsequent surveys confirmed the establishment and dispersal of only *P. relictus* in central California (Pickett et al., 2009; Pickett et al., 2013; Swezey et al., 2014). On the other hand, the univoltine *P. rubricollis* was introduced in North America to attack the exotic alfalfa plant bug *Adelphocoris lineolatus* Goeze (Day et al., 1992), although it has been also reared from *L. lineolaris* nymphs (Goulet & Mason, 2006). The parasitoid is found from Delaware to southern Quebec (Day et al., 1992; Day et al., 1998; Broadbent et al., 1999), and parasitism of the first generation of alfalfa plant bug has increased since its introduction (Day et al., 2005).

Despite variability in parasitism levels at various habitats, *P. digoneutis* has proved to have a significant impact on *Lygus* numbers in alfalfa (Day et al., 1990; Day, 1996), strawberries (Tilmon & Hoffmann, 2003; Day et al., 2004; Day & Hoelmer, 2012), apples (Crampton, 2007), and weeds (Day et al., 2004) in the northeast USA.

The introduction of the European *P. digoneutis* and *P. relictus* in North America is an example of new associations between parasitoids and their target hosts. The use of

Peristenus digoneutis to control Nearctic *Lygus* species is an example of the *neo-classical* biological control approach that has been effective on the target host, with no reported undesirable effects on non-target hosts. However, assessments should consider the native parasitoid community that may be affected by the presence of the new agent.

Organization of thesis

Chapter 2

Based on field and laboratory data collected over several years, this chapter describes the occurrence during the growing season of three native parasitoid species associated with *Lygus* bugs in Southern Alberta. *Peristenus braunae*, *P. carcamoi* and *P. broadbenti* were sampled from crop and non-crop habitats. Parasitism levels of *Lygus* spp. linked to these species were determined by rearing and dissecting *Lygus* nymphs. Overall seasonal parasitism did not exceed 16%. To predict time of appearance in the field, accumulated degree-days were calculated for the three species using field-collected adults. The three species required different degree-days throughout the season. In addition, the number of days for adult emergence after parasitoid attack was different for *P. carcamoi* and *P. broadbenti* under laboratory conditions.

Chapter 3

This chapter uses two known host plant species of *Lygus lineolaris*, to examine two main questions: (1) Does the European *Peristenus digoneutis* have a preference for a particular host plant to parasitize *L. lineolaris* nymphs, and (2) does the presence of the female parasitoid have an effect on the survival of *L. lineolaris* nymphs. The experiments were set up in choice and no-choice designs under greenhouse conditions. The results did

not show significant differences in the levels of parasitism of *L. lineolaris* between alfalfa and canola. However, more live nymphs were recaptured from alfalfa plants than canola, particularly when the parasitoid was present in the cage.

Chapter 4

This chapter is a general conclusion and synthesis of the information from Chapters 2 and 3 with the introductory literature. It highlights the achievement of the initial research goals and provides suggestions for future research directions that can supplement the known ecological interactions between *Lygus* spp. and *Peristenus* spp.

Chapter 2 - Seasonality and species composition of *Peristenus* Förster (Braconidae: Euphorinae) species in Southern Alberta, Canada.

Abstract

This chapter describes the seasonal variation in species composition of *Peristenus* species, and levels of parasitism of *Lygus* bug nymphs in southern Alberta. Plants sampled include alfalfa (*Medicago sativa* L.), canola (*Brassica napus* L.), and stands of different weed species, and grasses commonly found on crop field edges. Parasitism varied by year, site of collection, and host-plant species. Three native *Peristenus* species (*P. braunae*, *P. carcamoi* and *P. broadbenti*) were recorded over the years. *Peristenus carcamoi* and *P. broadbenti* differed in developmental time under laboratory conditions. Degree-days for field-collected individuals suggested a sequential occurrence during the growing season. *Peristenus braunae* appears early in the season, followed by *P. carcamoi*, and later on *P. broadbenti*. Studies of species composition and phenology of native parasitoids of insect pests provide valuable baseline information when considering introduction of exotic agents. It also contributes to the management and improvement of natural enemies in agricultural systems.

Introduction

Lygus bugs (Miridae) are plant-feeding insects with a broad host range (Wheeler, 2001), and some species are considered significant crop pests in temperate regions (Butts & Lamb, 1990b; Day et al., 1990; Day, 1996; Broadbent et al., 2001).

In the Prairies Ecozone of western Canada, the most common *Lygus* species in agro-ecosystems are *L. keltoni*, *L. borealis*, *L. elisus*, and *L. lineolaris*. The last of these is

the dominant *Lygus* species in the humid eco-regions of the Prairies (Cárcamo et al., 2002) and can cause severe damage to field crops such as canola (Butts & Lamb, 1990b; Butts & Lamb, 1991a; Turnock et al., 1995) and seed alfalfa (Butts & Lamb, 1991b). Control strategies rely heavily on insecticides, and economic thresholds are available (Wise & Lamb, 1998; Otani & Cárcamo, 2011). In some jurisdictions, overreliance on insecticides has led to the evolution of resistance (Snodgrass, 1996). Also, concerns about non-target effects of insecticides on beneficial insects (i.e., pollinators, natural enemies) and human safety make biological control an important alternative management strategy (Broadbent et al., 1999).

Nymphal parasitoid wasps in the genus *Peristenus* (Braconidae) attack early instars of *Lygus* spp. They lay an egg inside the host and the larva kills the *Lygus* nymph when it emerges to pupate. In North America, the impact of native parasitoids on *Lygus* populations in agro-ecosystems is too low for crop protection (~ 0-20% parasitism) (Braun et al., 2001; Matos & Obrycki, 2004; Day, 2005a; Rämert et al., 2005; Broadbent et al., 2006; Carignan et al., 2007). Nonetheless, up to 70% parasitism has been reported for *Lygus* spp. nymphs collected in alfalfa in Saskatchewan, Canada (Braun et al., 2001). However, this was the peak and the overall seasonal parasitism was under 10%. A similar situation occurred in a weedy alfalfa field in Ontario, where maximum parasitism was 50% (Broadbent et al., 2006). In contrast, the native *Peristenus howardi* (Day et al., 1999), present in the western US, is responsible for parasitizing between 44% and 80% of *Lygus* spp. nymphs in alfalfa seed in Idaho (Day et al., 1999; Seymour et al., 2005). This native parasitoid has 2-3 generations per year and produces mostly female offspring, which might explain its high level of parasitism on *Lygus* (Day et al., 1999).

Efforts to improve biological control of *Lygus* bugs in North America have led to the release of exotic parasitoids. The European *Peristenus digoneutis* Loan is a bivoltine parasitoid that was released in the northeastern US in the 1980s as a biological control agent of *Lygus lineolaris* in alfalfa fields, and has since spread successfully throughout the eastern United States (Day et al., 1990; Day et al., 2000; Day et al., 2008), and into southeastern Canada (Broadbent et al., 1999; Day et al., 2008). Previous attempts to introduce *P. digoneutis* in the western US (Pickett et al., 2009) and Canada have failed, possibly because of a low number of adults released, poor local adaptation (Broadbent et al., 2001), or environmental factors (Day et al., 2008). However, recent bioclimatic models suggest that *P. digoneutis* may establish in the Prairies of Canada in regions where *Lygus* bugs have two generations (Haye et al., 2013). Such establishment may affect western *Lygus* populations. Day (1996) reported that *P. digoneutis* reduced *L. lineolaris* numbers in alfalfa by up to 75% in the northeastern US where this parasitoid is well established.

Weather factors such as temperature influence insect emergence and developmental time. The use of degree-days allows prediction of the occurrence of pests and their natural enemies (Decker & Yeorgan, 2008; Chavalle et al., 2015), and helps determine phenological mismatch in the field (Evans et al., 2013). Synchrony between parasitoids and their hosts influences parasitoid survival and the effective control of the pest (Godfray et al., 1994). In southwestern Quebec, Carignan et al. (2007) reported that the native *P. pallipes* was active in the field at 57-289 degree days and *P. pseudopallipes* at 365-668 degree days. Their phenologies were well synchronized with the first and second generations, respectively, of their *Lygus* spp. hosts. Broadbent et al. (2006) found

similar results for these species in southern Ontario. Comparable studies are not yet available for the Prairies of Canada.

The objective of this study was to obtain baseline information on local *Peristenus* parasitoids associated with *Lygus* bug nymphs in Southern Alberta. I describe parasitoid species composition, parasitism levels of *Lygus* spp. in crop and non-crop systems, and the parasitoid phenology during the growing season in terms of the accumulated degree days required for appearance in the field and total developmental time under laboratory conditions. Part of the rationale for collecting this information was to assess the need to enhance biological control of *Lygus* bugs with an exotic species such as *Peristenus digoneutis*, and its potential risk to native parasitoid species.

Materials and Methods

The study was conducted over 12 years, although initially during some years the main objective was to provide specimens for a taxonomic revision (Goulet & Mason, 2006). Data include sampling by AAFC's researchers from previous years starting from 2003 and more recent field collected data from my sampling efforts in 2014 and 2015. Sampling intensity varied among years depending on resources available. Therefore, the years presented in each section were chosen because the data were the most complete and comparable to address the objectives stated above. Considering the variation in sampling methods and lack of data for some years, statistical analyses were not possible in all sections.

Study area

The sampling sites were located around Lethbridge (49°41'59" N, 112°49'06" W) and Vauxhall (50°05'74" N, 112°12'53" W) in southern Alberta within the Prairies Ecozone of Western Canada. Lethbridge is within the Moist Mixed Grassland Eco-region with dark brown chernozemic soil, mean annual temperatures around 5°C, and mean precipitation ranging from 350 to 400 mm (Marshall et al., 1996). The Vauxhall area is located within the semiarid Mixed Grassland Eco-region with a mean annual precipitation of 250-350 mm and a mean annual temperature of 3.5°C, but in some places it can exceed 5°C. The soils within this eco-region are mainly brown chernozemic with some solonchic zones (Marshall et al., 1996).

Study sites included cultivated systems (2006, 2007, 2015) at Lethbridge and Vauxhall, and a non-crop site (2015) in Peenaquim Park, Lethbridge.

Lygus and Peristenus rearing

Lygus. Nymphs used in laboratory trials were from a continuous laboratory colony of *Lygus lineolaris* maintained with organic romaine lettuce, water, and sprouted potatoes at the Lethbridge Research and Development Centre (LRDC). The colony was initiated in 2009 from field-collected adults from southern Ontario at various sites, and supplemented periodically with adults from northern or central Alberta collected in canola fields.

Peristenus. Following the protocol used by Whistlecraft et al. (2010) parasitized *Lygus* spp. nymphs were reared in cylindrical 4L polyethylene buckets (Plastipak Industries Inc.) with a screen top and a hard mesh (1-2 cm) glued half way to hold lettuce to feed them. The bottom was lined with vermiculite to provide a pupation substrate. About 50 to 100 nymphs were grouped per container and kept at $22 \pm 1^\circ\text{C}$, with an 18:6

light:dark regime. Once all nymphs were dead or developed to adults, the vermiculite with parasitoid pupae was transferred to petri dishes. Petri dishes were kept at room temperature until September of each year to allow parasitoid larvae to mature. Next, parasitoid cocoons were counted and cold-acclimated at 10°C and 5°C for 15 days at each temperature before overwintering them at $2 \pm 1^\circ\text{C}$ for 5-6 months.

The parasitoids used in the developmental time trials were obtained from *Lygus* spp. nymphs collected in the field the previous year and reared in the laboratory during the following field season.

Seasonal occurrence and species composition of Peristenus

In 2003-2007, 2014, and 2015, *Peristenus* wasps were collected directly from the field using sweep nets, yellow sticky cards (7.62 x 12.7 cm) or sometimes both. Where possible, sampling was carried out in the same site every season. However, sites were usually different (Table 1). When yellow sticky cards were used (2005-2007, 2015), cards were randomly set up in pairs with the sticky surface pointed in opposite directions within the field site. Twenty cards per field were used in 2015, and eight cards per field in the previous years. In the case of sweeping, sampling effort consisted of 200 sweeps per field. In total three fields were sampled. For 2005 and 2006, mass collections of over 10,000 sweeps were done by two collectors during 30 to 75 minutes per field. Net contents were emptied in a metallic dark box with a set of screens to separate insects by size. Small flying insects were collected in a bottle half filled with 70% ethanol attached to the exit of the dark box. The light at the exit attracts many flying insects, which accumulate faster than those that crawl. This apparatus was effective to obtain a sample of dead adult *Peristenus*. Parasitoids were also obtained through rearing of mass

collections of *Lygus* spp. and *Adelphocoris* spp. nymphs. *Peristenus* species, date of collection/emergence, and number of individuals were recorded.

Table 1. Methods and sampling effort used at various sites to collect *Peristenus* spp. adults in alfalfa, canola, weeds, and grasses during 2003-2007 and 2014-2015.

Year	Habitat	Site	Date	Method	Intensity- Effort
2003	Alfalfa	Wilmount Pavan Nichol Keffel	May 21 - Sep 25	Sweeping	100 sweeps/site
	Weeds	43St S LRC Fairfields Stirling	May 11 - Jul 23	Sweeping	100 sweeps/site
2004	Alfalfa	Stirling, Kaupp	May 15 - Sep 24	Sweeping	100 sweeps/site
		Victory church Wilmount IMP	Jun 1 - Jul 28	Dark box	2 collectors/30-75 minutes
	Weeds	Fairfields Stirling	May 7 - Jun 28	Sweeping	100 sweeps/site
		Peenaquim Park Pavan Park	Jun 1 - Jul 28	Dark box	2 collectors/30-75 minutes
2005	Alfalfa	Vauxhall Stirling, Kaupp Wilmount	May 20 - Oct 25	Sweeping	200 sweeps/site
	Weeds	Fairfields Kooy	May 5 - June 15	Sweeping	200 sweeps/site
		Peenaquim Park	May 25 - Aug 5	Dark box	2 collectors/30-75 minutes
	Canola	Vauxhall	Jun 28 - Aug 7	Sweeping	200 sweeps/site
	Grass	Jail site	May 25 - Aug 5	Dark box	2 collectors/30-75 minutes
May 4 - May 16			Sticky cards	8 cards/site	
2006	Alfalfa	IPM	May 4 - Aug 14	Sweeping Sticky cards	200 sweeps/site 8 cards/site
		Vauxhall	May 19 - Jul 26	Sticky cards	8 cards/site
			May 11 - Sep 28	Sweeping	200 sweeps/site
		Dairy Barn	Jun 2 - Aug 10	Sticky cards	8 cards/site
		Stirling, Kaupp	May 9 - Aug 3 May 25 - Jun 26		
	Weeds	Peenaquim Park	May 18 - Aug 9	Dark box	2 collectors/30-75 minutes
	Canola	IPM	Jun 30 - Aug 14	Sticky cards	8 cards/site
			Jun 26 - Jul 13	Sweeping	200 sweeps/site
		Vauxhall	May 19 - Jul 26 Jun 26 - Jul 13	Sticky cards Sweeping	8 cards/site 200 sweeps/site
	2007	Alfalfa	IPM	May 10 - Sep 26	Sweeping
Vauxhall			May 10 - Aug 29	Sticky cards	8 cards/site
Weeds		Peenaquim Park	May 7 - May 17	Sweeping	200 sweeps/site
Canola		IPM	Jun 25 - Aug 29	Sweeping	200 sweeps/site
	Vauxhall	Sticky cards		8 cards/site	
2014	Alfalfa	LRC	May 29 - Aug 27	Sweeping	200 sweeps/site
	Weeds	Peenaquim Park			
	Canola	Fairfields	Jun 23		
2015	Alfalfa	LRC	May 5 - Sep 18	Sweeping Sticky cards	200 sweeps/site 20 cards/site
		Vauxhall			
	Weeds	Peenaquim Park			

Parasitism of Lygus species nymphs

Parasitism was assessed and described only in the years 2006, 2007, and 2015 when field collection of nymphs was carried out on a weekly basis for comparisons. Seasonal sweep samples were taken from several habitats in 2006, 2007, and 2015 in southern Alberta. Field collections started as early as May 5 and ended as late as September 20 during the frost-free season. Sampling frequency varied depending on resources or specific objectives for the year. Sites included fields of canola (*Brassica napus* L.) and alfalfa (*Medicago sativa* L.), a weedy site with common weed species such as wild mustard (*Sinapis arvensis* L.), tumbling mustard (*Sisymbrium altissimum* L.), flixweed (*Descurainia sophia* L.), stinkweed (*Thlapsi arvense* L.), hoarycress (*Lepidium draba* L.), curly dock (*Rumex crispus* L.), ball mustard (*Neslia paniculata* L. Desv.), and a mixture of grasses.

For 2006, 2007, and 2015, *Lygus* spp. adults and nymphs were counted, and percentage of parasitism of *Lygus* nymphs was summarized graphically. In 2006 and 2007, canola and alfalfa were planted adjacent to each other. In 2015, sampling was carried out in alfalfa and weedy sites only. Sampling started early in the season for alfalfa and weedy sites (May 1-11). For canola fields, sampling started at the bud stage (June 26), and continued until senescence in August. Four samples of 50 sweeps each (180°) were taken using a standard 38 cm sweep net. Samples were collected in plastic bags, transported in a cooler, frozen, and processed later in the laboratory. *Lygus* bug species, stage, and number of individuals were recorded per sample.

To assess parasitism level, in 2006 and 2007 a subsample of 50 to 100 *Lygus* spp. nymphs were collected depending on field abundance. Nymphs, mostly between 3rd and 5th instars, were frozen at -20°C until dissection in the laboratory. In addition, mass

collections of ~100 to 200 *Lygus* nymphs were reared to identify parasitoid species and record parasitism levels. For 2015, percentage of parasitism was calculated using rearing and dissecting methods. When nymph abundances were low, additional sweeps were taken to increase the samples for rearing.

Developmental time of two Peristenus species

To determine development times, two species of *Lygus* nymphs (2nd or 3rd instars of *L. lineolaris* and *L. keltoni*) were exposed to *P. carcamoi* or *P. broadbenti* mated females in the laboratory. Ten *L. lineolaris* or *L. keltoni* nymphs were placed in a 35 mL snap-cap vial. In a single replicate, only 8 *L. keltoni* nymphs were used. Once nymphs were in the vial, a female of one of the *Peristenus* species was added to the vial for 24 hours, and then moved to a similar vial with 10 nymphs of the other *Lygus* species. Thirteen *P. carcamoi* and nine *P. broadbenti* females were used in the trials. In total, 790 *L. lineolaris* and 728 *L. keltoni* nymphs were exposed to the females. The sequence in which *Lygus* species were exposed to each parasitoid was determined randomly.

After exposure, the nymphs were reared as described in the *Lygus and Peristenus rearing* section to obtain parasitoid adults. The resulting parasitoid cocoons were cold-acclimated and then kept in $2 \pm 1^\circ\text{C}$ for six months until they were moved to room temperature ($20 \pm 1^\circ\text{C}$) the following year for adult emergence. All cocoons were moved out of the cold room on the same date. The number of emerged parasitoids, species, sex, *Lygus* host species, date of exposure, and date of emergence were recorded to calculate total developmental time. The offspring obtained in the experiment and the parental females were preserved in 70% ethanol and identified to species. Voucher specimens were kept in the Insect Pest Management Laboratory of the Lethbridge Research and

Development Centre and also at the Canadian National Collection of Insects, Arachnids, and Nematodes in Ottawa. Two-way ANOVA was used to compare mean developmental time with parasitoid species and sex (female and male) as main factors. *Lygus* host species was not included in this model because it had no influence on these variables. This was in agreement with past *Lygus* species host-range studies with another *Peristenus* species (Haye et al., 2005).

Degree-days of native Peristenus species

To predict flight periods of the three native *Peristenus* species (*P. braunae*, *P. carcamoi* and *P. broadbenti*) in the field, accumulated degree-day units (ADD) were determined for adult catches. In the case of *P. braunae*, the mean number of ADD was calculated for all 160 adults collected between May 1 and June 30, 2005. The same method was applied for the other two species. We calculated the units using the standard formula (Baskerville & Emin, 1969): $DD = [(max\ temperature - min\ temperature)/2] - base\ temperature$. We used the base temperature of 10.6° C for *Lygus lineolaris* (Carignan et al., 2007). Maximum and minimum daily temperatures were from the Government of Canada weather website, Lethbridge CDA station (49°41'42.000" N, 112°46'03.000" W). The annual mean of degree-days was calculated only for years (2005 and 2015) in which more than 10 *Peristenus* adults were collected from each species. Degree-days started to accumulate on January 1 of each year. Number of individuals collected, species and date of collection were recorded.

Results

Species composition of Peristenus

From 2003-2007 and 2014-2015, 1427 *Peristenus* adults were collected in the field and 719 were reared (2003-2014) from *Lygus* and *Adelphocoris* nymphs. Of the total 2146 adults, 67% were *P. carcamoi*, 17% were *P. broadbenti* and 16% were *P. braunae*. Forty-eight percent of the parasitoids were from alfalfa fields and 42% from weedy habitats. Less than 1% of the *Peristenus* wasps were collected from canola fields. The number of *Peristenus* adults varied depending on year, collection site, and method (sampling or rearing) (Table 2).

Generally, *Peristenus* adults appeared early in the season in May. However, in 2003 the first parasitoids were collected in late June, even though sampling started in early May. *Peristenus braunae* was the first species collected, usually at the beginning of the growing season with a peak of activity in late-May to early-June (Figure 1a). Only 8% of *P. braunae* adults were obtained in the laboratory and these came mostly from *Adelphocoris* nymphs (70%), which occur earlier than *Lygus* in the field.

Peristenus carcamoi was collected in the field from late May until late July with the largest number of individuals recorded at the end of May (Figure 1b). However, in 2005 it was active in early May and adults reared in the laboratory that year emerged from nymphs collected in mid to late June 2004. Based on laboratory emergence and field sampling, this species was the most abundant, making up more than 50% of the sampled individuals of the three species.

The activity of *P. broadbenti* started in late June and continued until late August with a maximum number of individuals collected in late August (Figure 1c). Most *P.*

broadbenti adults (76%) were obtained from laboratory rearing; the hosts were sampled after mid-June to late August, mostly from second-generation *Lygus* nymphs.

In 2005, on average, 20 individuals per collector were sampled when mass sweepings were taken from one site during 30 min and extracted using the dark box. In 2015, the maximum number of individuals collected in a single week per field was from yellow sticky cards during May 25 to June 2 at the weedy site in Peenaquim [22 ± 4.8 (SE)/card]. On May 29, 4 ± 1.1 (SE) individuals/50 sweeps were sampled using conventional sweeping at the alfalfa site in Lethbridge.

Parasitism

For 2006 and 2007, nymph numbers were similar in alfalfa and canola planted adjacent to one another. However, adult numbers varied depending on the crop. The peak number of *Lygus* adults was about six times higher in canola than alfalfa crop. A main peak of *Lygus* spp. nymphs occurred in alfalfa in mid-late June. A smaller peak of nymphs occurred later in the season in September (Figure 2a).

Conversely, a single peak of nymphs (2nd-4th instars) occurred in canola fields in mid to late July. Parasitized nymphs were collected from early June until early August in alfalfa and from mid-July in canola but at extremely low rates. Parasitism levels estimated by dissection reached up to 55% in alfalfa, whereas in canola it ranged from 0 to 3% (Figure 2b).

In 2015, the alfalfa site from Lethbridge had a long and abundant peak of *Lygus* nymphs starting from mid-May until early August, but they declined dramatically after this protracted peak (Figure 3a). At the Vauxhall site, *Lygus* spp. nymphs first appeared in mid-May and a peak occurred at the end of July (Figure 3b). Parasitized nymphs were

first collected at the end of May. Percentage of parasitism reached maxima of 25% and 30% for Lethbridge and Vauxhall sites, respectively. At the weedy site in Peenaquim Park, a single peak of *Lygus* nymphs occurred place early in the season (Figure 3c). Parasitized nymphs were also first collected at the end of May and the maximum observed parasitism value was 42%. Overall seasonal parasitism rate was 7.8% for the alfalfa site at Lethbridge, 15.6% for the alfalfa site at Vauxhall, and 15.5% for the Peenaquim uncultivated site.

Developmental time of two Peristenus species

Developmental time was calculated by counting the number of days since the parasitoids attacked the nymphs until parasitoid adults emerged from cocoons the following year.

In total, 118 *Peristenus* adults emerged at two periods of time, each corresponding to a different species: Fifty-four males and nine females of *P. carcamoi* emerged between June 2 to 13, and twenty-six males and twenty-nine females of *P. broadbenti* from July 7 to August 1. Fifty percent of individuals emerged by June 6 and July 17 for *P. carcamoi* and *P. broadbenti*, respectively.

There was a significant main effect of parasitoid species in developmental time ($F_{1, 114}=180.1, p= 2 \times 10^{-16}$, Two-way ANOVA) (Figure 4). The developmental time required for *P. broadbenti* (mean \pm SE: 363 days \pm 0.9) was longer than for *P. carcamoi* (350 days \pm 0.5). There was no significant main effect of sex (males- females) on developmental time ($F_{1, 114}= 0.13, p= 0.72$, Two-way ANOVA). Only in *P. carcamoi*, males emerged two days earlier (349 days \pm 0.5) than females (351 days \pm 1.7). For *P. broadbenti*, males emerged at 363 days \pm 1.4, and females at 363 days \pm 1.1 (Figure 4).

There was no interaction between parasitoid species and sex ($F_{1, 114}=0.79$, $p= 0.37$, Two-way ANOVA).

Degree-days of native Peristenus species

Accumulated degree-day values were based on field catches of at least 10 *Peristenus* adults per species in 2005 and 2015. *Peristenus braunae* was collected in the field when average accumulated degree-days were (mean \pm SE) 15 ± 1.5 (2005), and 23.9 ± 2.0 (2015). For *Peristenus carcamoi*, individuals were collected at 56.6 ± 2.7 (2005) and 38.3 ± 1.1 (2015). In the case of *P. broadbenti*, adults were found at 232.5 ± 16.9 and 269.7 ± 11.9 accumulated degree-days units for 2005 and 2015, respectively. The total grand average of accumulated degree-days was calculated for the three species including all years in which they were collected (2003-2007, 2014, 2015). The mean accumulated degree-days for *P. braunae*, *P. carcamoi* and *P. broadbenti* were 28.6 ± 2.2 , 59.4 ± 1.4 , and 249 ± 10 , respectively (Table 3).

Discussion

The long-term survey confirmed and provided details of the seasonal patterns of three dominant *Peristenus* species that attack *Lygus* bugs in the southern Prairies of Canada: *P. braunae*, *P. carcamoi*, and *P. broadbenti*. The three species can be distinguished in local collections based on morphological characters described by Goulet and Mason (2006). *Peristenus braunae* can be considered a “spring species” as it was recorded at the beginning of the growing season (Figure 1a). *Peristenus carcamoi*, an “early summer” species exhibited a broader range of seasonal activity and overlapped to

some extent with the other two species (Figure 1b). *Peristenus broadbenti* was caught later and can be considered a “late summer” species (Figure 1c).

The time of activity during the season suggests that these three species are univoltine, each with a distinct seasonal peak, but with some phenological overlap. In addition, this work demonstrated that the two summer species (*P. carcamoi* and *P. broadbenti*) differ in developmental times under laboratory conditions (Figure 4), and the three species have different degree-days requirements to appear in the field (Table 3).

Goulet and Mason (2006) documented that *P. braunae* is reared mainly from *Adelphocoris* hosts, suggesting that the early presence of this species in our survey matches with the presence of alfalfa plant bugs that overwinter as eggs and have small nymphs available for parasitoids early in the season. The period of activity for *P. braunae* and *P. broadbenti* in the field matches the description in the taxonomic review from Goulet and Mason (2006). However, *P. carcamoi*'s activity was longer in our study: starting from mid-May until late July instead of the end of May to late June documented by Goulet and Mason (2006). The differences are likely related to temperature at specific sites and years, and may disappear if converted to degree days. The specimens used in the taxonomic review were from both laboratory-reared and field-collected material. The field collections were done in June 2003 and based on limited-time sampling.

Although rearing and dissection have been the most common methods for assessing parasitism levels in *Lygus* spp. populations, both might underestimate actual values. Nymphs may die during the rearing process before parasitoid larvae are fully developed, and parasitoid eggs can be undetectable when early nymphal stages are dissected (Day, 2007). Dissection-based estimates of parasitism of *L. lineolaris* nymphs can be 44% higher than when nymphs are reared (Day, 1994). However, Garipey et al.

(2005) reported similar parasitism levels of field-collected *Lygus rugulipennis* nymphs when determined through rearing, dissection, and a multiplex PCR approach. From these results and ours, it appears that dissection is an adequate method to obtain a reasonably accurate estimate of *Lygus* nymph parasitism.

In this study, parasitism levels in alfalfa fields for 2006 and 2007 reached 60% by dissection, a lower value compared to those of Braun et al. (2001), who noted that parasitism peaked at 70% in alfalfa fields in Saskatchewan. At the urban park habitat (Peenaquim), a similar level of parasitism near 70% was noted on 22 June 2005 (Cárcamo, Herle and Goulet, unpublished data). In 2006 and 2007, parasitism in canola fields had similar low values with those reported by Braun and colleagues. For 2015, the percentage of parasitism combining dissected and reared nymphs had a maximum peak of 33% for alfalfa sites, and up to 43% in the weedy site at Peenaquim Park. Despite moderate peaks of parasitism in alfalfa and uncultivated sites, the peaks of parasitized *Lygus* in each generation remain low in canola (<3%).

Differences in developmental time for *P. carcamoi* and *P. broadbenti* in this study confirmed field data showing that parasitoid species emerge at different times during the season. This difference may be associated with the appearance of the appropriate generation and host stage in the field. It might also contribute to reduced extrinsic competition when searching for hosts. Similar activity for two indigenous *Peristenus* spp. was reported in southern Ontario and southwestern Quebec (Broadbent et al., 2006; Carignan et al., 2007). Likewise, differences among the time of emergence of seven *Peristenus* species may be related to the presence of their main host species in the field (Day, 1999; 2005b). Additionally, the degree-day requirement for the three species also showed a sequence of occurrence in the field.

Unlike Canadian native *Peristenus*, *P. digoneutis* is a bivoltine parasitoid. This feature allows it to produce a non-diapausing first generation that attacks the second generation of *Lygus* nymphs. A few individuals of the second generation emerge to attack a small third generation of *Lygus* bugs, but the majority undergo diapause until the following spring (Day, 2005b). According to Haye et al. (2013), the westward expansion of *P. digoneutis* in Canada might depend more on the presence of two host generations than cold stress. Although ecoclimatic values define the Prairies Ecozone as marginal or unfavorable for the establishment of *P. digoneutis*, the presence of two generations of *Lygus* bugs in Southern Alberta may allow the parasitoid to establish in the area (Haye et al., 2013).

The partial temporal overlap of *P. digoneutis* with native species may pose a moderate threat for native parasitoids. In a sequential larval host-competition study, Lachance et al. (2001) exposed *L. lineolaris* nymphs to an exotic and a native parasitoid. They found that the European wasps *P. digoneutis* and *P. relictus* (syn. *P. stygicus*) successfully produced offspring regardless of the order of attack with the native species (*Leiophron lygivorus*, *P. pallipes* and *P. pseudopallipes*). *Peristenus digoneutis* was only tested against *Leiophron lygivorus*, resulting in 45% and 41% of emergence of *L. lygivorus* and *P. digoneutis*, respectively, when the *L. lygivorus* attacked first. In contrast, 84% of *P. digoneutis* and 6% of *L. lygivorus* offspring emerged when *P. digoneutis* attacked first. Although it is still uncertain whether attacks either from native or exotic females resulted in actual ovipositions, it seems that *P. digoneutis* is a better intrinsic competitor than *L. lygivorus*. This possibility may be applied to native *Peristenus* in Alberta, although the parasitoid community is different, and outcomes are hard to predict

until competition trials are carried out. A methodology of intrinsic competition was tested between native *Peristenus* and *P. digoneutis* (see Appendix 1).

Peristenus digoneutis has a narrow host range and a preference for *Lygus* spp. (Mason et al., 2011). In no-choice tests, non-target mirids exposed to *P. digoneutis* were attacked at a lower rate than the target host *L. lineolaris*, but the parasitoid was equally able to attack and parasitize *L. borealis*, *L. keltoni*, and *L. elisus* when presented as non-target hosts (Cárcamo and Herle, unpublished data; (Mason et al., 2011). Even with a narrow host range, *P. digoneutis* has the potential to impact non-economically important *Lygus* species.

In addition, competition between exotic and native parasitoids is possible due to temporal and spatial overlapping. However, knowing that *P. digoneutis* attacks the two main generations of *L. lineolaris* (Day, 1999), the presence of *P. digoneutis* in the Canadian Prairies may improve biological control of *Lygus* spp. in crops such as canola, where native *Peristenus* are not present. The second generation of the parasitoid will be present when the single peak of nymphs in canola occurs in mid-late July. Native *Peristenus* are present in agricultural ecosystems. However, weedy habitats like Peenaquim Park that harbour these species can potentially contribute to their maintenance in the presence of *P. digoneutis*.

Furthermore, the improvement of the biological control of *Lygus* using *P. digoneutis* may lead to the reduction of insecticide application to control *Lygus* populations, and its associated non-target environmental impacts on other beneficial arthropods. However, competition tests between *P. digoneutis* and local native *Peristenus* species of western Canada need to be carried out to assess the possibility of displacement, and to contribute to a risk-benefit analysis of releasing *P. digoneutis* in the region.

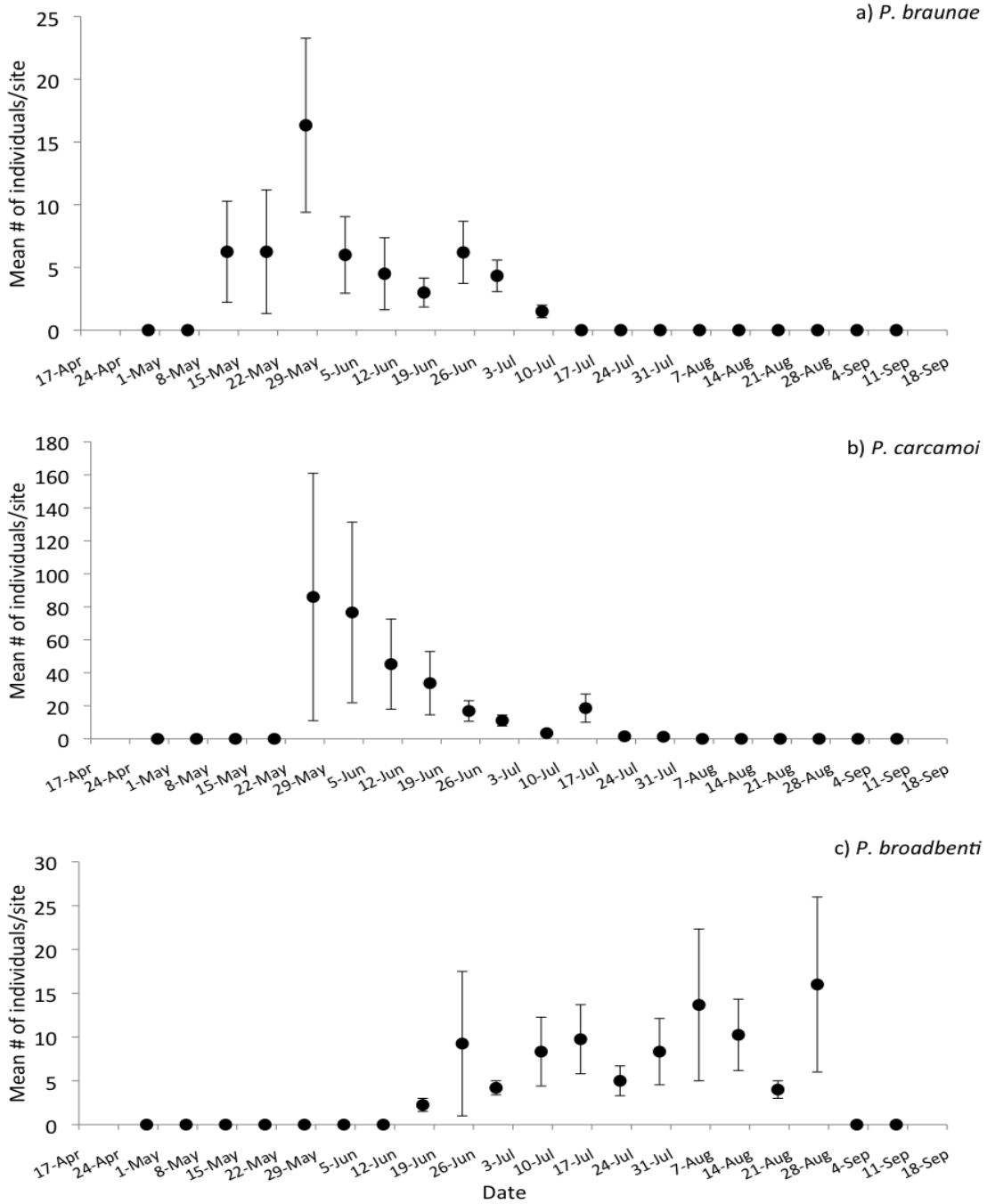


Figure 1. Numbers (mean \pm SE) of the three native parasitoid adults [*P. braunae* ($n=206$), *P. carcamoi* ($n=1228$), and *P. broadbenti* ($n=369$)] collected at various fields in southern Alberta or reared from nymphs in the laboratory during the growing season from 2003 to 2015. The x axis is a composite phenology, “Date” shows the date when parasitoids were found in the field for field samples, and the date when reared nymphs were collected in the field the previous year for lab-reared samples. Data included years in which >5 individuals per species were collected or reared, and it is aggregated on a weekly basis.

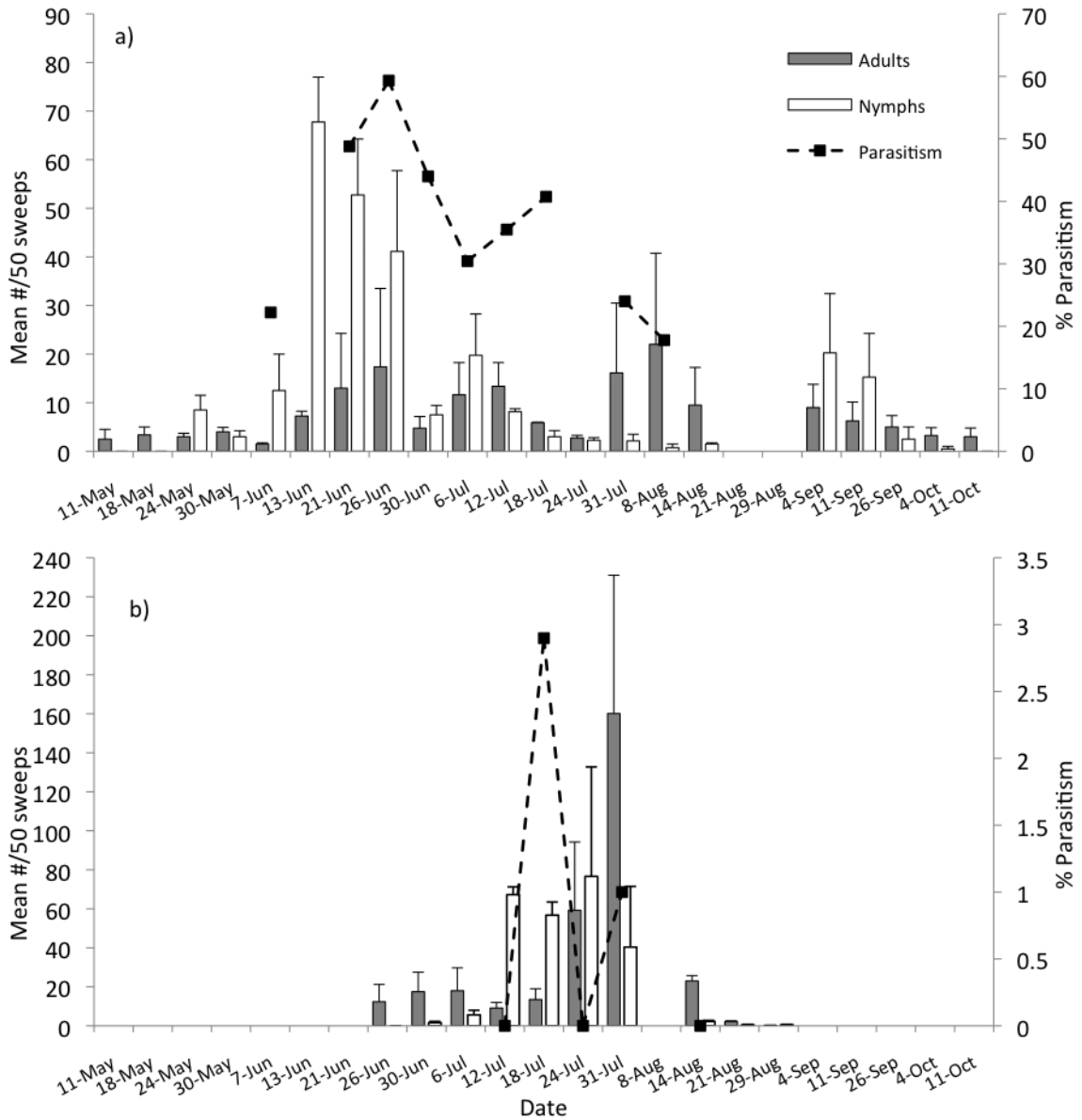


Figure 2. Numbers (mean \pm SE) of *Lygus* spp. nymphs and adults collected in sweep nets, and parasitism level determined by dissection from collections in a) alfalfa (total nymphs dissected=439), and b) canola (total nymphs dissected=290). Field plots were planted adjacent. Data pooled for 2006 and 2007, and aggregated on a weekly basis. Gaps indicate sampling did not occur for that date.

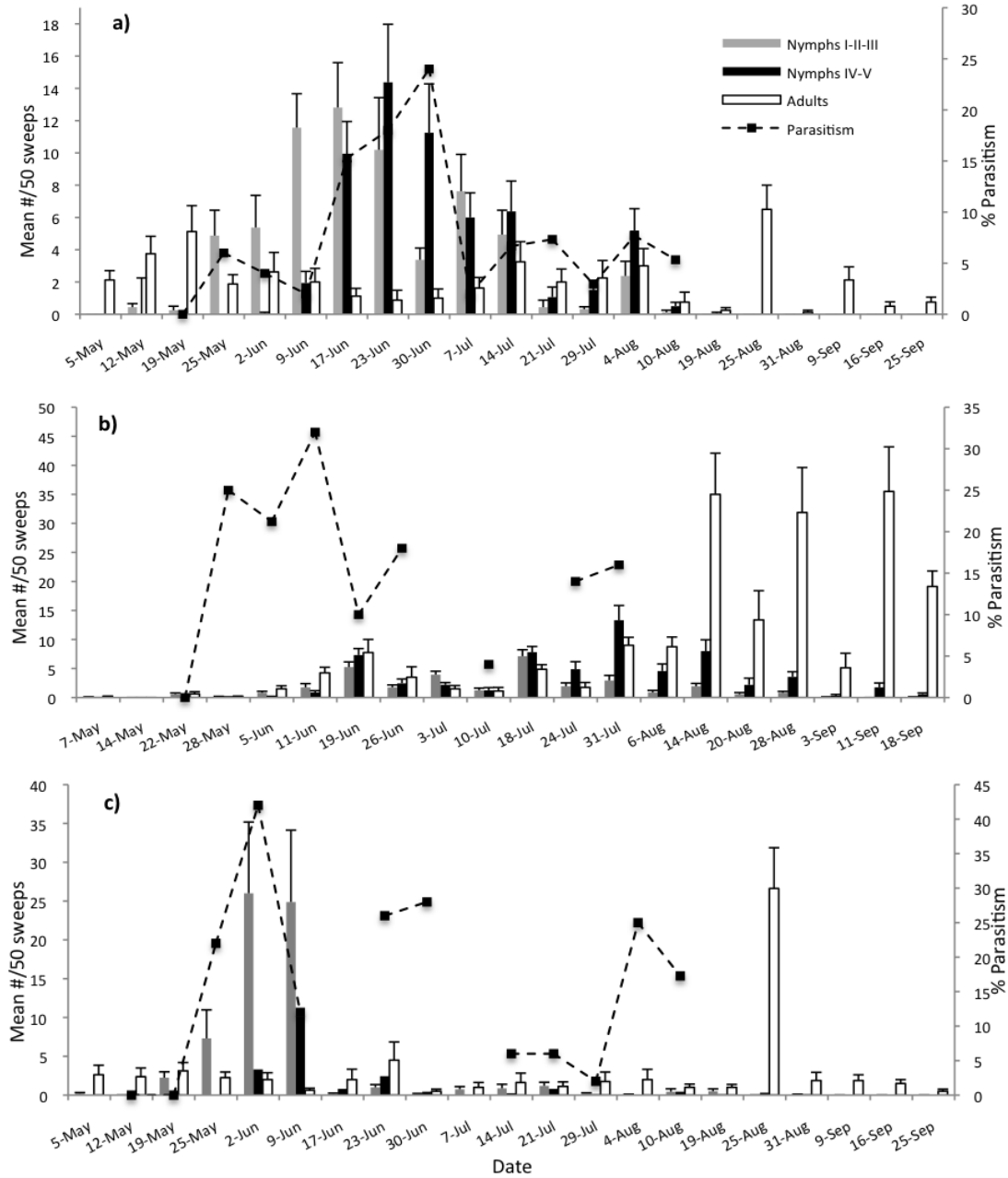


Figure 3. Numbers (mean \pm SE) of *Lygus* spp. nymphs and adults collected in sweep nets, and parasitism level from samples in 2015. Sites include alfalfa at a) Lethbridge (total nymphs reared and dissected= 1146), b) Vauxhall (total nymphs reared and dissected= 425), and weedy site at c) Peenaquim Park, Lethbridge (total nymphs reared and dissected=905). Note that parasitism was determined by rearing and dissection methods combined, and additional sweeps were taken when nymph numbers were low to increase sample size for rearing. When number of nymphs (reared+dissected) in a given date was >50 , values are reported in the graph. Data were aggregated on a weekly basis. Gaps indicate sampling did not occur for that date.

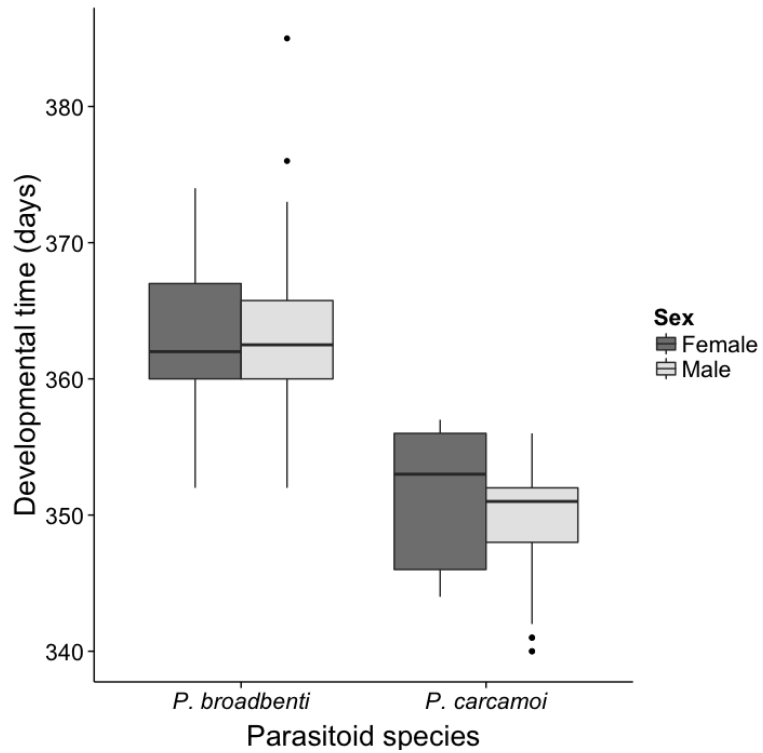


Figure 4. Box plot showing the time (days) required to develop and emerge as adults for *P. broadbenti* ($n=26♂$; $29♀$) and *P. carcarnoi* ($n=54♂$; $9♀$) under laboratory conditions.

Days were counted from the day the parasitoid attacked the nymph until the adult emerged the following year. All cocoons were moved out of overwintering at the same time.

Table 2. Total numbers of the three native *Peristenus* species adults field-collected and laboratory-reared from alfalfa, canola, weed, and grass sites from 2003 to 2015. Percentages of total number are given in parentheses. Dashed lines indicate lack of data. *Adults were collected using conventional sweeping, time-intense sweeping (dark box extraction), and sticky cards.

Year	Habitat	Field*			Laboratory		
		<i>P. broadbenti</i>	<i>P. carcamoi</i>	<i>P. braunae</i>	<i>P. broadbenti</i>	<i>P. carcamoi</i>	<i>P. braunae</i>
2003	Alfalfa	0	1 (0.05)	2 (0.09)	1 (0.05)	3 (0.14)	0
	Weeds	0	0	6 (0.28)	4 (0.19)	42 (1.96)	0
2004	Alfalfa	0	13 (0.61)	2 (0.09)	30 (1.4)	59 (2.75)	1 (0.05)
	Weeds	0	7 (0.33)	2 (0.09)	0	16 (0.74)	0
2005	Alfalfa	2 (0.09)	112 (5.22)	13 (0.61)	37 (1.72)	65 (3.03)	5 (0.23)
	Weeds	16 (0.74)	10 (0.46)	23 (1.07)	16 (0.74)	33 (1.54)	0
	Canola	0	0	1 (0.05)	1 (0.05)	0	0
	Grass	0	0	123 (5.73)	-	-	-
2006	Alfalfa	3 (0.14)	106 (4.94)	11 (0.51)	0	13 (0.61)	6 (0.28)
	Weeds	3 (0.14)	55 (2.56)	13 (0.61)	12 (0.56)	0	0
	Canola	0	0	0	0	0	0
2007	Alfalfa	0	100 (4.66)	3 (0.14)	39 (1.82)	28 (1.3)	19 (0.88)
	Weeds	0	0	0	2 (0.09)	38 (1.77)	1 (0.05)
	Canola	0	0	0	0	0	0
2008	Alfalfa	-	-	-	30 (1.4)	13 (0.6)	8 (0.37)
	Weeds	-	-	-	14 (0.65)	15 (0.7)	0
2009	Alfalfa	-	-	-	6 (0.28)	3 (0.14)	10 (0.47)
	Weeds	-	-	-	0	3 (0.14)	0
2010	Weeds	-	-	-	37 (1.72)	24 (1.12)	0
2011	Alfalfa	-	-	-	0	15 (0.7)	0
	Weeds	-	-	-	35 (1.63)	5 (0.23)	0
2013	Alfalfa	-	-	-	7 (0.33)	0	6 (0.28)
	Weeds	-	-	-	3 (0.14)	4 (0.19)	0
2014	Alfalfa	4 (0.19)	1 (0.05)	1 (0.05)	4 (0.19)	3 (0.14)	0
	Weeds	3 (0.14)	1 (0.05)	0	0	3 (0.14)	0
	Canola	1 (0.05)	0	0	0	0	0
2015	Alfalfa	23 (1.07)	241 (11.23)	53 (2.47)	-	-	-
	Weeds	37 (1.72)	407 (18.96)	28 (1.3)	-	-	-
TOTAL		92 (4.28)	1054 (49.12)	281 (13.09)	278 (12.96)	385 (17.94)	56 (2.61)

Table 3. Accumulated degree-days (mean \pm SE) (base 10.6°C) for the three native *Peristenus* species. Mean was calculated for years when more than 10 individuals were collected in the field. *A total great average was for calculated the years with available data.

Year	<i>P. broadbenti</i>	<i>n</i>	<i>P. carcamoi</i>	<i>n</i>	<i>P. braunae</i>	<i>n</i>
2005	232.5 \pm 16.9	18	56.6 \pm 2.7	122	15 \pm 1.5	160
2015	269.7 \pm 11.9	60	38.3 \pm 1.1	648	23.9 \pm 2.0	81
(2003-2007, 2014-2015)*	249 \pm 10	92	59.4 \pm 1.4	1054	28.6 \pm 2.2	281

Chapter 3 - Host-plant effects: parasitism success of *Peristenus digoneutis* on *Lygus* bugs in canola and alfalfa

Abstract

The ability of parasitoids to find and attack insect herbivores can be influenced by their host plants. This study uses greenhouse experiments to test whether the non-native parasitoid *P. digoneutis* has a preference to attack and parasitize *Lygus lineolaris* nymphs feeding on canola (*Brassica napus*) or alfalfa (*Medicago sativa*). Also, the effect of plant species on *Lygus* nymph recapture was tested without parasitoids. The study was conducted in *choice* and *no-choice* experimental designs under greenhouse conditions. The results showed that *P. digoneutis* attacks and parasitizes *L. lineolaris* nymphs regardless of the host plant. Fewer nymphs were recaptured alive from canola than alfalfa plants when the parasitoid was present. These results may underestimate parasitism level if parasitized nymphs did not survive long enough to assess parasitism.

Introduction

Interactions between parasitoids and their herbivore hosts can be influenced by the host plant (Barbosa, 1998). Chemical compounds, plant resources, plant architecture, and the spatio-temporal distribution of host plants are among the factors that determine host exploitation by a parasitoid (Godfray, 1994; Wajnberg & Colazza, 2013; Moreira et al., 2015). The variability in host-plant traits can impact parasitoid fitness indirectly (Hawkins & Sheehan, 1994).

Most of the research on parasitoid foraging behaviour has focused on volatile compounds released by the host plant. Phytochemical cues can be constitutively released,

which means that the cues are already present in the plant, or induced by the presence of a herbivore (Hawkins & Sheehan, 1994). In the latter instance, plants react to herbivory by releasing volatiles which attract parasitoids that can limit damage to the plant (Thaler, 1999; Dicke, 2009). Concurrently, parasitoids are rewarded with hosts, nectar, and pollen, which enhance their survival and success (Jervis et al., 1993). These tri-trophic interactions have been the focus of much basic and applied research (Barbosa, 1998). In biological control programs, host plants are a key factor that influence parasitoid attack on a target pest (Godfray, 1994; Barbosa, 1998).

The most common parasitoids of *Lygus* are wasps of the genus *Peristenus* that attack the juvenile stages (Loan, 1974a, 1974b; Loan & Craig, 1976; Loan et al., 1980). *Lygus* bugs comprise a complex of phytophagous insects associated with more than 300 host plants in agricultural and non-agricultural systems (Young, 1986; Wheeler Jr, 2000; Wheeler, 2001). This generalist-feeding behaviour increases the challenge of controlling *Lygus* using *Peristenus* in terms of crop exploitation. *Peristenus* spp. attack *Lygus* bugs on several plant species, but parasitism levels are variable (Braun et al., 2001; Rämert et al., 2005; Broadbent et al., 2006; Carignan et al., 2007).

The European species *Peristenus digoneutis* was first introduced to North America to control *L. lineolaris* populations in alfalfa fields (Day, 1996) and it is an effective biological control agent in this crop (Day et al., 1990; Day, 1996). Additionally, its efficacy has been demonstrated in strawberries (Tilmon & Hoffmann, 2003), vetch, red clover, and weeds (Day et al., 2004). However, information regarding its performance in canola crops is lacking, probably because canola is not a dominant crop within its current range (Day et al., 1998; Broadbent et al., 1999). If present in the Canadian

Prairies, the parasitoid may attack *Lygus* on canola and contribute to reduced *Lygus* numbers in this crop.

Knowing that *P. digoneutis* successfully attacks *Lygus* bugs in alfalfa, the objective of this study was to determine if *P. digoneutis* had a preference to parasitize *L. lineolaris* nymphs feeding on alfalfa or canola under controlled conditions in a greenhouse. This information will help establish the potential of this parasitoid to reduce *Lygus* bug populations in canola in the field.

Materials and Methods

Parasitoids, nymphs, and host-plants source

Parasitoid wasps were obtained from cocoons reared at the Ottawa Research and Development Centre. Cocoons came from parasitized *L. lineolaris* nymphs collected in alfalfa, vetch, and clover sites in the Ottawa region in 2014 and overwintered in petri dishes with vermiculite at 2°C for about eight months (Whistlecraft et al., 2010). The cocoons were shipped to the Lethbridge Research and Development Centre (LRDC) in the spring of 2015 and kept at 22 ± 2 °C and 18:6 light:dark regime until emergence. Upon emergence, parasitoids were identified to species and females and males were kept in pairs in snap-cap vials (1.5 mL) at room temperature for 24 hours for mating; a dental wick with honey solution was provided as a food source.

Lygus nymphs were from a continuous laboratory colony of *L. lineolaris* initiated in 2009 at the LRDC. The adults were field collected at different sites in Ontario and periodically supplemented with individuals from Alberta. *Lygus* adults were reared in a cage with sprouted potatoes, lettuce, and water following the procedure described by

Whistlecraft et al. (2010). Lettuce and tubers were replaced twice a week to stimulate oviposition. Newly hatched nymphs were kept in plastic containers (1 L) and fed lettuce.

Canola (*Brassica napus*) plants were grown individually from treated commercial seeds of InVigor L150 (Bayer CropScience) and alfalfa (*Medicago sativa*) from bare seed AC Bluejay (Agriculture and Agri-Food Canada). The active ingredients in the canola seed were the insecticide Clothianidin (290 g/L) and the fungicides Penflufen (10.7 g/L), Trifloxystrobin (7.15 g/L) and Metalaxyl (7.15 g/L). Seeds were planted in plastic pots (12 cm diameter x 13 cm tall) using a mix of peat moss, Turface, vermiculite, and fertilizer (18-6-12 Osmocote). Pots were kept under greenhouse conditions (18:6 light:dark, 22 ± 2 °C). Plants were used when buds or flowers were present and were 50-80 cm tall. For alfalfa, this occurred between 59-64 days after seeding; canola plants were ready to use between 48-57 days after planting.

Choice and no-choice trials

Using *choice* and *no-choice* experiments with a female parasitoid, trials were designed to test whether *P. digoneutis* had a preference to parasitize *L. lineolaris* nymphs feeding on alfalfa or canola plants. However, differences in the number of live *L. lineolaris* nymphs collected from each plant at the end of the trials made it necessary to test if this difference was associated with the parasitoid or with the host plant species. Therefore, the same experimental set ups were repeated upon completion of the first set of trials but without the parasitoid. Ideally, *choice* and *no-choice* experiments with and without the female parasitoid should be conducted simultaneously.

Choice trials. Each replicate consisted of one plant of each species enclosed inside a 1 m³ cage. The two plants were randomly assigned to opposite corners of the cage to avoid touching each other. To facilitate nymph recapture, plant pots were set up inside cardboard boxes, and their walls were sprayed with Insect-a-Slip-Fluon (BioQuip Products Inc. California, USA) solution to prevent nymphs from crawling out of the box. Also, plants were tied up using plastic fasteners to avoid having nymphs fall out of the box. Fifty *L. lineolaris* nymphs (2nd – 3rd instar) were placed horizontally on each plant in 10 mL vials with the lids removed to allow nymphs to move out of the vial on their own. Thirty minutes later, a mated female *P. digoneutis* was released inside the cage. After 48 hours, the parasitoid was aspirated and nymphs were carefully removed from each plant and cardboard box. Female parasitoids were used between days 1 and 4 after emergence. The experiment was replicated 15 times under the same greenhouse conditions (18:6 light:dark, 22 ± 2 °C).

Nymphs were reared for 5-7 days then dissected in 70% ethanol under a microscope at 10X magnification. If the parasitoids were found dead in the cage, and, after dissection, none of the recaptured nymphs were parasitized, trials were not included in the analysis. The same experimental set up under the same greenhouse conditions was replicated 17 times, but with no female wasp released within the cage.

No-choice trials. The experimental set up, conditions, and data recorded were similar to the choice design described above, but in this case the two plants inside the cage were the same species. The study was replicated nine times for alfalfa and eight for canola. A similar experiment without *P. digoneutis* in the cage was replicated 10 times for alfalfa and 12 times for canola.

Response variables and statistical analyses

For both experimental designs when *P. digoneutis* was present or absent in the cage, the response variables recorded from each plant species were: proportion of live nymphs recaptured, proportion of dead nymphs found, and proportion of nymphs that were neither found alive nor dead = missing nymphs. For the trials when the parasitoid was present, proportion of nymphs parasitized from each plant was also recorded. Proportion of parasitism was calculated by dividing the total number of parasitized nymphs by the total number of recaptured nymphs from each plant.

Choice trials. Mixed Two-way ANOVAs were used to test if proportions of live nymphs recaptured, found dead, and missing were different between plant species in the absence or presence of *P. digoneutis*. Data were arcsine-square root transformed to promote normality. Parasitoid presence/absence was used as the between-subjects effect, and plant species as within-subjects effect; subject (i.e., individual cage), nested within parasitoid presence/absence was included as a random effect. To test differences in proportion of parasitism between host plants, the non-parametric Friedman's test for repeated measures was used.

No-choice trials. To test whether the proportion of live nymphs recaptured, found dead, and missing was different between plant species when *P. digoneutis* was present or absent, a Two-way ANOVA was used. Proportions of recaptured and dead nymphs were normal. However, proportion of missing nymphs required arcsine-square root transformation to meet the assumptions of normality. To test differences in the proportion of parasitism between host plants, the non-parametric Kruskal-Wallis test was applied.

Results

Choice trials. *Peristenus digoneutis* presence did not significantly affect the proportion of nymphs recaptured at the end of the trial (Mixed Two-way ANOVA, $F_{1,30} = 1.69$, $p = 0.20$). However, plant species had a significant main effect on the proportion of nymphs recaptured. A lower proportion of live nymphs were recaptured from canola (0.61) than from alfalfa (0.82) (Mixed Two-way ANOVA, $F_{1,30} = 40.14$, $p = 5.47 \times 10^{-7}$). Furthermore, parasitoid presence/absence interacted with plant species when fewer nymphs were found alive in canola than alfalfa (Mixed two-way ANOVA, $F_{1,30} = 4.08$, $p = 0.04$) (Figure 5a).

The parasitoid did not have a significant effect on the proportion of dead nymphs between plant species (Mixed two-way ANOVA, $F_{1,30} = 1.68$, $p = 0.20$). However, host plant species had a significant main effect on the proportion of dead nymphs (Mixed two-way ANOVA, $F_{1,30} = 19.93$, $p = 1.05 \times 10^{-4}$). A greater proportion of nymphs were found dead in canola (0.22) than alfalfa (0.11). There was no significant interaction effect between parasitoid presence/absence and host plant species (Mixed two-way ANOVA, $F_{1,30} = 0.02$, $p = 0.88$) (Figure 5b).

The proportion of missing nymphs was higher in cages with the parasitoid (0.159) than those without it (0.059) (Mixed Two-way ANOVA, $F_{1,30} = 10.42$, $p = 0.003$), and from canola (0.154) than from alfalfa (0.058) plants (Mixed Two-way ANOVA, $F_{1,30} = 43.5$, $p = 2.63 \times 10^{-7}$). A significant interaction effect was found between *P. digoneutis* and host plant for the proportion of missing nymphs (Mixed Two-way ANOVA, $F_{1,30} = 14.16$, $p = 7.28 \times 10^{-4}$). Missing nymphs in canola increased when *P. digoneutis* was present inside the cage (Figure 6).

Mean proportion of parasitism was higher in alfalfa (0.19 ± 0.05 SE) than canola (0.1 ± 0.03 SE), but values were not significantly different (Friedman's test, $X^2_1 = 1.14$, $n = 15$, $p = 0.28$) (Figure 7).

No-choice trials. The number of live nymphs recaptured was not related to the presence of *P. digoneutis* inside the cage (Two-way ANOVA, $F_{1, 35} = 0.64$, $p = 0.42$). A greater proportion of live *L. lineolaris* nymphs were recaptured from alfalfa than canola plants (Two-way ANOVA, $F_{1, 35} = 13.77$, $p = 7.12 \times 10^{-4}$). Also, a significant interaction was found when *P. digoneutis* was present in the cage and fewer nymphs were recaptured from canola than alfalfa (Two-way ANOVA, $F_{1, 35} = 5.15$, $p = 0.02$) (Figure 8a).

The presence of *P. digoneutis* did not have a significant effect on the proportion of dead nymphs (Two-way ANOVA, $F_{1, 35} = 0.59$, $p = 0.44$). A larger proportion of dead nymphs was collected from canola than alfalfa (Two-way ANOVA, $F_{1, 35} = 9.82$, $p = 3.48 \times 10^{-3}$). There was no significant interaction between plant species and parasitoid in terms of the proportion of dead nymphs (Two-way ANOVA, $F_{1, 35} = 3.16$, $p = 0.08$) (Figure 8b).

More nymphs were missing when *P. digoneutis* was released in the cage (Two-way ANOVA, $F_{1, 35} = 4.6$, $p = 0.03$). A larger number of nymphs were missing from canola plants than alfalfa (Two-way ANOVA, $F_{1, 35} = 10.30$, $p = 2.84 \times 10^{-3}$), but there was no significant interaction between plant species and parasitoid was observed (Two-way ANOVA, $F_{1, 35} = 1.91$, $p = 0.17$) (Figure 9).

Mean proportion of parasitized nymphs was not significantly different between alfalfa (0.2 ± 0.07 SE) and canola (0.15 ± 0.04 SE) plants (Kruskal-Wallis test, $X^2_1 = 0.33$, alfalfa $n = 9$, canola $n = 8$, $p = 0.56$) (Figure 10).

Discussion

The objective of this study was to determine if *P. digoneutis* had a preference to attack *L. lineolaris* nymphs feeding on alfalfa or on canola plants. Choice and no-choice designs showed that *P. digoneutis* attacks and successfully parasitizes *L. lineolaris* nymphs that were feeding on alfalfa or canola. In contrast to Canada, *Lygus* bugs are not part of the main insect pests of canola in Europe where *P. digoneutis* is native (Ulber et al., 2010). This may explain the lack of information about European *Peristenus* parasitizing *Lygus* spp. in canola. In an extensive survey, Haye (2004) reported *P. digoneutis* parasitizing European *Lygus* spp. nymphs in chamomile and red clover sites.

In the current study, *P. digoneutis* parasitized *Lygus* nymphs in canola and alfalfa, and there was no significant difference between host plants. Parasitoids can be attracted to the floral resources. However, floral preference or attraction to either plant species has not yet been determined. Parasitoids exploit the floral resources of the food plants of their hosts. Using a Y-tube olfactometer and five *Lygus* host plant species, Shahjahan (1974) showed that females of the univoltine *P. pseudopallipes* were highly attracted to the odors of *Erigeron* spp. flowers. The author suggested a preference related to floral resources. In a similar design, Halloran et al (2013) found that females of *P. relictus* were attracted to *Erigeron* plants with host feeding damage and to undamaged plants. These are examples that parasitoids are attracted to habitats where hosts are not present to fulfill their needs, other than hosts (Barbosa 1998).

Field-recorded parasitism levels attributed to *P. digoneutis* are variable among host plants (Day, 1996; Tilmon & Hoffmann, 2003; Day et al., 2004; Pickett et al., 2007; Day & Hoelmer, 2012). This difference in parasitism may be related to features such as patch size, diversity, and continuity of the habitat. Streams et al. (1968) noted variable

levels of parasitism of *L. lineolaris* nymphs attributed to *Leiophron pallipes* (syn. *P. pallipes*, *P. mellipes*) in different host plants in several habitats. Habitats with high levels of parasitism were dominated by one or two species of *Erigeron* plants, which may suggest a preference for this group.

Another important factor to consider is the architecture of the host plants. Even though plants at the same stage (buds or flowers present) were used and equally tied up, alfalfa and canola are structurally different. Canola has a main stem with a simple structure of fewer branches and nodes than alfalfa, but it has a hairy surface. The hairs may hinder parasitoids searching on the plant. On the other hand, alfalfa is denser than canola and has a bushy appearance, which may provide shelter and hiding places for hosts and parasitoids. Based on these features, plant architecture can influence parasitoids' behaviour and level of parasitism. Consequently, more detailed studies under even more simplified conditions are required to elucidate these factors.

Lygus lineolaris is a generalist feeder, although it has preferences for certain host plants in the field, and also in the laboratory (Curtis & McCoy, 1964; Gerber, 1997). The results of this study showed that host plant species had an effect on the recapture of live *L. lineolaris* nymphs and this difference increased when *P. digoneutis* was present. A greater number of nymphs was recaptured from alfalfa than canola plants. However, in a study without parasitoid wasps, Butts and Lamb (1990a) found that *Lygus* spp. survive better in canola than in alfalfa in laboratory trials. They tested the suitability of alfalfa (*M. sativa*, Beaver) and canola (*B. napus*, Andor) in the development of three *Lygus* spp. including *L. lineolaris*. Their results showed that survival of *Lygus* spp. nymphs fed on canola buds was higher than on alfalfa. However, field-collected adults from alfalfa were heavier than the individuals reared in the laboratory, and not different from reared or

collected adults in canola. In another study, Gerber and Wise (1995) found that four species of canola (*B. napus*, *B. carinata*, *B. juncea* and *B. rapa*) are adequate hosts to support *L. lineolaris* populations. Based on these results, I would expect to collect more nymphs in canola than in alfalfa, or at least similar numbers. However, a significant number of dead nymphs were associated with canola regardless of the parasitoid presence/absence. This difference can be associated with the treatment present in the seed coat of canola. The treatment is a combination of fungicide and insecticide that contains clothianidin, a neonicotinoid insecticide. The treatment protects canola seedlings against some fungal diseases and insect pests. Although the target pest of the insecticide treatment is flea beetles (*Phyllotreta* spp.) not *Lygus* bugs, the insecticide in the seed coat may have an effect on *Lygus* nymphs' survival. Dosedall (2009) reported a reduction in the number of the largest larval stages of cabbage seedpod weevil in canola associated with neonicotinoids (clothianidin and imidacloprid) present in the treated seeds. Most of the commercial canola used in Western Canada is treated with fungicide, insecticide or a combination of both. Studies have shown that treatments in canola seed coat benefit its establishment and are important for yield crop (Soroka et al., 2008; Clayton et al., 2009). Further trials comparing proportion of recaptured nymphs alive from seed treated with a neonicotinoid insecticide (i.e. clothianidin) and bare canola did not show significant difference between these plant treatments. Therefore, it is possible to suggest that nymphs' mortality feeding on canola plants is associated with the presence of *P. digoneutis*.

For the missing nymphs, their numbers increased more on canola than alfalfa when *P. digoneutis* was present. If missing nymphs were also dead, we may think that the presence of *P. digoneutis* has an effect on the mortality of *L. lineolaris* nymphs feeding

on canola. Possibly, the female parasitoid attacked those missing nymphs and they did not survive the attack. These missing nymphs may have died during the experiment and dropped inside the plant pot where they were hard to find. However, mortality induced by parasitoids is hard to determine because dead individuals are hard to find or if already dead, causes cannot be attributed to the parasitoid itself.

In the present study, *P. digoneutis* parasitized *L. lineolaris* nymphs feeding on two different plant species, and no preference was found. When *P. digoneutis* was present, the number of live *L. lineolaris* nymphs was reduced in both plants but the reduction was significantly larger in canola than alfalfa. Likewise, the number of missing nymphs increased in canola when the parasitoid was present. Due to the nature of the experiments it is hard to observe actual attack of *P. digoneutis* on *Lygus*, but testing the presence/absence of *P. digoneutis* in the cage, we may indirectly infer that the parasitoid has an effect on *Lygus* nymphs' survival

On the other hand, perhaps when *P. digoneutis* attacks *L. lineolaris*, some of the nymphs do not survive the attack and actual level of parasitism may be underestimated due to early mortality of the host. In competition trials, when a single nymph is exposed to a single parasitoid in small containers, the parasitoid's attack is easily observed. Sometimes, exposed nymphs die immediately after the attack, even when they are 2nd and 3rd instars (Fernández and Herle unpublished data).

Canola is an extensive crop in the Canadian Prairies and the present study suggests that if *P. digoneutis* was present in the area, it should attack *Lygus* in canola. However, the extent of the effect in the field is unknown. Haye (2004) suggested that it is difficult to predict the efficacy of European parasitoids in crop habitats, where host plants rotate yearly. Canola harbours different stages of *Lygus* spp. nymphs (Cárcamo et al.,

2003) that *P. digoneutis* can exploit, but the overwintering parasitoid generation may emerge in a habitat such as a cereal crop with few *Lygus* bugs the following year. It is unknown if these new adults will be able to follow their hosts to a new habitat. However, habitats like alfalfa close to canola fields can be used as reservoir for *P. digoneutis*.

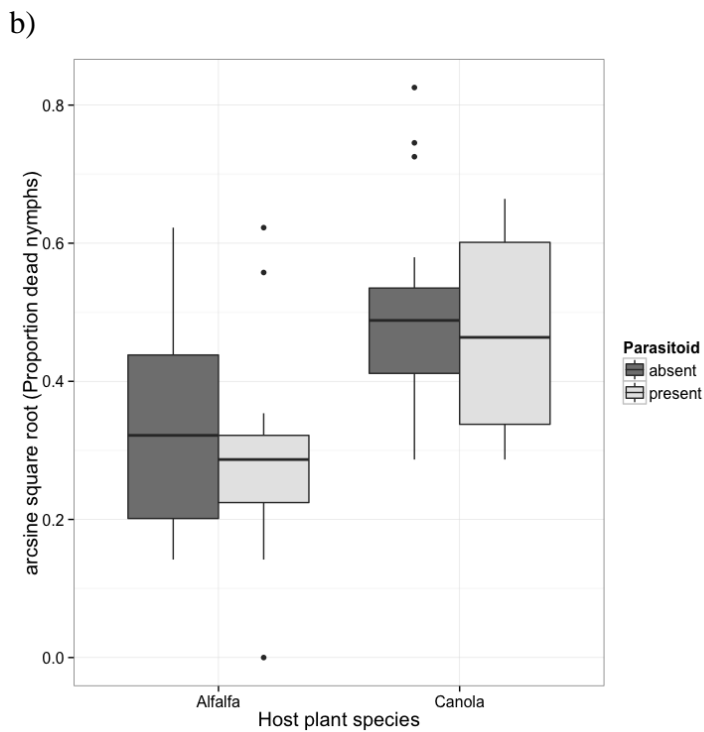
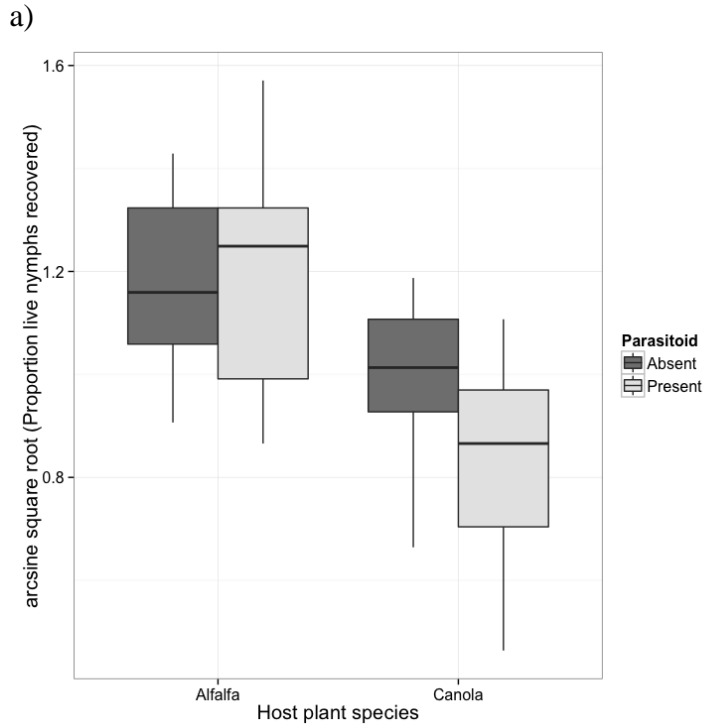


Figure 5. Proportion of *L. lineolaris* nymphs a) alive and b) dead from alfalfa, and canola plants in the *choice* test. Dark boxes refer to the absence of *P. digoneutis* in the cage; light boxes to presence of *P. digoneutis* in the cage. *Choice*/absent ($n=17$), *choice*/present ($n=15$). Data were arcsine root transformed. Box= lower and upper edges are the 25th and 75th quartiles, horizontal line refers to the median, whiskers refer to the maximum and minimum observations that are not extreme, dots are extreme values.

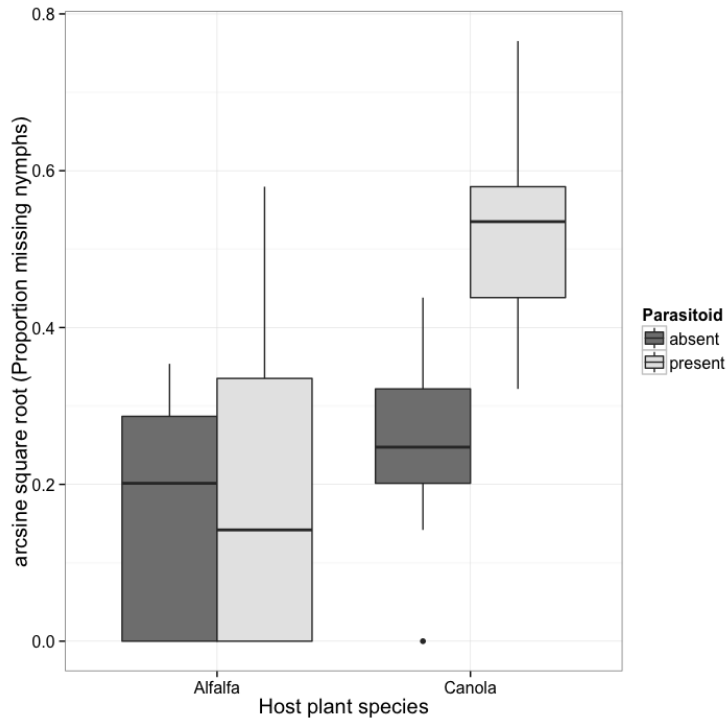


Figure 6. Proportion of *L. lineolaris* nymphs missing in alfalfa and canola plants in the choice test. Dark boxes refer to the absence of *P. digoneutis* in the cage; light boxes to presence of *P. digoneutis* in the cage. Choice/absent ($n=17$), choice/present ($n= 15$). Data were arcsine root transformed. Box= lower and upper edges are the 25th and 75th quartiles, horizontal line refers to the median, whiskers refer to the maximum and minimum observations that are not extreme, dots are extreme values.

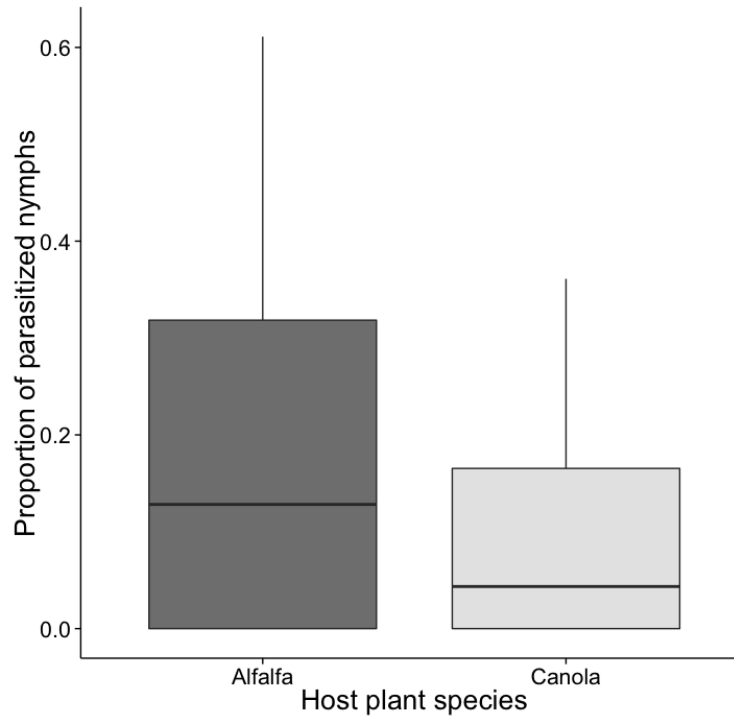


Figure 7. Proportion of *L. lineolaris* nymphs parasitized from alfalfa and canola plants. Results are from nymphs recaptured in the *choice* experiment when *P. digoneutis* was present in the cage ($n= 15$). Box= lower and upper edges are the 25th and 75th quartiles, horizontal line refers to the median, whiskers refer to the maximum and minimum observations that are not extreme.

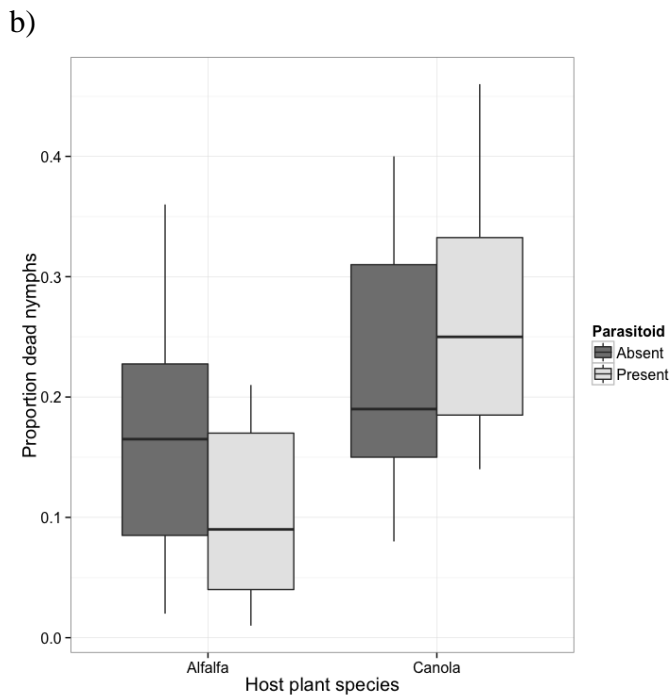
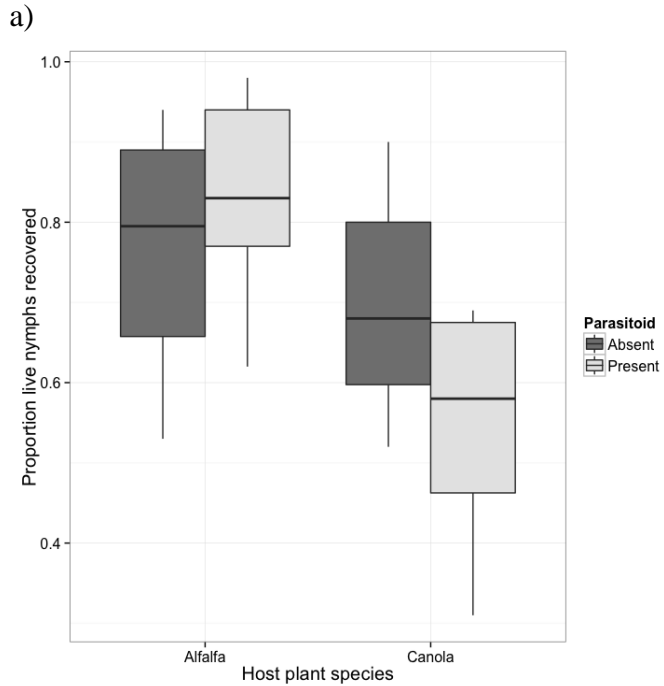


Figure 8. Proportion of *L. lineolaris* nymphs a) alive and b) dead from alfalfa, and canola plants in the *no-choice* test. Dark boxes refer to the absence of *P. digoneutis* in the cage; light boxes to presence of *P. digoneutis* in the cage. Absent (alfalfa $n=10$, canola $n=12$), present (alfalfa $n=9$, canola $n=8$). Box= lower and upper edges are the 25th and 75th quartiles, horizontal line refers to the median, whiskers refer to the maximum and minimum observations that are not extreme.

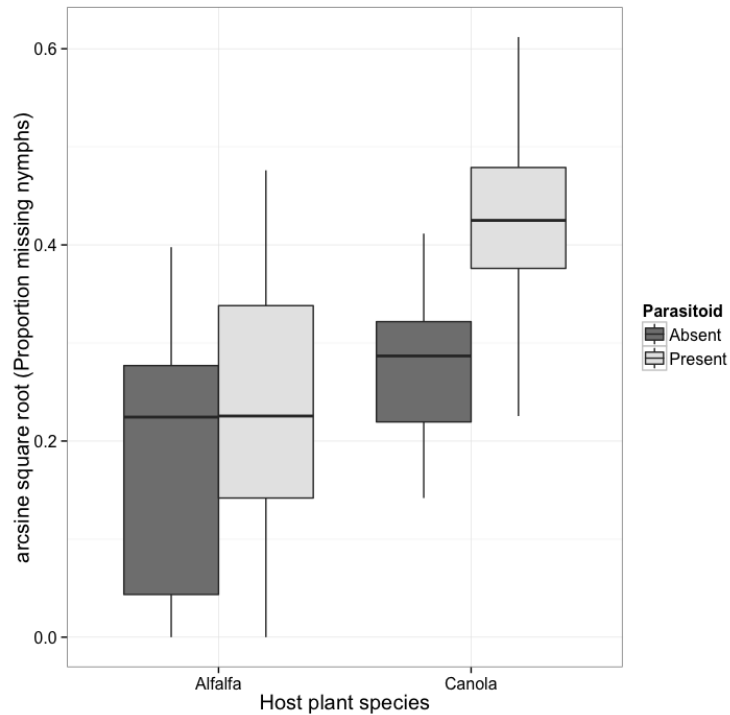


Figure 9. Proportion of *L. lineolaris* nymphs missing from alfalfa, and canola plants in the *no-choice* test. Dark boxes refer to the absence of *P. digoneutis* in the cage; light boxes to presence of *P. digoneutis* in the cage. Absent (alfalfa $n=10$, canola $n=12$), present (alfalfa $n=9$, canola $n=8$). Data were arcsine root transformed. Box= lower and upper edges are the 25th and 75th quartiles, horizontal line refers to the median, whiskers refer to the maximum and minimum observations that are not extreme.

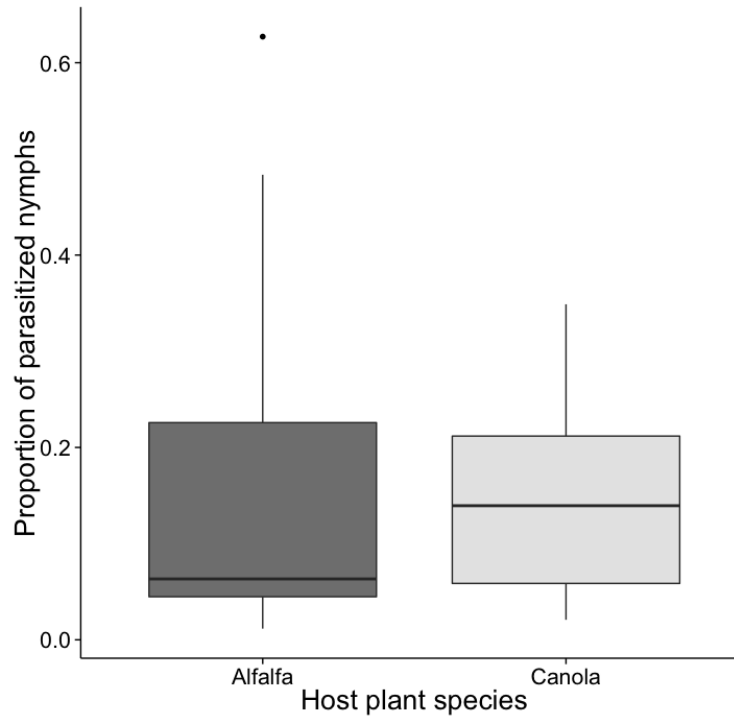


Figure 10. Proportion of *L. lineolaris* nymphs parasitized from alfalfa and canola plants. Data is from *no-choice* experiments when two plants of the same species were presented to a female *P. digoneutis* per cage (alfalfa $n=9$; canola $n=8$). Box= lower and upper edges are the 25th and 75th quartiles, horizontal line refers to the median, whiskers refer to the maximum and minimum observations that are not extreme, dots are extreme values.

Chapter 4 - General discussion and conclusions

Ecological interactions among host plants, herbivores, and parasitoids are the basis of biological control programs that include parasitoids as agents of control. It is important to understand these complex relationships, so adequate management can be carried out. Furthermore, prior to the introduction of foreign agents into a new ecosystem, assessments of the possible implications are essential. Information regarding the ecological host range of the new natural enemy, possible displacement of native parasitoid community members, and the predicted effect on the target pest need to be assessed beforehand.

This thesis gives information about the state of *Peristenus* species associated with *Lygus* spp. in Southern Alberta, their possible interaction with the foreign *P. digoneutis* if present in the zone, and the potential effects of *P. digoneutis* on the control of *Lygus* bugs in canola fields.

Summary

Chapter 2. Seasonality and species composition of Peristenus Förster (Braconidae: Euphorinae) species in Southern Alberta, Canada.

This chapter uses field and laboratory data to describe the phenology of three nymphal parasitoids of *Lygus* spp. during the growing season. The three species (*Peristenus braunae*, *P. carcamoi*, and *P. broadbenti*) appear sequentially from early May until late August. Similar results were described by Goulet and Mason (2006) for *P. braunae* and *P. broadbenti* in a recent review for the Nearctic *Peristenus*. Although the period of activity of *P. carcamoi* was shorter in their study than the current one, the

difference may be due to differences in time and effort invested in sampling.

Accumulated degree-days for field-collected parasitoid adults showed that the species have different degree-day developmental requirements, and the use of these values may predict the appearance of each species in the field. In addition, developmental time of *P. carcamoi* and *P. broadbenti* in the laboratory showed that these two species differ in time of emergence since the female has attacked the *Lygus* nymph.

All three species were found parasitizing early instars of *Lygus* spp. feeding on alfalfa, canola, or weeds; although in canola crops their presence was rare. Overall seasonal parasitism levels did not exceed 16% for alfalfa and weedy sites, and 3% for canola. Similar values have been reported throughout Canada (Braun et al., 2001; Broadbent et al., 2006; Carignan et al., 2007), and are considered inadequate to keep *Lygus* numbers under the economical threshold (Wise & Lamb, 1998; Braun et al., 2001; Matos & Obrycki, 2004; Rämert et al., 2005; Broadbent et al., 2006; Carignan et al., 2007; Otani & Cárcamo, 2011).

To increase the suppression of *Lygus* spp., introduction of the European *P. digoneutis* has been considered. This parasitoid is already established in eastern Canada (Broadbent et al., 1999; Day et al., 2008), and it may disperse to the west part of the country. Its presence in the western Provinces may improve the control of *Lygus*. Although competition between the exotic agent and native *Peristenus* is a possibility, *P. digoneutis* may exploit host and food sources where native parasitoids are not present or are rare.

Chapter 3. Host-plant effects: parasitism success of Peristenus digoneutis on Lygus bugs in canola and alfalfa

This study uses greenhouse trials to test if *P. digoneutis* prefers to attack and parasitize *L. lineolaris* nymphs feeding on alfalfa or canola plants. Initially, the experiments were designed to test differences in parasitism levels between plant species. However, differences in the resulting number of live nymphs from each plant led to test whether the presence of *P. digoneutis* had an effect on number of live nymphs recaptured, and the same experimental set up was carried out without the female parasitoid. Two different experimental designs were conducted: 1) *Choice* and 2) *No-choice* designs.

The experiments found no evidence that *P. digoneutis* has a preference for *L. lineolaris* nymphs using alfalfa or canola as hosts. The parasitoid was able to attack and successfully parasitize nymphs on both plants. This indicates the potential of *P. digoneutis* to exploit canola fields when searching for hosts. However, results showed that the presence of *P. digoneutis* had an effect on the survival of *Lygus* nymphs. Fewer nymphs were collected alive from canola plants than alfalfa when the parasitoid was present. *P. digoneutis* may cause the early mortality of *L. lineolaris* nymphs, and the actual parasitism level may be underestimated. However, it is not clear if nymph' mortality is resulting from the neonicotinoid insecticide that is routinely coated to canola seeds as used in this experiment.

Future research and directions

More questions emerged out of this work than the ones that were answered. Information about the biology of native *Peristenus* is scarce. Perhaps the impact of native parasitoids on *Lygus* spp. populations in the Prairies is related to the use of different mirid

species as hosts. It would be interesting to see if *P. carcamoi* and *P. broadbenti* attack other mirid species available at the time they emerge. *Peristenus braunae* uses *Adelphocoris* nymphs as its primary host (Goulet & Mason, 2006) and a similar case may be occurring with the other species. Field-collected non-*Lygus* nymphs that co-occur with *Lygus* spp. can be reared following the same protocol described by Whistlecraft et al. (2010). This will contribute to determining if the native parasitoids have other preferred host species.

In chapter 3, the experiments did not detect any *P. digoneutis*' preference to parasitize *L. lineolaris* in alfalfa or canola. However, the result can be biased due to nymphal mortality in canola plants. To clarify this result, experiments can be reproduced using treated and untreated canola seeds, so nymphal mortality can be assessed. In addition, host plant preference can be tested using untreated canola seeds.

Competition trials between *P. digoneutis* and native *Peristenus* need to be carried out in a more natural and feasible scale (e.g. greenhouse trials that test parasitoid searching for hosts). Besides intrinsic competition that it is usually between parasitoids' young instars (Godfray, 1994), competition also happens between adults. To test possible interactions between the exotic and native parasitoids, adults of both species can be allowed to attack *Lygus* nymphs simultaneously under greenhouse conditions. With this design, it would be possible to describe a more realistic outcome if *P. digoneutis* interacts with the local species.

Dissection and rearing of nymphs are two complementary methods to assess parasitism (Garipey et al., 2008c). Dissection gives an immediate estimate of the parasitism level, whereas rearing provides information of the parasitism level and also allows identification of the parasitoid species. However, each one has its limitations. It

would be useful to implement molecular techniques already developed for a similar system (Bon et al., 2008; Garipey et al., 2008a; Garipey et al., 2008b) to supplement the information of host-parasitoid interactions in the *Lygus-Peristenus* system in Southern Alberta.

Conclusion

In biological control programs, the interactions among the living organisms involved are the main consideration before any action is taken. However, it is equally important to assess indirect effects. Based on the information available and prior assessments, undesirable outcomes can be anticipated and successful programs are possible. This thesis provides baseline information of the current state of local *Peristenus* spp., and the possible interactions to occur among native, exotic parasitoids, and their shared host in Southern Alberta. The results gathered in this document are the starting point and more research needs to be carried out to anticipate possible outcomes of releasing the exotic parasitoid.

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Appendix 1 - Methodology to assess interspecific larval competition of *Peristenus* spp. in *Lygus* bugs

Abstract

This appendix describes a trial intended to assess the intrinsic competition between native and non-native parasitoid species when they exploit the same host species. The system includes the exotic parasitoid *Peristenus digoneutis*, its congeners occurring locally in southern Alberta (*Peristenus carcamoi*, *P. broadbenti*, and *P. otaniae*), and the shared host, *Lygus lineolaris*. Trials were performed under laboratory conditions exposing a single nymph to the native and exotic females alternately. To test larval competition, a nymph was exposed to a female parasitoid and immediately after the attack, the same nymph was exposed to a second heterospecific female. Availability of native parasitoid females to perform enough replicates was an important constraint during the experiment. Nymphs were individually exposed to the different treatments, and only one cocoon resulted at the end but no parasitoid adults emerged. One *P. digoneutis* larva emerged from its host, and the remaining nymphs turned into *Lygus* adults. Considering the low success of the experiments, this appendix presents a preliminary approach to assess intrinsic competition between native and non-native *Peristenus* in *Lygus* nymphs.

Introduction

In parasitoid communities, interspecific competition can occur when species that overlap in space and time exploit the same host species and stage (egg, larva, pupae, adult) (DeBach & Sundby, 1963; Van Nouhuys & Punju, 2010; Teder et al., 2013). Competition can be *extrinsic* when adult parasitoids forage for hosts or *intrinsic* when multiple larvae develop within the same host (Godfray, 1994; Harvey et al., 2013). In intrinsic competition, one of the competitors defeats another through physiological suppression and/or physical attack (Godfray, 1994). When physiological suppression occurs, one of the competitors produces changes in the host's hemolymph that reduces the development or survival of the second parasitoid (Salt, 1961; Vinson, 1976). However, the mechanisms involved in this suppression are not entirely understood (Godfray, 1994). In some cases, early larval instars of different species are adapted for combat with strong mandibles or caudal appendages that allow them to fight intruders (Chau & Maeto, 2008; Harvey et al., 2013). The outcome of the intrinsic interaction has significant implications on biological control programs with insect parasitoids. Competition between parasitoids controlling the same pest can occur, leading to the displacement of the less competitive agent. Also, failure of the pest management program is possible, if the less competitive parasitoid is the new introduced agent.

Lygus species is a group of piercing-sucking insects in the family Miridae that feed on more than 300 plant species (Curtis & McCoy, 1964; Craig & Loan, 1969; Fye, 1982; Young, 1986; Wheeler Jr, 2000; Wheeler, 2001), and are considered important pests in North America. Natural enemies of *Lygus* occurring in North America include the nymphal parasitoids *Peristenus*. This group attacks juveniles of *Lygus* (2nd and 3rd instars) that eventually die when the larval parasitoid emerges from its host. Their effect on *Lygus* numbers is considered inadequate to keep the pest below thresholds for economic damage (Lim & Stewart, 1976b; Braun et al., 2001; Carignan et al., 2007). Efforts to improve control and reduce *Lygus* pests in the USA led to the introduction of European *Peristenus* parasitoids. *Peristenus digoneutis* was first introduced in the north East US to control *L.*

lineolaris in alfalfa fields, and has spread in cultivated and non-cultivated plant species (Day, 1996; Day et al., 2004; Day & Hoelmer, 2012). The parasitoid has also established on the *Lygus* populations of eastern Canada (Broadbent et al., 1999; Day et al., 2000; Day et al., 2008; Garipey et al., 2008a) and may spread westward (Haye et al., 2013).

The European parasitoid *P. digoneutis* and native *Peristenus* species exploit the same *Lygus* bug stage and if they co-occur in a patch, competition may take place. First instar larvae of *P. digoneutis* have sclerotized mandibles (Carignan et al., 1995). This trait may pose an advantage in case of intrinsic competition. However, the trait is also present in the native *P. pseudopallipes* (Lim & Stewart, 1976a), which suggests a character present in the genus. Data associated with the interaction between native and exotic *Peristenus* are scarce. Lachance et al. (2001) tested *P. digoneutis* against the native parasitoid *Leiophron lygivorius* in Ontario. They found that *P. digoneutis* offspring emerged 80% of the times when they attacked first, and 40% when attacked second. The native parasitoid emerged 45% and 6% when they attacked first and second, respectively. However, the female parasitoids were not tested after each attack to prove if they were laying eggs effectively.

The purpose of this appendix is to describe preliminary trials to determine if *P. digoneutis* is a better competitor when its larval stage develops in the presence of a second heterospecific larva. Trials were designed to test if the order of parasitoids' encounter with the host determined the outcome of the offspring in sequential attacks (i.e. exotic attacks first or native attacks first). The exotic *P. digoneutis* was tested against the native *P. otaniae*, *P. carcamoi* and *P. broadbenti* present in southern Alberta.

Materials and Methods

Parasitoids and nymphs source

The exotic parasitoid *P. digoneutis* was obtained from cocoons reared in Ottawa and shipped to the Lethbridge Research and Development Centre (LRDC). Cocoons came from parasitized *Lygus lineolaris* nymphs collected in alfalfa, vetch, and clover sites. The native parasitoid species (*P. carcamoi*, *P. broadbenti*) were reared from *Lygus* spp. nymphs field-collected at several sites in Vauxhall and Lethbridge, Alberta. Individuals of *P. otaniae* were obtained from cocoons reared in Beaverlodge, Alberta, and shipped to the LRDC. All were kept in Petri dishes with vermiculite as a substrate until parasitoid emergence (18:6 light:dark, 22±1 °C). Upon emergence, parasitoids were identified and sexed with a dissecting microscope. Newly emerged females and males of the same species were kept in pairs in a snap-cap vial (1.5 mL) at room temperature for 24 hours for mating; a dental wick with honey solution was provided as a food source.

Nymphs used in the experiment were obtained from a continuous laboratory colony of *L. lineolaris* at the LRDC. The colony was initiated from adults collected in Ottawa in 2009, and supplemented with adults field-collected from around Alberta. *L. lineolaris* adults were kept in plastic oviposition cages and fed with lettuce and water. Sprouted potatoes were provided as oviposition substrate and changed twice a week. Potatoes from the oviposition cage were set aside in plastic containers (4 L) with lettuce for the newly hatched nymphs. The colony was kept at 18:6 light:dark regime, 22±1 °C.

Host rearing

Once nymphs were exposed to a specific treatment, attacked nymphs were set aside in plastic vials (35 mL) and reared using vermiculite as a substrate for pupation.

Vial lids were perforated and had a piece of fabric to allow ventilation. Small pieces of lettuce were provided twice a week to feed them.

Competition trials

One healthy second-instar *L. lineolaris* nymph was exposed to a mated parasitoid female inside a snap-cap vial 1.5 mL, and removed immediately following a single attack. After the first attack the once-attacked nymph was immediately exposed to a second parasitoid female species in a different vial until a single attack was observed. The time between the first and second attack was just the necessary to remove the nymph from one vial and put it inside the second container. Once the nymph was attacked by the second parasitoid, it was set aside in a rearing container. If no attack was observed within the next 15 minutes, the nymph was replaced. Trials were carried out using plastic snap-cap vials (Eppendorf).

Two different treatments were designed and each treatment had a control trial for the native and the exotic wasps. A complete round consisted of a healthy nymph attacked by both female parasitoids, and each female attacking a new healthy nymph as control. In a round, each female parasitoid attacked a total of two nymphs (Figure 11). Female parasitoids were used until no interest and consequently no attack in the nymph was observed even when the nymph was replaced with a new one. New female wasps were used in each treatment. All trials were performed under laboratory conditions at 22 ± 1 °C.

Treatment 1 - Native first, exotic second (N/E). One native parasitoid female was introduced in a plastic snap-cap vial with a 2nd - 3rd instar nymph. After the attack was observed, the nymph was removed and exposed to the exotic parasitoid female. Right after the second attack, the nymph was set aside in a rearing container. Once the twice-attacked nymph was set aside, a new healthy nymph was exposed to the same Native female and set aside after the attack (Control Native). The same procedure was conducted with a new nymph exposed to the Exotic female (Control Exotic). This treatment was completed with two nymphs ($n=2$).

Treatment 2 - Exotic first, native second (E/N). A new exotic parasitoid female was introduced in a plastic snap cap vial with a 2nd - 3rd instar nymph. After the attack was observed, the nymph was removed and exposed to a new native parasitoid female. Right after the second attack, the nymph was set aside in a rearing container. Two complete rounds were performed ($n= 2$). After the twice-attacked nymph was set aside, a new nymph was exposed to the Exotic female and set aside after the attack (Control Exotic). The same procedure was conducted using a new nymph exposed to the Native female (Control Native).

Results

Treatment 1 - Native first, exotic second (N/E). Two healthy *L. lineolaris* nymphs were exposed in this trial and went through a full exposure round. Each nymph was used in a different round. Nymphs were exposed to the native *P. otaniae* and *P. carcamoi* first. Afterwards, each nymph was exposed to a different *P. digoneutis* female. No control exposures to confirm activity of the parasitoid were possible because female parasitoids did not show interest in healthy nymphs. Attacked nymphs turned into adults and no cocoons were formed.

Treatment 2 - Exotic first, native second (E/N). Only one cocoon was built when *P. digoneutis* attacked first against *P. otaniae*. The control of the exotic female in this trial

produced a parasitoid larva, although the larva did not spin a cocoon. In addition, the control of the native parasitoid turned into a *Lygus* adult. In a second round, a new healthy nymph was exposed to a new *P. digoneutis* female first and later to a *P. broadbenti* female. In this case, the twice attacked nymph turned into adult. The controls were carried out but both nymphs died during the rearing process.

Summary

Based on the small sample size and results, it is impossible to tell if any of the parasitoid species tested is a better competitor than the other. Numbers of native parasitoids was a constraint. Females were scarce and, usually, males and females of the same species were not available for mating. After the treatments, some of the nymphs (2nd-3rd instar) did not survive the parasitoid attack, or mortality was a factor due to handling. In some cases the parasitoid did not show any interest in the nymph.

The methodology proposed in this document is a first step to test heterospecific larval competition within the host. However, in the case of *Peristenus* species the methodology is not as straightforward as expected. Native parasitoids have one generation per year and cannot be reared easily to get enough numbers for replicates. Synchrony in emergence among the species to test can be manipulated under laboratory conditions, but differences will affect the experiment if number of individuals is not enough. Usually, mating occurred immediately after males and females were put together in the vial. However, females did not allow the male to approach when more than five days had passed after emergence. Ideally, thirty complete rounds would be a desirable number to test larval competition.

As an option to circumvent these constraints, the competition experiment can be carried out at a different level. A pair (male and female) of native and a pair of exotic parasitoids can be placed with the hosts for 48 hours. The nymphs will be recaptured at the end of the trial and reared in small groups for cocoon formation. Dissecting the cocoons when the pupae is fully formed, allows species identification (exotic from native). Parasitoids will be checked for the presence of an edge (carina) on the posterior aspect of the parasitoid's head. The carina is complete in the native species and incomplete in *P. digoneutis* (Figure 12).

This modification in the experimental design will test competition between species; although it will be uncertain if the competition is *intrinsic* or *extrinsic*. However, for practical purposes in terms of making decisions about potential displacement of natives by exotic wasps, the mechanism is not the primary concern and this test would provide important information to support the decision to release or not release the exotic species.

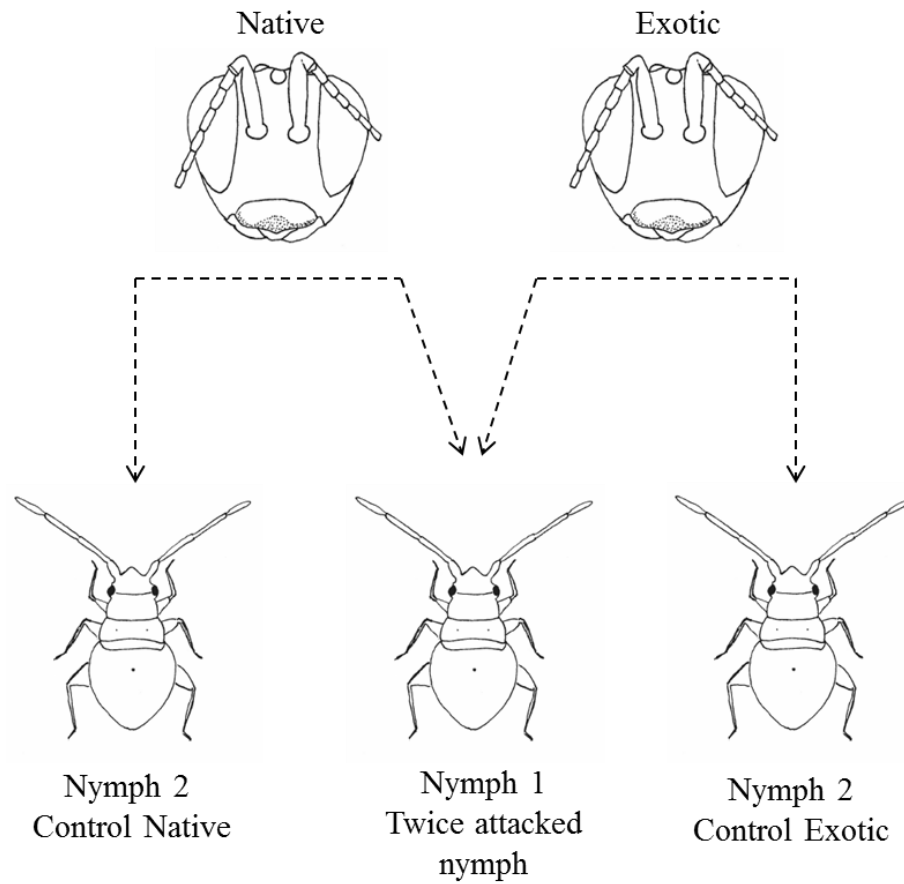
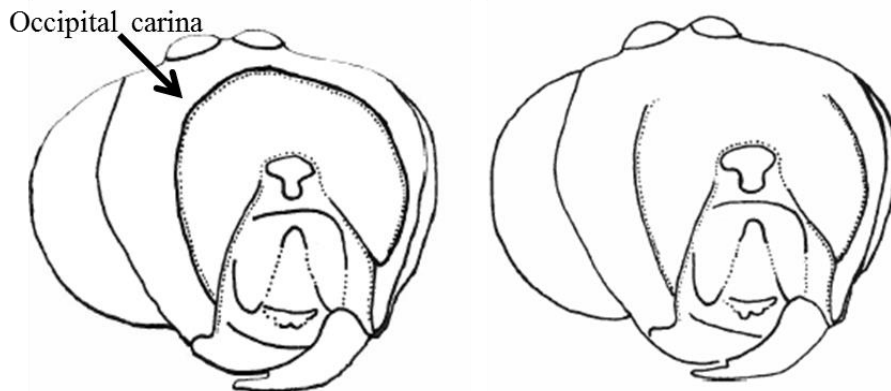


Figure 11. Method to test interspecific larval competition between native and exotic *Peristenus*.



Modified from Hymenoptera of the world (Goulet & Huber 1993)

Figure 12. Posterior view of a parasitoid's head showing the complete occipital carina (left) present in *P. digoneutis*, and an incomplete occipital carina (right) characteristic of native *Peristenus* species (Goulet & Huber, 1993).