ECOLOGY AND MANAGEMENT OF *LYGUS* AND THE DISTRIBUTION AND SPECIES COMPOSITION OF *PERISTENUS* SPP. IN WASHINGTON

By

TIMOTHY D. WATERS

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

DOCTOR OF PHILOSOPHY

WASHINGTON STATE UNIVERSITY
Department of Entomology
MAY 2009

To the Faculty of Washington State University:

The members of the Committee appointed to examine the dissertation of TIMOTHY DAVID WATERS find it satisfactory and recommend that it be accepted.

_____
Douglas B. Walsh, Ph.D., Chair

_____
Richard S. Zack, Ph.D.

_____
Keith Pike, Ph. D.

_____
David Horton, Ph.D.

ACKNOWLEDGEMENTS

I would like to take this opportunity to thank some of the individuals who have been of assistance through this process. First of all, I would like to thank Dr. Doug Walsh for his patience and willingness to serve as a mentor to me for the last several years. I would also like to thank Dr. Rich Zack for his advice and overseeing my affairs on campus and serving on my committee. I would like to thank my committee members Dr. Dave Horton and Dr. Keith Pike for assistance with the project and prompt revision recommendations on my dissertation. Thanks also go out to those who assisted me with the laboratory portion of my work including Dr. Tom Unruh, Kelly Thomson-Archer, and Dr. Laura Lavine. I would also like to acknowledge the numerous laboratory assistants who painstakingly assisted in the often tedious sample collection and analysis. I would like to thank my parents, Frank and Linda Waters for their support throughout my education endeavors. I would also like to thank Carin and Owen Waters for their support and encouragement to complete this project.

ECOLOGY AND MANAGEMENT OF *LYGUS* AND THE DISTRIBUTION AND SPECIES COMPOSITION OF *PERISTENUS* SPP. IN WASHINGTON

Abstract

by Timothy D. Waters, Ph. D.
Washington State University
May 2009

Chair: Douglas B. Walsh

Lygus hesperus Knight (Hemiptera: Miridae) is the perennial key direct pest in alfalfa seed production in the western US. *Lygus* populations left uncontrolled reduce seed yields and quality. *Lygus* spp. may colonize alfalfa fields from non-agricultural habitats and host plants adjacent to fields. We investigated if candidate host plants found in non-crop areas near alfalfa seed fields allowed reproduction and nymphal development by *L. hesperus*. Canada thistle (*Cirsium arvense* Scop.) and stinging nettle (*Urtica dioica* L.) were the plants that *Lygus* were able to complete their life cycle on more frequently than other plants evaluated. In a diverse agricultural landscape such as the Columbia Basin of central Washington, plants that are found in non-crop areas are not the only potential source for pest insect refuge. As such, several cover crop blends were evaluated for their potential to serve as host sources for *Lygus*. The highest numbers of *Lygus* spp. were collected from alfalfa *(Medicago sativa L.)* and northern birdsfoot trefoil (*Lotus corniculatus* L.), plots whereas very few were collected from perennial ryegrass (*Lolium perenne* L.) plots. This information could be quite useful to serve as an information guide in cropping systems where *Lygus* is a major pest and where integrated pest management plans are being implemented. An important parasitoid of *Lygus* in Washington state is the wasp

Peristenus howardi Shaw (Hymenoptera: Braconidae) and its congener species. Here we report parasitism rates of *Lygus* in alfalfa seed production and other habitats in Washington state, and provide preliminary methods to identify *Peristenus* spp. from larval parasitoids obtained in parasitized nymphs of *Lygus*. We collected *Lygus* nymphs from sites throughout southeastern Washington State to determine parasitism rates by *Peristenus* spp. in alfalfa seed and other crops. Over 15,000 *Lygus* nymphs were collected, and dissected to determine parasitoid presence in different geographic regions and on different host plant species. The dissections yielded small larval parasitoids that were not identifiable morphologically to species level. Therefore, we used PCR to amplify a region of the mitochondrial cytochrome oxidase 1 gene for species identification. We identified three species of *Peristenus* from *Lygus* nymphs in our samples.

TABLE OF CONTENTS

Page

LIST OF TABLES AND FIGURES

Host Plant Suitability for *Lygus* spp.

Distribution and Species Composition of *Peristenus*

Important Insects in Alfalfa Seed in Eastern Washington

Background

Alfalfa (*Medicago sativa* L.) is an introduced species to North America. It originated in Iran nearly 10,000 years ago, and its association with *Lygus* may be that old, as *Lygus* is still considered a major pest of alfalfa in Iran (Mirab-balou and Khanjani 2008). Alfalfa was brought to Europe and South America by invading armies, explorers, and missionaries as a valuable source of animal feed, and in 1850 Spanish cultivars of alfalfa germplasm were introduced into the southwestern United States from South America (Edminster et al. 2001). Between 1858 and 1910, three winter-hardy germplasm sources from Europe and Russia were brought into the upper midwestern United States and eastern Canada. There were two intermediate winter-hardy introductions, one from Iran, Afghanistan and Turkey between 1898-1925 and the other from France in 1947 (Edminster et al. 2001).

Over 74,000 acres of alfalfa is grown for seed annually in the United States with an average production of 79 million lbs. of seed (Anonymous 2005). Most of this production takes place in the western United States. Alfalfa seed is considered a minor crop, but it is the foundation of the alfalfa forage industry. Forage alfalfa ranks third in planted agricultural acres in the United States at 22.9 million acres annually (Anonymous 2005). In Washington State, 14,000 acres of alfalfa seed and 446,000 acres of alfalfa forage are planted annually (Schreiber 2008). The alfalfa seed crop is worth $14 million annually, while the alfalfa forage crop is worth $267 million annually (Schreiber 2008).

Fall dormancy is an important trait of alfalfa varieties, reflecting the crops growth in the fall in response to decreasing temperatures and day length (Teuber et al., 1998). Fall dormancy scores range from 1 to 11, with the lower-numbered varieties exhibiting less growth in the fall. Typical adaptation for alfalfa varieties are as follows: Fall dormancy (FD) scores of 1-4 for colder regions (Intermountain, northern US); FD 5-7 scores are typical for mild temperate regions (e.g. Sacramento Valley); varieties with FD 7-9 for warmer and Mediterranean regions (e.g. southern San Joaquin Valley); FD 8-11 varieties for hot desert zones (e.g. Arizona, Imperial Valley, Mexico) (Malinowski et al. 2007). Fall dormancy clearly influences stand persistence, adaptation, and performance (Malinowski et al. 2007). For example, non-dormant (FD 8-11) varieties will typically be killed by temperatures that occur in winters of the northern USA, whereas more dormant varieties typically have greater persistence (Malinowski et al. 2007). In Washington State, all dormancy groups of alfalfa seed can be produced (Wagoner pers. comm.), which is clearly an advantage to Washington alfalfa seed producers as few other regions have a climate that permits such a broad range of fall dormancy groups.

In Washington State, alfalfa for seed is planted in August - September or February - March with the first seed crop then harvested the following August (Hirnyck and Downey 2005). The crop is sown at a rate of 1-2 pounds of seed per acre and is planted with 22 or 30 inch row spacing. A stand may be viable for up to 5 years; stand longevity is most often dictated by the seed company which has contracted the sale of

the seed. Alfalfa seed is rotated with grains, dry peas, hay or vegetables (Wagoner pers. comm.). The crop is watered heavily in autumn and early spring with little to no irrigation occurring during the summer. Lack of irrigation prompts maximum flowering and reduces the likelihood of fungal plant pathogens. Traditionally, crop residue after harvest was burned the following winter, but that practice has become less utilized due to air quality concerns associated with burning. Alternatives for remaining crop residue are currently being investigated. Most herbicide applications are made in the fall, but a few late winter and early spring applications are made as well. The early spring applications not only control weeds, but also set the flowering period of the crop back for better synchrony with insect pollinator activity. This synchronization formerly was accomplished by swathing the crop early in the year, but this practice is not often utilized in Washington State since it is thought to lower yields.

Alfalfa seed production is generally limited by three major factors: arthropod pests, insect pollinators, and water, although adverse weather may override these factors (Baird et al. 1991). Cool windy weather can alter cross pollination of alfalfa seed, and can also significantly reduce bee activity and pollination of the crop. Heavy rain in July can reduce alkali bee populations, and heavy rain in August and September can significantly impact harvest of the crop by making the seed pods too wet to combine (Baird et al. 1991). Alfalfa seed production requires that growers not only produce a productive crop of alfalfa, but they must also successfully propagate pollinators for the seed crop. Conserving pollinators and protecting the crop from its most

devastating pest, *Lygus*, requires an effective integrated pest management (IPM) scheme. When seed prices are high, alfalfa seed production can be quite profitable, but when prices are static and input costs increase, profitability and sustainability of production are at risk.

The use of IPM principles in alfalfa seed was first implemented in the early 1970's (Baird et al. 1991). Producers and fieldmen had become convinced that the way they managed pests and beneficial insects in the past was not sustainable. The use of insecticides such as parathion and DDT was discontinued as newer products were identified that were safer to the beneficials while still proving lethal to *Lygus*. Sampling procedures and economic pest population levels were identified to further support the use of selective insecticides (Baird et al. 1991). IPM programs in alfalfa seed differed from most other IPM programs in that pest control efforts had to compatible with pollinator protection for producers to be successful.

Pollinators

The principle pollinators in alfalfa seed are the alfalfa leaf-cutter bee *Megachile rotundata* (F.) (Hymenoptera: Megachilidae) and the alkali bee *Nomia melanderi* Cockerell (Hymenoptera: Halictidae) (Peterson et al. 1992). Alfalfa leaf-cutter bees are used most as their biology is well understood, they are easily propagated and their emergence can be synchronized with crop flowering by controlling temperature and humidity of overwintered captive bees. Alkali bees are used less frequently as they are ground nesting bees that occur only in certain production regions, and

management and biology of the bees is still not well understood (Frick et al. 1960). Both species provide higher yields in alfalfa seed compared with the honey bee *Apis melifera* L. (Hymenoptera: Apidae), which is a rather ineffective pollinator of alfalfa (Menke 1954). Honey bees typically account for about 200 lbs. per acre of alfalfa seed yield, while growers who utilize alfalfa leaf-cutter bees or alkali bees average 1,000 - 2,000 lbs. of seed per acre (Wagoner pers. comm.). In the Touchet River Valley of Washington State, where a large portion of the states alfalfa seed is grown, some fields benefit from both alkali and alfalfa leafcutter bees and typically these fields are among the highest yielding regions for alfalfa seed production in the United States. Because the bees have such a significant impact on yield, it is imperative that growers understand the biology of the bees and carefully monitor bee-activity prior to applying insecticides. When bees are actively visiting alfalfa seed fields, growers generally apply insecticide during the evening to avoid poisoning them. Producers must also be aware of the mode of action of the insecticides they use and its persistence to avoid bee poisoning.

Alkali Bee *Nomia melanderi* Cockerell (Hymenoptera: Halictidae)

The alkali bee is the most efficient pollinator of alfalfa seed in the Pacific Northwest (Bohart 1950). Where alkali bees are abundant in south-central Washington, alfalfa seed yields of up to 1,800 pounds per acre have been reported from commercial fields (Frick et al. 1960). Alkali bees trip over 95% of the flowers they visit as where honey bees trip less than 1% (Menke 1954). Alkali bees show a preference for alfalfa blooms compared to blooming weeds (Menke 1954).

The alkali bee is native to the northwestern United States and occurs in arid areas west of the Rocky Mountains. The alkali bee is a solitary bee, but quite often is a gregarious nester; as many as 100 nests per square foot have been found in some areas (Frick et al. 1960). The alkali bee thrives in areas of alkali soil that receive subsurface irritation causing the upper surface of the soil to crust (Cane 2007). Adult alkali bees are slightly smaller than honey bees and have bright metallic green or blue bands on the abdomen (Frick et al. 1960). The prepupal phase of the alkali bee overwinters in the soil anywhere from 2 to 10 inches beneath the surface (Frick et al. 1960). The prepupae develop as soil temperatures warm and pupate in late May to early June. Adults begin to emerge in late June, with peak emergence occurring during the early part of July. Adult life span is 4 to 6 weeks (Stephen 1959). Nesting occurs in July and August. Cells are dug into the soil, and eggs are deposited upon a ball of pollen provided by the female. Females produce between 12 and 16 broods, of which about half become males. Alkali bees are limited to one generation per year in Washington and adults have a range of up to 5 miles (Stephen 1959).

Most alfalfa seed producers who have active alkali bee beds work to maintain the basic pH of the soil, control vegetation on the bed, control predators, and provide adequate subsurface irrigation. Predators and parasites that attack alkali bees include mice, skunks, birds, the tiger beetle (*Cicindella haemorrhagica* Lecarte), beefly (*Heterostylum robustum* (Osten Stacken), and conopid flies (*Zodion obliquefasciatum* Macquart) (Frick et al. 1960).

Leaf-cutter Bee *Megachile rotundata* (F.) (Hymenoptera: Megachilidae)

The leaf-cutter bee is an important pollinator of alfalfa seed in the western United States due to its preference for alfalfa flowers and relative ease of propagation (Stephen 1962). The leaf-cutter bee is believed to be native to Eurasia and was first recorded in the United States in 1937 (Stephen 1962).

Each female leaf-cutter bees is capable of pollinating up to 1/4 lb. of seed. Yields of up to 1,963 lbs. per acre are possible with this bee (Baird et al. 1991). Leaf-cutter bees prefer not to fly extreme distances for food; therefore, when producers place them near an alfalfa field in flower they can be sure that the bees are pollinating the field and not flying to others (Hobbs 1967). The alfalfa leaf-cutter bee, unlike the honey bee, is undeterred by the tripping mechanism in alfalfa flowers and prefers alfalfa over competing bloom from nearby crops or weeds (Peterson et al. 1992). Growers prefer leaf-cutter bees in most situations, because they can incubate them after a sufficient cold period to synchronize their emergence with flowering of the alfalfa seed crop (Baird et al. 1991).

Female leaf-cutter bees are about ¼ inch long, and black in color with short white hairs on the body (Baird et al. 1991). Male leaf-cutter bees are smaller than females and have buff colored hair with two light spots on the rear of their abdomen. Like the alkali bee, the leaf-cutter bee is a solitary bee that nests gregariously (Peterson et al. 1992). They overwinter in a pre-pupal stage and begin to emerge as adults in early

June (Klostermeyer 1964). Males usually emerge a week before the females (Baird et al. 1991). A few days after emergence, mating begins and females nest using existing holes about 3/16 of an inch in diameter in trees, soil, fence posts, or the pre-drilled bee boards (Klostermeyer 1964). The female chews circular disks off of the edges of leaves and manipulates these discs at the nest hole to form a thimble shaped cell. She then places nectar and pollen in each cell before laying an egg and closing the cell with a round leaf disc (Baird et al. 1991). Each tunnel has four to seven cells before the female moves on to nest in another tunnel. Adults can live up to two months and during this time the females produce 35 to 40 eggs. Leaf-cutter bees are mostly univoltine, but about 10-20% of the population emerges as a second generation late in summer (Baird et al. 1991).

Over 20 species of insects can prey upon leaf-cutter bees including minute chalcids (*Tetrastichus megachilidis* Burks), checkered flower beetles (*Trichodes ornatus* Say), and carpet beetles (*Trichoderma* spp.) (Baird et al. 1991). The most devastating pest of leaf-cutter bees, and the reason that producers do not keep artificial bee boards for more than two seasons, is chalkbrood fungus (Wagoner pers. comm.). Additionally, leaf-cutter bees are more susceptible to insecticides than are alkali or honey bees (Walsh unpubl.).

Pests

Lygus Bugs *Lygus hesperus* Knight (Hemiptera: Miridae)

Lygus hesperus attacks a broad range of hosts in the Pacific Northwest including fruit, vegetable, seed, tree, and fiber crops (Kelton 1975, Scott 1977, Johnson and Lyon 1988, Mayer et al. 1998) in addition to their persistence on a number of native and weedy non-crop plants (Berry 1998, Barlow et al. 1999, Goodell 2001). Once alfalfa fields are cut for hay, *Lygus* often fly to nearby fields to feed (Stoltz and McNeal 1982, Scott 1987). Forty-seven insecticides are registered for *Lygus* control on over one hundred different crops in Washington (Daniels 2005, Hollingsworth 2008).

Lygus is the primary insect pest of alfalfa seed in the Pacific Northwest, causing reduced seed yields if not controlled (Sorenson 1936). Feeding by *Lygus* causes several types of injury to alfalfa. *Lygus* feed by injecting their mouthparts into plant tissues and ingesting the contents of the tissue (Mayer and Johansen 1991). Feeding on flowers may cause floral bud abortion, while feeding on developing seed pods may make seeds unviable (Strong 1970). Seed feeding constitutes the major crop loss caused by *Lygus* in the alfalfa seed production system (Mayer and Johansen 1991). Late instar nymphs have been noted to feed significantly more than adults (Rosenheim et al. 2004). Additionally, female adult *L. hesperus* feed more than males (Rosenheim et al. 2004).

Lygus are a serious crop pest with cosmopolitan distribution (Gupta et al. 1980). In the western U.S., *Lygus hesperus* Knight (Hemiptera: Miridae) is the dominant species in

a complex that includes *L. elisus* Van Duzee*, L. keltoni* Schwartz and Footit, and *L. lineoloaris* (Palisot de Beauvois) (Clancy and Pierce 1966, Kelton 1982, Goodell 2001). Adult *L. hesperus* are a greenish brown, oval shaped insect about 1/4 inch long and somewhat dorsal ventrally flat (Kelton 1975). Immature forms are usually pale green or brown with later nymphs exhibiting five distinct black spots located dorsally on the abdomen (Kelton 1975). Nymphs resemble adult aphids in shape and size, but can be distinguished by their ability to move more quickly than aphids and they lack cornicles. *Lygus hesperus* over-winter as adults in the crown of short plants or in underlying debris which at time males are mahogany in color, while females take on a reddish hue (Kelton 1975). Overwintered adults become active in early spring. After mating, females oviposit their curved shaped eggs into the tissue of leaf midribs and stems of host plants (Day 1996). Populations of *L. hesperus* produce 3-6 generations per year.

Monitoring or assessing *Lygus* numbers in the field is done using a sweep net. Several 180° sweeps are taken then the net is opened and the nymphs and adults are counted and the average per sweep is determined. Economic thresholds in alfalfa seed crops change through the growing season. The established threshold in the Pacific Northwest is 3-5 *Lygus* per 180° sweep before seeds harden, to as many as 15 per 180° sweep after seeds have hardened (Mayer and Johansen 1991).

In alfalfa seed crops, insecticide applications solely targeting *Lygus* are typically recommended three times per season, one prior to alfalfa bloom, one during the

bloom, and the final after bloom during seed set and maturation. When *Lygus* numbers reach the established economic threshold, insecticide applications should be made using a ground sprayer at 20 gallons of water carrier per acre. Prior to bloom, applications can be made during the day, but as bloom begins, applications should be made exclusively in the evening after sunset to avoid killing leafcutter or alkali bees. Alfalfa seed producers must balance *Lygus* control with pollinator safety. This concern leads to the use of chemicals with shorter residual and reduced *Lygus* efficacy.

Alfalfa weevil *Hypera postica* (Gyllenhal) (Coleoptera: Curculionidae)

The alfalfa weevil is native to Europe and was first collected in the United States in 1904 (Baird et al. 1991). Both adults and larvae feed upon leaves and buds of alfalfa. Early instar larvae feed on the stem and work their way to the tip of the plant to feed on buds. Alfalfa produces secondary buds as a response to alfalfa weevil feeding, but with heavy infestations leaves are skelotonized and plant growth is stopped (Baird et al. 1991). Alfalfa weevil is most often a problem in areas with a short growing season.

Adult alfalfa weevils are light brown, 1/4 to 3/10 of an inch long, with a dark line down the center of the dorsum. Eggs are shiny, yellowish translucent and are laid 3-6 inches above the ground in the alfalfa stem in a hole chewed by the female (Baird et al. 1991). Larvae are dark green with a white stripe extending the length of the dorsum. Alfalfa weevil overwinter in the adult stage in field debris or other protected areas and become active as early as mid-February in the Columbia Basin (Baird et al.

1991). After mating, eggs are laid along the stem of alfalfa in clusters ranging from 10-40. Each female can lay from 600-800 eggs during her life. Three weeks is required for larvae to develop from an egg to pupa, and pupation takes approximately 10 days. Adults continue to feed on alfalfa throughout the summer until hibernation in early fall. The species is univoltine.

Alfalfa weevil is monitored using a sweep net in the same manner as described above for *Lygus*. The economic threshold for alfalfa weevil in alfalfa is 20 larvae per sweep (Hirnyck and Downey 2004). Third and fourth instar larvae cause most of the economic damage, so initiating sampling at the peak occurrence of second instars should be targeted (Blodgett and Peairs 2006). Development of alfalfa weevil increases at a constant rate as temperature rises above 48°F; it is recommended that sampling commence at 148 degree days base 48°F (Blodgett and Peairs 2006). Once the economic threshold is reached, alfalfa weevil can be controlled with pyrethroid insecticides (Hollingsworth 2008).

Pea Aphid *Acyrthosiphon pisum* (Harris) (Hemiptera: Aphididae)

Pea aphid is a native to Europe and was first detected in the United States in 1879 (Baird et al. 1991). Pea aphid prefers pea as a host, but will also feed on a number of other plants including alfalfa. Severe infestations of pea aphid on alfalfa seed will cause plants to wilt and turn yellow or in extreme cases kill the plant. In instances where more than 1,000 pea aphids per plant occur, flowers on the plant may drop.

Adult pea aphids are about 1/8 of an inch long, and are green, yellow-green, or pink in color (Baird et al. 1991). Pea aphids overwinter as eggs. Once they hatch, the nymphs molt four times before adulthood. In some warm regions, nymphs and adults may survive the winter. The number of offspring and rate of fecundity is dependent upon temperature. A female will produce as many as 150 offspring in her life time (Hirnyck and Downey 2005). In cool regions, males appear in the fall and mate with egg-producing females that in turn lay eggs on the stem of the alfalfa plant that overwinter. Overwintered eggs begin to hatch in March, and become stem mothers that produce winged females that eventually migrate or spread the population (Baird et al. 1991). Several generations per year are produced.

In alfalfa seed, pea aphid is monitored by sweep net or beating sheet. It has been estimated that the damage of 40 pea aphids per sweep is equal to that of one *Lygus* per sweep (Baird et al. 1991). Therefore, economic thresholds could be extrapolated as 120-200 aphids per sweep prior to seeds hardening. Pirimicarb (Pirimor) has been utilized for aphid control when chemical control is necessary.

Spotted Alfalfa Aphid *Therioaphis trifolii* (Monell) (Hemiptera: Aphididae)

Spotted alfalfa aphid prefers to feed on alfalfa, although they will infest some clovers. The aphid injects a toxic salivary secretion into the plants while feeding, which causes yellowing at the feeding site and along the veins of the leaf (Sloderbeck and Whitworth 2008). Spotted alfalfa aphid is the most important aphid pest on alfalfa grown for seed. Aphid feeding may kill seedlings, and will severely damage

established stands in susceptible varieties (Baird et al. 1991). In addition, the honeydew secreted during feeding leads to development of sooty mold on leaves and subsequent reduced plant productivity (Sloderbeck and Whitworth 2008).

Spotted alfalfa aphid is about 1/16 of an inch long and is pale yellow to gray in color with 4-6 rows of raised dark spots with small spines on the dorsum. Females produce up to 100 offspring, which develop through four nymphal instars in as few as five days. In Washington, spotted alfalfa aphids overwinter as adult females or nymphs on the taproot of alfalfa as deep as 4-5 inches below the soil surface (Baird et al. 1991). Males are produced in late fall. Maximum development rates occur at 85°F and 60-70% relative humidity. Winged forms have smoky areas along the veins of the wings. Spotted alfalfa aphid prefers lower portions of the alfalfa plant and can be found on the underside of the leaves (Sloderbeck and Whitworth 2008). Several overlapping generations per year are produced in Washington (Baird et al. 1991).

Monitoring is done with sweep net or beating sheet; examination of stems is used to sample for economic threshold densities. Thresholds for seedlings are 2-3 aphids per seedling; for established stands the threshold is as many as 20-40 aphids per stem (Baird et al. 1991).

Two-spotted spider mite *Tetranychus urticae* Koch (Acari: Tetranychidae)

Two-spotted spider mite is a pest of a large number of crops in the United States. Damage levels in alfalfa seed vary from season to season. Two-spotted spider mite

damage can be extensive with hot dry weather, and ecological disruption as a result of the use of broad spectrum insecticides directed to other pests. Alfalfa seed is prone to two-spotted spider mite infestation since the crop is only irrigated in the early spring and is allowed to dry in the summer. Plants infested with two-spotted spider mite will have a characteristic stippling on the leaves from the mite feeding. When infestations are severe, the silken webs produced by the mite may cover leaves, flowers and buds, and interfere with crop pollination (Baird et al. 1991), and lead to severe wilting or death (Berry 1998).

Two-spotted spider mites are about 1/50 of an inch and range in color from light yellow or green to dark green or brown. The mite has two distinct dark spots on the cephalothorax. Males are more active, and are smaller than females with a narrower body with a more pointed abdomen and longer legs (Berry 1998). Females lay their eggs in webbing on the underside of the leaves. They produce up to 20 eggs per day and up to a total of 120 eggs, depending on the host plant and temperature. The spherical eggs are transparent when first deposited, but gradually turn yellowish (Baird et al. 1991). Eggs hatch in 3-15 days producing six-legged colorless nymphs. The next two nymphal instars are pale yellowish to green with 8 legs, and with two dark spots visible. Mites mature into adults in as few as 5 days during hot, dry weather. Adults live about two weeks during the summer, but much longer as temperatures decline (Baird et al. 1991). Multiple overlapping generations are produced each season. Two-spotted spider mites overwinter as non-feeding females,

and are bright orange or red. Development stops when temperatures drop below about 55°F.

Mites should be chemically controlled before they reach high populations densities, since high densities are difficult to control with any treatments. For monitoring and assessing mites in alfalfa grown for seed, leaflets should be removed and the underside of leaves examined with a hand lens to look for the presence of mites. Infestations in late spring and early summer are of most concern, whereas as later season (late July) outbreaks should not have significant impact on the crop. Producers and fieldmen can also watch for stippled leaves and webbing as an indicator of mite activity, but mites will be much more difficult to manage if they are allowed to reach populations that are observed this readily. When viewing mites, it is important to note the stage and be able to discern predatory mites from two-spotted spider mite. Avoiding broad spectrum insecticide use early in the season will help conserve natural enemies and decrease the likelihood of a mite outbreak.

Predators

Minute pirate bug *Orius tristicolor* (White) (Hemiptera: Anthocoridae)

Minute pirate bug is an important predator of mites and thrips in alfalfa seed fields in Washington State. This predator also feed on small aphids and early instar *Lygus* nymphs (Baird et al. 1991). It can be abundant if care is taken when choosing insecticides for pest insect control.

The minute pirate bugs are small (1/16 inch long), a true bugs with a distinctive checkered white and black pattern as an adult; nymphs are orange and extremely small and difficult to see. Both adults and late instar nymphs are predacious and grasp their prey with their front legs, utlilizing piercing sucking mouthparts to extract the contents of their prey (Schuh and Slater 1995). Minute pirate bugs overwinter as mated adult females in and around sheltered areas such as trees, buildings, and plant material. In early spring, females deposit eggs clear to white in color in alfalfa plant tissues (Wright 1994). Each female can produce up to 100 eggs. The eggs hatch within 5 days, and develop through 5 nymphal instars before becoming adults (Schuh and Slater 1995). This process takes anywhere from 9-25 days depending on temperature. Adults live between 3-4 weeks (Wright 1994). There are four generations per year on alfalfa seed in Washington State (Baird et al. 1991).

Western Bigeyed Bug *Geocoris pallens* Stål (Hemiptera: Geocoridae)

Western bigeyed bug is the most common bigeyed bug and the most important predator of *Lygus* in alfalfa seed in Washington State (Baird et al. 1991). The bugs appear in alfalfa seed in early summer when *Lygus* populations begin to reach economically damaging levels (Baird et al. 1991). Western bigeyed bug is an important predator of *Lygus,* aphids, and leafhoppers in alfalfa seed, but may also feed on spider mites. The bugs seem to prefer aphids, so when aphid numbers build, the effectiveness of predation on *Lygus* is diminished (Baird et al. 1991).

Western bigeyed bug is 1/8 inch long, dull brown to grey in color, with large protruding eyes (Schuh and Slater 1995). It overwinters as an adult in and around sheltered areas such as trees, buildings, and plant material (Baird et al. 1991). In Washington State, there are three generations per year. The eggs are somewhat oval shaped, grayish-white with red eyespots that become noticeable just prior to hatching (Schuh and Slater 1995). Eggs are laid singly on leaves or stems and hatch in approximately one week. They have five nymphal instars, each lasting from 4 to 6 days (Schuh and Slater 1995). Adults live approximately one month and a female can lay up to 300 eggs during her life. Both adults and nymphs are predacious and grasp their prey with their front legs and utilize piercing-sucking mouthparts to extract the contents of their prey (Schuh and Slater 1995).

Crab spiders (Aranae: Thomisidae)

Crab spiders are the most commonly occurring spiders in alfalfa seed fields (Jorgensen 2005). While crab spiders consume *Lygus*, there is little information on the quantity of *Lygus* they consume in alfalfa seed fields. It is likely that they consume a large number of pollinators in addition to pest insects. In many terrestrial communities crab spiders are among the dominant predators and exert significant pressure on populations of specific prey (Young and Edwards 1990).

Crab spiders are free living and as the name implies somewhat resemble crabs in shape having short wide bodies with large forelegs that are held outward. Most crab spiders are cursorial hunters which do not use silk for prey capture, but instead

ambush and wait until prey comes within reach (Kaston 1953). Though their jaws are small and slender, many crab spiders possess potent venoms that quickly immobilize their prey. Most crab spiders are less than 2/5 inch long and can move forward, backward, or sideways much like crabs do (Coddington and Levi 1991).

Beneficial Parasitoids

***Peristenus* spp. (Hymenoptera: Braconidae)**

Peristenus spp. are parasitoids of *Lygus* in the Pacific Northwest. Surveys conducted in Idaho in 1997 and 1998 reported parasitism rates as high as 81% by *P. howardi* Shaw on *L. hesperus* nymphs in alfalfa grown for seed (Day et al. 1999). Surveys were also conduced in Oregon and Washington in 1996 and 1997. No parasitoids were collected in Oregon, but parasitism rates ranged from 28 to 70% in Washington (Mayer et al. 1998). Further work in Idaho in 1999 showed parasitism rates as high as 75% (Colgrove and Bitner 1999). Intentional releases of *P. digoneutis* Loan took place in the eastern United States as early as 1979, where they were shown to reduce some *Lygus* populations (Coulson 1987). Intentional releases of *P. digoneutis* and *P. relictus* also took place in California in 1998, 1999, and 2000 and the two species were successfully established and reduced *Lygus* numbers (Picket et al. 2007). Results from a 2000 survey of *Lygus* parasitism by *Peristenus* spp. in Idaho and Nevada were less-than-promising. About 10% of the *Lygus* nymphs collected in Idaho and none of those collected in Nevada showed evidence of parasitism (Ball et al. 2001). High parasitism rates, as shown by Colgrove and Bitner (1999) in Idaho, naturally led researchers to speculate on the practical application of the *Braconid* wasp as a biological control

agent for *Lygus* in alfalfa seed. There has been speculation that introductions of non-native *Peristenus* could displace the native *Peristenus* in different regions where biological control of *Lygus* is desirable (Mayer et. al 1998, Broadbent et. al 2006). It has also been found that *Lygus* living in weedy areas are more heavily infested with *Peristenus* than *Lygus* found in alfalfa (Lim and Stewart 1976). Though, as larvae, the species of *Peristenus* cannot be discerned morphologically (Goulet and Mason 2006), there are molecular methods available for separating species (Tilmon et al. 2000, Mowry and Barbour 2004, Zhu et al. 2004).

Previously, there was some confusion as to whether the parasitoid in Washington was *Peristenus howardi* Shaw or *Peristenus pallipes* (Curtis) (Mayer et al. 1998). Larval and pupal forms are structurally similar between species and therefore impossible to differentiate morphologically (Bilewicz-Pawinska 1974, Carnigan et al. 1995). *Peristenus* spp. are similar in biology and life history. Adult parasitoids emerge in late spring and insert eggs into first to third instar *Lygus* nymphs (Lim and Stewart 1976). The larvae feed internally in the host's abdomen, but do not cause mortality until their hosts begin to transition from fifth instar to adult. The parasitoid then emerges from the host and falls to the ground to form a pupal cocoon in ground debris (Brindley 1939). *Peristenus howardi* Shaw is a recently described species believed to be native to the Pacific Northwest (Goulet and Mason 2006). In Idaho, where it was originally discovered, *P. howardi* produces three generations per year (Day et al. 1999). *Peristenus howardi* is a small black wasp about 1/10 of an inch in length with a large proportion of recorded adults being females (Ball et al. 2001). Adults of *P. howardi* are

readily discernible from adults of *P. pallipes* (Goulet and Mason 2006). The reproductive biology and life stages of *P. howardi* are believed to be similar to those in *P. pallipes*, although more studies are required for a more complete understanding.

Monitoring *Peristenus* in the field is done in a couple different ways. Adults are collected using sweep nets, but due to the small size and low population density of the wasp, detection can be difficult. A better approach to determining abundance is to collect late instar *Lygus* nymphs and dissect them for the presence of larval *Peristenus*.

References Cited

Anonymous 2005. United States Department of Agriculture, National Agricultural Statistics Service. Retrieved from World Wide Web on December 30, 2005: http://www.nass.usda.gov/Statistics_by_State/index.asp

Baird, C. R., J. Berg., R. M. Bitner, G. Fisher, C. Johansen, D. A. Johnson, L. P. Kish, J. Krall, R. Lauderdale, D. F. Mayer, J. H. O'Bannon, R. Parker, R. Peaden, C. M. Rinker, W. P. Stephen, and J. Undurraga, 1991. Alfalfa seed production and pest management. Western Region Extension Publication 12.

Ball, J. C., C. H. Pickett, C. R. Baird, and J. Knight. 2001. Search for Native Parasitoids of *Lygus hesperus* in Idaho and Nevada. In Biological Control Program Annual Summary, 2000, Dale M. Woods, Ed. California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, California. 78 pp. Retrieved from World Wide Web on January 10, 2008: http://www.cdfa.ca.gov/phpps/ipc/biocontrol/annuals/2000annual/2000annual.pdf.

Barlow, V. M., L. D. Godfrey, and F. E. Norris. 1999. Population Dynamics *of Lygus hesperus* (Heteroptera: Miridae) on Selected Weeds in Comparison with Alfalfa. Journal of Economic Entomology. 92: 846-852.

Berry, R. E. 1998. Insects and Mites of Economic Importance in the Northwest. 2nd Ed. 221 p.

Bilewicz-Pawinska, T. 1974. Larvae of *Peristenus* Foerster (Hymenoptera: Braconidae) parasitic on *Lygus rugilipennis* Popp. (Heteroptera: Miridae). Polskie Pismo Entomologiczne. 44: 759-764.

Blodgett, S. and F. Peairs. 2006. Alfalfa Weevil Management. Montana State University Extension Publication. Alfalfa X-1 Retrieved from World Wide Web on February 26, 2009:http://ipm.montana.edu/Training/PMT/2006/AlfalfaWeevil.pdf

Bohart, G. E., 1950. The alkali bee Nomia meladeri Ckll., a native pollinator of alfalfa. Reprint 12th Alfalfa Improvement Conference, Lethbridge, Canada.

Brindley, M. D. 1939. Observations on the life-history of *Euphoris pallipes* (Curtis) (Hymenoptera: Braconidae) a parasite of Hemiptera-Heteroptera. Proceedings of the Royal Entomological Society of London. (A) 14: 51-56.

Broadbent, A., S. Lachance, M. Sears, M., & H. Goulet. 2006. Native Braconid parasitism of the Tarnished plant bug (Hemiptera: Miridae) in Southern Ontario. Biocontrol Science & Technology, 16(7), 687-698.

Cane, J.H. 2007. Alkali Bee. p.11-12. In: Shimanuki, H., Flottum, K., Harman, A., editors. ABC & XYZ of Bee Culture. 41st edition. Medina, OH. A.I. Root Company.

Carnigan, S., G. Boivin, and R. K. Stewart. 1995. Developmental Biology and Morphology of *Peristenus digoneutis* Loan (Hymenoptera: Braconidae: Euphorinae). Bioligical Control 5: 553-560.

Clancy, D. W., and H. D. Pierce. 1966. Natural enemies of some *Lygus* bugs. Journal of Economic Entomology 59: 853-858.

Coddington, J.A. and H.W. Levi. 1991. Systematics and evolution of spiders (Araneae). Annual Review of Ecology and Systematics 22:565–592.

Colgrove, E. and R. Bitner. 1999. Idaho *Lygus* Parasites: *Lygus* Parasitoid Progress Report for Summer 1999. International Pollination Systems. Retrieved from World Wide Web on February 26, 2009: http://www.pollination.com/PublicationsResearch/idaho*Lygus*parasites.htm.

Coulson, J. R. 1987. Studies on the biological control of plant bugs (Heteroptera: Miridae): an introduction history, 1961-1983, pp. 1-12. In Economic importance and biological control of *Lygus* and *Adelphocorus* in North America, R.C. Hedlund and H. M. Graham. U. S. Department of Agriculture, Agricultural Research Service 64. National Technical Bulletin, Springfield, VA.

Daniels 2005. Pesticide Information Center On-Line (PICOL). Search titled: Chemicals Registered for *Lygus*. Retrieved from World Wide Web on November 4, 2005: http://picol.cahe.wsu.edu/labels/Labels.php

Day, W. R. 1996. Evaluation of the biological control of the tarnished plant bug (Heteroptera: Miridae) in alfalfa by the introduction of *Peristenus digoneutus* (Hymenoptera: Braconidae). Environmental Entomology 25: 512-518.

Day, W., C. Baird, and S. Shaw. 1999. New, native species of Peristenus (Hymenoptera: Braconidae) parasitizing *Lygus hesperus* (Heteroptera: Miridae) in Idaho: biology, importance, and description. Annals of the Entomological Society of America, 92: 370-375.

Edminster, C., D. Miller, and J. Moutray. 2001. Importance of Alfalfa. *in* Proceedings China Alfalfa Development Conference. China Grassland Society, Beijing Agriculture Committee. May 10-14, 2001.

Frick, K. E., H. Potter, and H. Weaver. 1960. Development and Maintenance of Alkali Bee Nesting Sites. Washington Agriculture Experiment Stations Institute of Agricultural Science. Stations Circular 366: 10 pp.

Goodell, P. B. 2001. Managing *Lygus* in the landscape. Kerney Agricultural Center Plant Protection Quarterly 11: 8-11.

Goulet, H. and P. G. Mason. 2006. Review of the Neartic Species of *Leiophron* and *Perisentus* (Hymenoptera: Braconidae) parasitizing *Lygus* (Hemiptera: Miridae: Mirini). Zootaxa 1323: 118 pp.

Gupta, R. K., G. Tamaki, and C. A. Johansen. 1980. *Lygus* bug damage, predator-prey interaction and pest management implications on alfalfa grown for seed. Washington State University Technical Bulletin 92. 18 pp.

Hirnyck, R. and L. Downey. 2005. Pest Management Strategic Plan for Western U. S. Alfalfa and Clover Seed Production. A summary of a workshop conducted February 18, 2004. Boise, ID.

Hobbs, G. A. 1967. Domestication of alfalfa leaf-cutter bees. Canada Department of Agriculture. Publication 1313: 19 pp.

Hollingsworth, C. S. 2008. Pacific Northwest Insect Management Handbook. Oregon State University. 665 pp.

Johnson, W. T. and H. H. Lyon. 1988. *Trees and Shrubs*. Second Edition. Ithaca, New York: Cornell University Press.

Jorgensen, A. E. 2005. Conserving Natural Enemies to control Lygus is Washington State Alfalfa Fields. Masters Thesis, Washington State University, Department of Entomology.

Kaston, B. J. 1953. How to know the spiders. WM. C. Brown Company Publishers. Dubuque, IA. 220 pp.

Kelton, L. A. 1975. The *Lygus* bugs (genus *Lygus* Hahn) of North America (Heteroptera: Miridae). Memoirs Entomological Society of Canada. 95.

Kelton, L. A. 1982. Plant bugs on fruit crops in Canada (Heteroptera: Miridae). Agriculture Canada Monograph No. 24. 201 pp.

Klostermeyer, E. C. 1964. Using alakali and leaf-cutter bees to pollinate alfalfa. Washington State University Agricultural Experiment Station Circular 442: 8 pp.

Lim, K. P. and R. K. Stewart. 1976. Parasitism of the Tarnished Plant Bug, *Lygus lineolaris* (Hemiptera: Miridae), by *Peristenus pallipes* and *P. Pseudopallipes* (Hmenopotera: Braconidae). Canadian Entomologist 108: 607-608.

Malinowski, D. P., W. E. Pinchak, B. A. Kramp, H. Zuo, and T. J. Butler. 2007. Supplemental Irrigation and Fall Dormancy Effects on Alfalfa Productivity in a

Semiarid, Subtropical Climate with a Bimodal Precipitation Pattern. Agron J. 99: 621-629.

Mayer, D. F., and C. Johansen. 1991. Integrated insect control practices, *In* Alfalfa Seed Production and Pest Management, Western Regional Extension Publ. 12.

Mayer, D. F., C. R. Baird, and B. Simko. 1998. Parasitism of *Lygus* spp. (Hemiptera: Miridae) by *Peristenus* (Hymenoptera: Braconidae) in the Pacific Northwest. Journal of the Entomological Society of British Columbia 95: 53-57.

Menke, H. F. 1954. Insect Pollination in Relation to Alfalfa Seed Production in Washington. Washington Agriculture Experiment Stations State College of Washington. Bulletin 55: 24 pp.

Mirab-balou, M. and M. Khanjani. 2008. Harmful Hemiptera of *Lygus* genus (Miridae, Hemiptera) on alfalfa (*Medicago sativa* L.) in Hamedan Province (Western Iran). Journal of Plant Protection Research 48 (3): 313-322.

Mowry, T. M., J. D. Barbour. 2004. Distinguishing the Parasitic Wasp, *Peristenus howardi*, From Some of its Congeners Using Polymerase Chain Reaction and Restriction Endonuclease Digestion. Journal of Insect Science 4:25.

Peterson, S. S., C. R. Baird, and R. M. Bitner. 1992. Current status of the alfalfa leaf-cutter bee, *Megachile rotundata*, as a pollinator of alfalfa seed. Bee Science 2: 135-142.

Pickett, C.H., R. Rodriguez, J. Brown, D. Coutinot, K. A. Hoelmer, U. Kuhlmann, H. Goulet, M. D. Schwartz, and P. D. Goodell. 2007. Establishment of *Peristenus digoneutis* and *P. relictus* (Hymenoptera: Braconidae) in California for the control of *Lygus* spp. (Heteroptera: Miridae). Biocontrol Science and Technology 17: 261–272.

Rosenheim, J. A., R. E. Gomez, and E. F. Thatcher. 2004. Omnivore or herbivore? Field observations of foraging *Lygus hesperus* (Hemiptera: Miridae). Environmental Entomology 33(5): 1362-1370.

Scott, D. R. 1977. An annotated listing of host plants of *Lygus hesperus* Knight. Bulletin of the Entomological Society of America 23: 19-22.

Scott, D. R. 1987. Biological control of *Lygus* bugs on vegetable and fruit crops, pp. 40-47. *In* R.C. Hedlund and H. M. Graham, Economic importance and biological control of *Lygus* and *Adelphocorus* in North America. U. S. Department of Agriculture, Agricultural Research Service 64. National Technical Bulletin 15: 159-169.

Schuh, R.T., and J. A. Slater. 1995. True Bugs of the World (Hemiptera: Heteroptera*)*. Cornell University Press.

Screiber, A. S. 2008. Alfalfa and Alfalfa Seed Production. In The Washington Ag. Project. Retrieved from World Wide Web on January 12, 2009: http://www.snakeriver.org/wscpr/index.cfm.

Sloderbeck, P. E. and R. J. Whitworth. 2008. Alfalfa Insect Management – Spotted Alfalfa Aphid – Kansas State Research and Extension Bulletin. 3 pp.

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

Stephen, W. P. 1959. Maintaining alkali bees for alfalfa seed production. Oregon State College. Station Bulletin 568: 23 pp.

Stephen, W. P. 1962. Propagation of the leaf-cutter bee for alfalfa seed production. Oregon State College. Station Bulletin 586: 16 pp.

Stoltz, R. L., and C. D. McNeal, Jr. 1982. Assessment of Insect Emigration from Alfalfa Hay to Bean Fields. Environmental Entomology 12: 578-580.

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

Teuber, L.R., K. L. Taggard, L. K. Gibbs, M. H. McCaslin, M. A. Peterson, and D.K. Barnes. 1998. Fall dormancy. *In* C.C. Fox et al. (ed.) Standard tests to characterize alfalfa cultivars. Retrieved from World Wide Web on February 20, 2008: www.naaic.org/stdtests/Dormancy2.html North American Alfalfa Improvement Conference.

Tilmon, K. J., B. N. Danforth, W. H. Day, and M. P. Hoffman. 2000. Determining Parasitoid Species Composition in a Host Population: A Molecular Approach. Annals of the Entomological Society of America 93(3): 640-647.

Wagoner, M. Conversation March 7, 2006. Discussing alfalfa seed production history in the Touchet River Valley, Touchet, WA.

Wright. R. 1994. Know Your Friends: Minute Pirate Bugs. Midwest Biological Control News. Vol. 1 No. 1, p. 3.

Young, O.P. & G.B. Edwards. 1990. Spiders in United States field crops and their potential effect on crop pests. Journal of Arachnology 18:1–27.

Zhu, Y. C., E. W. Riddick, L. Williams, III, D. J. Schotzko, A. L. Guillermo, and C. G. Jackson. 2004. Potential Detection and Identification of Nymphal Parasitoids (Hymenoptera: Braconidae) of *Lygus* Bugs (Heteroptera: Miridae) by Using Polymerase Chain Reaction and ITS2 Sequence Analysis Techniques. Annals of the Entomological Society of America. 97(4): 743-752.

Host Plant Suitability for *Lygus* spp.

Abstract

Lygus hesperus Knight (Hemiptera: Miridae) is the perennial key pest in alfalfa seed production in the western United States. *Lygus* populations left uncontrolled have been documented to reduce seed yields and quality. *Lygus* spp. may colonize alfalfa fields from non-agricultural habitats and host plants adjacent to fields. We investigated if candidate host plants found in non-crop areas near alfalfa seed fields allowed reproduction and nymphal development by *L. hesperus*. The plant species monitored were selected on the basis of preliminary studies where both nymphs and adults of *Lygus* had been collected. Replicated plots of host plants were established at the Washington State University Irrigated Agriculture Research and Extension Center (IAREC) near Prosser, Benton County, WA. Adult *L. hesperus* were introduced onto caged plants and monitored to determine if a second generation was produced. Canada thistle (*Cirsium arvense* Scop.) and stinging (*Urtica dioica* L.) nettle were the plants that *Lygus* were able to complete their life cycle on more frequently than other plants evaluated. In a diverse agricultural landscape such as the Columbia Basin of central Washington, plants that are found in non-crop areas are not the only potential source for pest insect refuge. As such, several cover crop blends were evaluated for their potential to serve as host sources for *Lygus*. The highest numbers of *Lygus* spp. were collected from alfalfa *(Medicago sativa L.)* and northern birdsfoot trefoil (*Lotus corniculatus* L.), plots whereas very few were collected from perennial ryegrass (*Lolium perenne* L.) plots. This information could be quite useful to serve as an information guide in cropping systems where *Lygus* is a major pest and where

integrated pest management (IPM) plans are being implemented, both locally and in area wide management programs.

Introduction

Over 30,000 ha of alfalfa seed are produced annually in the United States with an average production of 36 million kg of seed (Anonymous 2005). Most of this production takes place in the western United States. Alfalfa seed is considered a minor crop, but it is the foundation of the alfalfa forage industry which ranks third in planted agricultural acres in the United States at 9.3 million ha annually and subsequently impacts the dairy and beef cattle industries as a major feedstock (Anonymous 2005).

Lygus spp. are serious crop pests, many species with cosmopolitan distributions (Gupta et al. 1980). In the western United States, *Lygus hesperus* Knight (Hemiptera: Miridae) is the dominant species in a complex that includes *L. elisus* Van Duzee*, L. keltoni* Schwartz and Footit, and *L. lineoloaris* (Palisot de Beauvois) (Goodell 2001). Species of Lygus are established pests of a number of agronomic crops in Washington. *Lygus hesperus* is known to attack a broad range of host plant species in the Pacific Northwest including fruit, vegetable, seed, tree, and fiber crops (Kelton 1975, Scott 1977, Johnson and Lyon 1988, Hollingsworth 2008) in addition to their persistence on a number of native and weedy non-crop plants (Fye 1980, Berry 1978, Barlow et al. 1999, Goodell 2001). Forty-seven insecticides are registered for *Lygus* control on over one hundred different crops in Washington (Daniels 2005). *Lygus*

bugs are the primary insect pests of alfalfa seed in the Northwest U.S., causing reduced seed yields if not controlled. Feeding on alfalfa flowers may cause floral bud abortion, while feeding on developing seed pods may make seeds unviable. Seed feeding constitutes the major crop loss caused by *Lygus* in the alfalfa seed production system (Mayer and Johansen 1991). In Washington State many crop and non-crop host plants of species of *Lygus* grow in close proximity to alfalfa seed fields. Small streams and rivers often are found near alfalfa seed fields, and the riparian areas adjacent to the waterways are vegetated with non-crop plants for erosion control and other reasons. Many of the plants selected for these studies are found in the riparian areas. Some of these plants have been noted as hosts for species of *Lygus* while others have not (Scott 1977). Alfalfa forage fields and flowering seasonal weeds may be particularly problematic. When alfalfa forage fields are cut, dry out, or disturbed by spraying or mowing, adult *Lygus* will migrate to alfalfa seed production fields when present. We assessed host plant suitability of *Lygus* in alfalfa seed producing regions in the Pacific Northwest to provide growers with an understanding of which plants and habitats may provide refuge for *Lygus* populations. Improved understanding of source habitats and host plants from which *Lygus* colonize alfalfa is intended to assist growers in controlling the pest, by allowing them to make informed decisions about managing non-crop habitats or timing insecticide applications.

Materials and Methods

Riparian *Lygus* Host Plant Studies

Seventeen plant species including forbs, grasses, and woody perennials that commonly occur in Eastern Washington State riparian habitats that border alfalfa fields were chosen to assess their suitability for *Lygus*. The plants evaluated were: dogwood *(Cornus alba* L.*)*, willow *(Salix pur* L.*)*, Woods rose (*Rosa woodsi* Lindl.), rugosa rose (*Rosa rugosa* Thunb.), Nutkana rose (*Rosa nutkana* Presl), clematis (*Clematis ligusticifolia* Nutt.), stinging nettle (*Urtica dioica* L.), yarrow (*Achillea millifolium* L.), sagebrush (*Artemisia tridentata* Nutt), green rabbitbrush (*Chrysothamnus viscidiflorous* Nutt.), gray rabbitbrush (*Chrysothamnus nauseosus* Britt.), Oregon grape (*Mahonia repens* Lindl.), Canada thistle (*Cirsium arvense* Scop.), reed canary grass (*Phalaris arundinacea* L.), chokecherry (*Prunus virginiana* L.), honey suckle (*Lonicera tatarica* L.), and saltbush (*Atriplex canesenes* Nutt.). Nursery stock of some of the plants chosen from the study were obtained (Lawyer Nursery, Connell, WA) and planted in one meter square replicated blocks in June 2003. The remaining plants, not available through commercial sources, were transplanted from riparian habitats. The plants were placed (caged) in six replicated blocks located on the Washington State University Irrigated Agriculture Research and Extension Center in Prosser, Benton County, WA (WSU IAREC) and irrigated as needed through a low pressure micro-sprinkler irrigation system. The plots were one meter square, separated from each other by one meter. The plants were periodically pruned to keep the vegetation within the cages. The plots were maintained and utilized for the *Lygus* host plant study during the 2005 and 2006 field seasons.

Lygus used in the study were collected from an alfalfa forage field at the WSU IAREC. A sub-sample of specimens were taken from the source population and identified as *L. hesperus*. However, other species of Lygus may have been in the samples used in these studies. Several weeks prior to *Lygus* introductions, individual plants were caged with wire and covered with Econet LS insect-proof mesh (Ludvig Svenson Econet LS U.S. Global Resources, Seattle, WA). Forty-eight hours prior to *Lygus* introductions, cages were opened from the top and treated with naled (Dibrom 8E) (Amvac Chemical Corporation, Newport Beach, CA) insecticide at a rate of 1.17 L/ha in order to eliminate any insects that were present. Cages were closed to prevent insect invasion or colonization. After 48 hours, *Lygus* were introduced into cages at a rate of fifty adults (25 male, 25 female) per cage on two dates (July 1, 2005 and July 31, 2006). Previous studies by Pickel et al. (1990) showed that the number of degree days required for *Lygus* to develop from the egg to the adult stage was 623 using 54 degrees F as the threshold for development. For this study, 500 degree days were used for the evaluation since there was only enough time for mating, oviposition, and development of nymphs, but not enough time for adult development to occur. After 500 degree-days an Allen Vac (Osborne and Allen 1999) was used to aspirate insects from the cages and determine the presence or absence of second-generation *Lygus*. The Allen vac was operated in each cage for one minute. The collection of *Lygus* nymphs from the specific species of caged plants implied that development of *Lygus* was feasible on that plant species in these no-choice tests.

The numbers of *Lygus* collected were compared among plant species using one-way ANOVA with Minitab 14.0 Software. When the ANOVA was significant, means were separated using Fishers PLSD. Data were transformed for analysis using a Log(x+1) transformation in order to normalize the distribution of the data. Means and standard errors are reported as they were before transformation.

Cover Crop *Lygus* Host Plant

During the spring of 2003, twelve different cover crops were established at WSU IAREC. These included: Mammoth red clover (*Trifolium pratense* L.), northern birdsfoot trefoil (*Lotus corniculatus* L.), a native grass blend *(Stipa comata* Trin. & Rupr.*, Panicum occidentale* Scribn.*, Festuca idahoensis* Elmer*, Agropyron spicatum* Scribn. & Smith*),* perennial ryegrass (*Lolium perenne* L.), strawberry clover (*Trifolium fragiferum* L.), white Dutch clover (*Trifolium repens* L.), alfalfa (*Medicago sativa* L.), alfalfa and perennial ryegrass mix, alsike clover (*Trifolium hybridium* L), delar small burnett (*Sanguisorba minor* Scop.), ladino clover (*Trifolium repens* L), and Bug-n-breakfast (a blend of barley (*Hordeum vulgare* L.), rape (*Brassica napus* L.), and buckwheat (*Fagopyrum esculentum* Moench). Seed was obtained from Grasslands West (Clarkston, WA) and Integrated Fertility Management (Wenatchee, WA) and planted at suggested rates using a Scotts Brand broadcast fertilizer spreader and soil incorporated just beneath the soil surface with a standard tine harrow. Plots were irrigated using solid set hand lines applying 2 cm of water once per week from April 1 through October 15. During the 2004 and 2005 seasons, when the majority of the plots reached a meter in height, they were mowed to

approximately 16 cm using a tractor mounted rotary mower. Plots were 7.6 x 10.6 m, replicated five times in a randomized complete block design with 2 m separating plots. During the establishment season, selective herbicides were applied in order to achieve weed control and ensure a uniform stand. No fertilizers or insecticides were applied to the plots during the experiment.

Every other week during the growing seasons (April to October) of 2004 and 2005 each plot was sampled for *Lygus* by sweeping a 38 cm diameter heavy duty sweep net five times (180° sweep) through each plot. Samples were placed in a brown paper bag and stapled shut. Samples were then labeled with date and location, and placed on ice for transportation to the lab where they were stored in a -4°C freezer until the contents could be sorted. The sorting was done under a 0.7-30x Stereo-Zoom® stereo-scope (Bausch & Lomb Inc.) after opening the bag and placing the contents into a 100 mm diameter Petri dish. The number of *Lygus* adults and nymphs were recorded for each sample.

The numbers of *Lygus* collected were compared among plant species using one-way ANOVA with Minitab 14.0 Software. When the ANOVA was significant, means were separated using Fishers PLSD. Data were transformed for analysis using a Log(x+1) transformation in order to normalize the distribution of the data. Means and standard errors are reported as they were before transformation.

Results

Riparian *Lygus* Host Plant Study.

During the 2005 field season, significantly more *Lygus* were collected from cages containing Canada thistle and stinging nettles (Fig. 1) than other plants in the study (F = 3.95; df = 16, 67; P= 0.0001). During the 2006 field season, the number of *Lygus* collected did not differ statistically among plant species (Figure 2; F = 0.81; df = 16, 67; P= 0.666). *Lygus* nymphs were collected from Canada thistle, clematis, coyote willow, dogwood, rugosa rose, sagebrush, and stinging nettle plots during the 2006 season (Fig. 2).

Cover Crop *Lygus* Host Plant Study.

During the 2004 field season, significantly more *Lygus* nymphs were collected from some cover crops than others (F = 6.38; df = 11, 538; P= 0. 01). During 2004, significantly more *Lygus* nymphs were collected from the alfalfa and northern birdsfoot trefoil than the other cover crops (Fig. 3). Moderate numbers of *Lygus* nymphs were collected from alfalfa/ryegrass, northern birdsfoot trefoil, Bug-n-breakfast, delar small burnettt, ladino clover, mammoth red clover, strawberry clover, and white Dutch clover plots during 2004 (Fig. 3). The native grass and perennial ryegrass plots hosted significantly fewer *Lygus* nymphs during 2004 than did the other treatments (Fig. 3). The abundance of *Lygus* nymphs also differed significantly during the 2005 field season (F = 4.66; df = 11, 711; P= 0.002). During 2005, significantly more *Lygus* nymphs were collected from the alfalfa and northern

birdsfoot trefoil plots (Fig. 4). The perennial ryegrass plots hosted the fewest *Lygus* nymphs during 2005, though results were not statistically significant from the other plants (Fig. 4). All other plots hosted a moderate number of *Lygus* nymphs during 2005 but did not differ statistically from the other plants evaluated (Fig. 4).

Discussion

Results in Figs. 1 and 2 indicate that *Lygus* spp. are capable of completing their development on other non-crop plants than have been previously investigated, though their reproductive potential seems to be less than one would see on a preferred host such as alfalfa. During the 2005 study of non-crop plants, Canada thistle, gray and green rabbitbrush, reed canary grass, and stinging nettles appeared to serve as hosts for *Lygus*, enabling them to complete their life cycle. During the experiment, *Lygus* reproduction was sustained on sage brush and coyote willow. Plants, such as Canada thistle that sustain bloom over a long period of time, may be a better quality food source for *Lygus* than plants such as dogwood or honeysuckle that were not blooming during the experiment. The presence of bloom though cannot be the sole factor that makes a plant a better source than another plant for *Lygus*. The three rose species studied (woods, nutkana, and rugosa rose) and the yarrow were in bloom during the testing period and did not serve as a viable host for *Lygus* reproduction. Also, the application of the insecticide did not likely have an effect on *Lygus* ability to reproduce within the cages since the insecticide used had a very short residual activity. Further, it is unlikely that *Lygus* consumed one another in the cage since it has been show that they are not likely predaceous (Rosenheim et al. 2004).

Many of the plant species listed that we studied are commonly encountered in riparian buffers and field margins in Eastern Washington State. Eliminating species that serve as a source for *Lygus* reproduction could result in a lowering of resident *Lygus* that might otherwise migrate into adjacent agricultural fields. Conversely, establishing plants that did not serve as hosts for *Lygus* reproduction would serve to benefit for the adjacent agricultural lands. The three rose species that did not serve as *Lygus* hosts have been shown to be good habitat for beneficial insects and other wildlife (Suszkiw 2004). Though there is no data available on the other plant species, it is likely that increased plant cover and increase plant species diversity would help enhance beneficial insect populations.

During the 2004 and 2005 studies, the alfalfa and northern birdsfoot trefoil cover crop plots were superior hosts for *Lygus* (Figs. 3 & 4). During 2004, the Alsike clover plots also served as a superior *Lygus* hosts (Fig. 3). This indicates that alfalfa, northern birdsfoot trefoil, and Alsike clover should not be planted near crops that are highly susceptible to *Lygus* feeding. In areas where alfalfa and cotton are planted in adjacent fields, alfalfa is a preferred host to cotton. But, when alfalfa is swathed, *Lygus* migrate from alfalfa to adjacent cotton fields and inflict damage (Sevacherian and Stern 1975, Ellsworth and Jones 2001). If crops such as alfalfa and northern birdsfoot trefoil were harvested or mowed, *Lygus* likely would migrate into other susceptible crops. There is the possibility of using certain Lygus preferred crops as trap crops near higher value but less preferred crops.

Conversely, the perennial ryegrass plots hosted significantly fewer *Lygus* during both seasons of the study and the native grass hosted significantly fewer *Lygus* during 2004 (Figs. 3 & 4). These data indicate that perennial ryegrass and the native grass blend would be suitable to plant near crops that are highly susceptible to injury from *Lygus* feeding.

All other plants in the study hosted moderate populations of *Lygus* indicating that their impact as a source for *Lygus* is possible, but not as problematic as alfalfa, northern birdsfoot trefoil, and alsike clover.

These likelihood studies are intended to serve as a guide in cropping systems where *Lygus* is a major pest and where integrated pest management (IPM) plans are being implemented, both locally and in area wide management programs. For example, in south central Washington, alfalfa seed is the major crop grown and *Lygus* are the key pests. Producers could cooperate with local conservation organizations that have been removing farmlands adjacent to local streams and vegetating with woody perennial plants to minimize erosion. A recommendation would be to plant rose species and yarrow since they do not serve as a *Lygus* hosts, but could enhance beneficial insect populations. Additionally, one would recommend that they avoid rabbit brush, reed canary grass, and weeds such as Canada thistle since they serve as *Lygus* hosts.

The information obtained from the cover crop study is also useful in area wide pest management programs. Plantings of forage alfalfa and northern birdsfoot trefoil were excellent *Lygus* hosts and should be avoided in areas near *Lygus* susceptible crops, unless used as trap crops. The perennial ryegrass did not serve as a *Lygus* host and therefore could be utilized as a cover crop or seed crop that would not contribute to the problem. Alfalfa seed was the subject of this study, but the implications of this research may also prove useful in other cropping systems such as vegetable seeds, canola, and tree fruit where *Lygus* is an established pest. In tree fruits, this information could be useful when establishing cover crops on the orchard floor in regions where *Lygus* cause damage to the fruit. Implementation of these recommendations could result in improved insect control to susceptible crops and a reduction in the use of insecticides to control *Lygus*.

References Cited

Anonymous 2005. United States Department of Agriculture, National Agricultural Statistics Service. Retrieved from World Wide Web on December 30, 2005: http://www.nass.usda.gov/Statistics_by_State/index.asp

Barlow, V. M., L. D. Godfrey, and F. E. Norris. 1999. Population dynamics *of Lygus hesperus* (Heteroptera: Miridae) on selected weeds in comparison with alfalfa. Journal of Economic Entomology 92: 846-852.

Berry, R. E. 1978. Insects and Mites of Economic Importance in the Northwest. Oregon State University Book Stores, Inc. 2nd Ed. 221 pp.

Daniels 2005. Pesticide Information Center On-Line (PICOL). Search titled: Chemicals Registered for *Lygus*. Retrieved from World Wide Web on November 4, 2005: http://picol.cahe.wsu.edu/labels/Labels.php

Ellsworth, P. C., and J. S. Jones. 2001. Cotton IPM in Arizona: A decade of research, implementation & education, pp.1088 -1096. *In* P. Dugger and D. Richter [eds.], Proceedings Beltwide Cotton Conferences. National Cotton Council, Memphis, TN.

Fye, R. E. 1980. Weed Sources of *Lygus* Bugs in the Yakima Valley and Columbia Basin in Washington. Journal of Economic Entomology 73:469-473.

Goodell, P. B., 2001. Managing *Lygus* in the landscape. Kerney Agricultural Center Plant Protection Quarterly. 11: 8-11.

Gupta, R. K., G. Tamaki, and C. A. Johansen. 1980. *Lygus* bug damage, predator-prey interaction and pest management implications on alfalfa grown for seed. Washington State University Tech. Bull. 92. 18 pp.

Hollingsworth, C. S. 2008. Pacific Northwest Insect Management Handbook. Oregon State University. 665 pp.

Johnson, W. T. and H. H. Lyon. 1988. *Insects that feed on Trees and Shrubs*. Second Edition. Ithaca, New York: Cornell University Press. 556 pp.

Kelton, L. A. 1975. The *Lygus* bugs (genus *Lygus* Hahn) of North America (Heteroptera: Miridae). Memoirs of the Entomological Society of Canada. 95.

Mayer, D. F., and C. Johansen. 1991. Integrated insect control practices, *In* Alfalfa Seed Production and Pest Management, Western Regional Extension Publ. 12.

Osborne, K. H. and W. W. Allen. 1999. Allen-Vac: An internal collection bag retainer allows for snag-free arthropod sampling in woody scrub. Environmental Entomology 28(4): 594-596.

Pickel, C., N. C. Welch, and B. D. Walsh. 1990. Timing Lygus sprays using degree-days in central coast strawberries. Santa Cruz County Agricultural Extension Leaflet. University of California. 6 pp.

Rosenheim, J. A., R. E. Gomez, and E. F. Thatcher. 2004. Omnivore or herbivore? Field observations of foraging *Lygus hesperus* (Hemiptera: Miridae). Environmental Entomology 33(5): 1362-1370.

Scott, D. R. 1977. An annotated listing of host plants of *Lygus hesperus* Knight. Bull. Entomol. Soc. Am. 23: 19-22.

Sevacherian, V. and Stern, V. M. 1975. Movement of *Lygus* between alfalfa and cotton. Environmental Entomology 4: 163-165.

Suszkiw, J. 2004. Rose gardens make fruit orchards more inviting to friendly wasps. Ag. Res. 52; 12-15.

Figure 1. Mean number of *Lygus* nymphs aspirated from plants in the Riparian Lygus Host Plant Study.

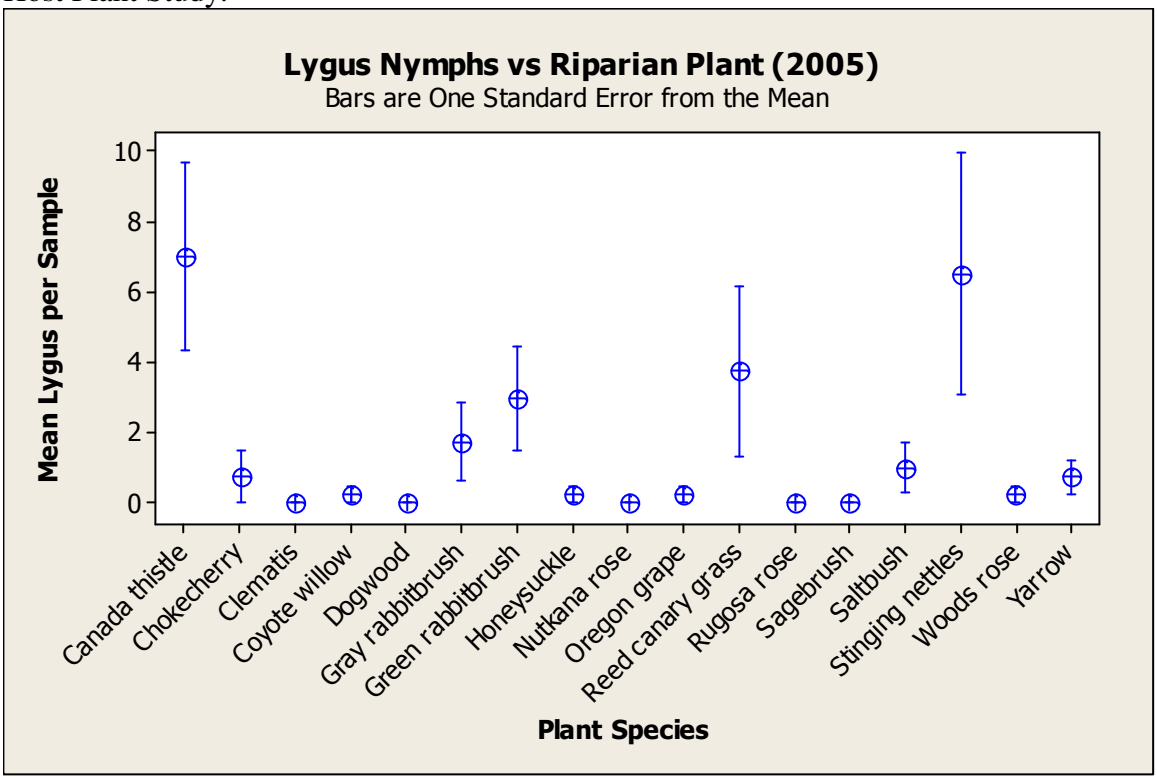


Figure 2. Mean number of *Lygus* nymphs aspirated from plants in the Riparian Lygus Host Plant Study.

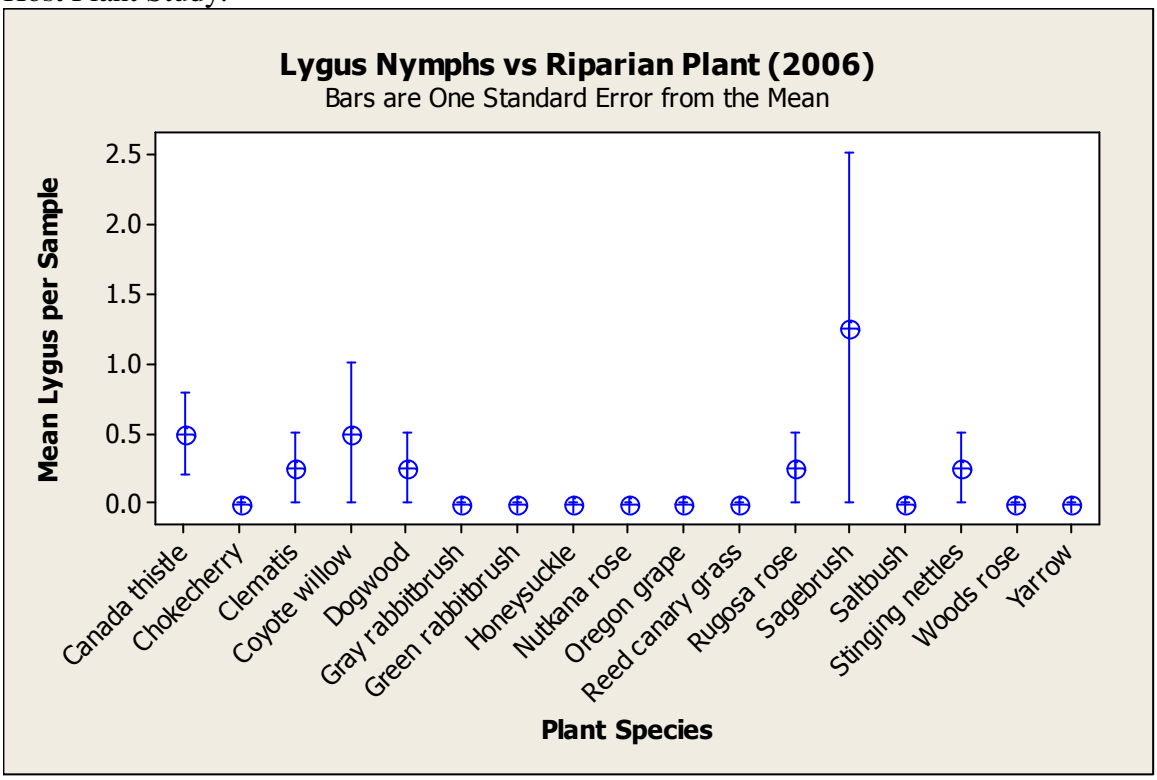
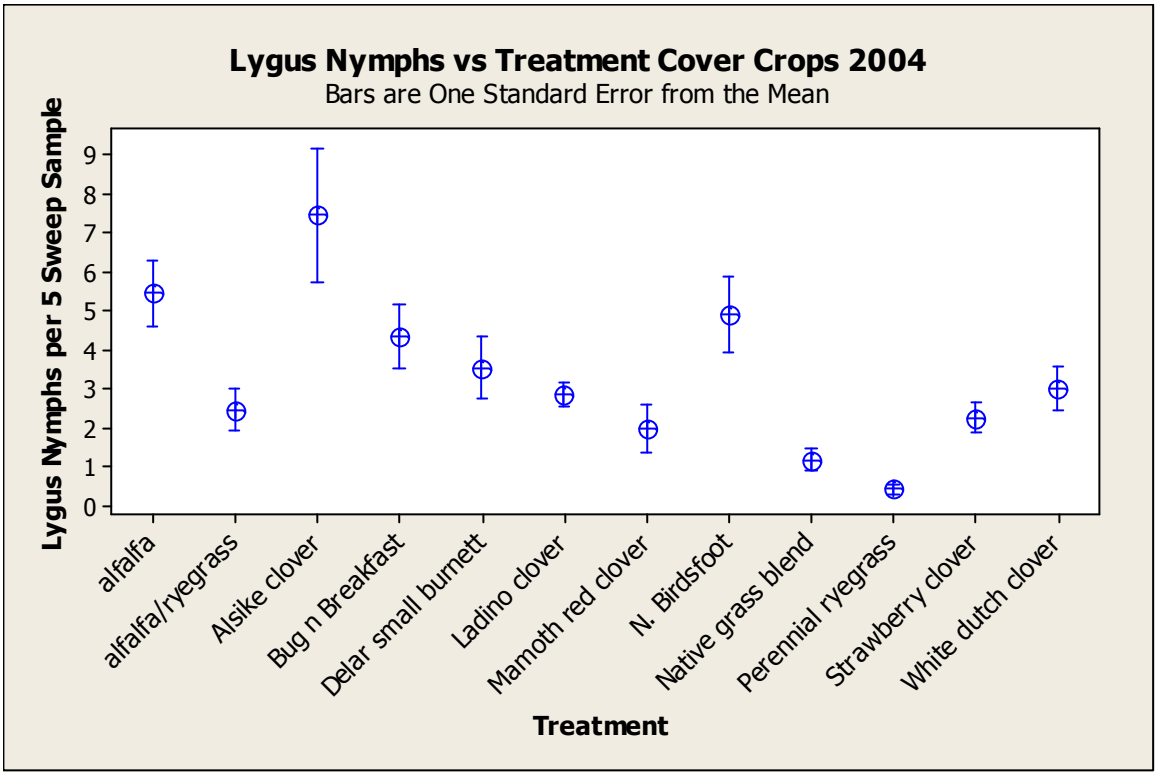
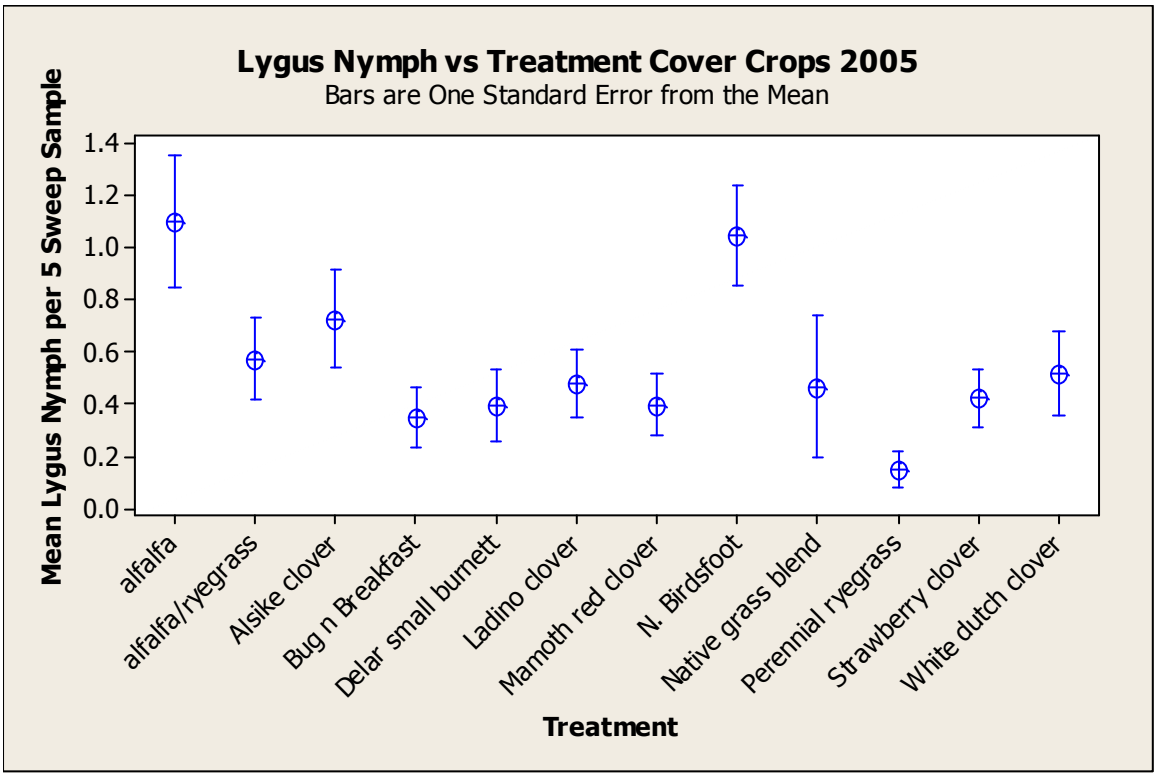


Figure 3. Mean number of *Lygus* nymphs per 5 sweep sample for the cover crop study 2004.



.

Figure 4. Mean number of *Lygus* nymphs per 5 sweep sample for the cover crop study 2005.



Distribution and Species Composition of *Peristenus*

Abstract

Lygus hesperus Knight (Hemiptera: Miridae) is the perennial key direct pest in alfalfa seed production in the western US (Mayer 1991, Mueller 1998, Hussman 1999). *Lygus* populations left uncontrolled reduce seed yields (Klostermeyer 1962) and quality (Sorenson 1936). An important parasitoid of *Lygus* in Washington state is the wasp *Peristenus howardi* Shaw (Hymenoptera: Braconidae) and its congener species. Here we report parasitism rates of *Lygus* in alfalfa seed production and other habitats in Washington state, and provide preliminary methods to identify *Peristenus* spp. from larval parasitoids obtained in parasitized nymphs of *Lygus*. We collected *Lygus* nymphs from sites throughout southeastern Washington State to determine parasitism rates by *Peristenus* spp. in alfalfa seed and other crops. Parasitoid surveys were conducted annually between 2002 and 2006. Over 15,000 *Lygus* nymphs were collected, and dissected to determine parasitoid presence in different geographic regions and on different host plant species. The dissections yielded small larval parasitoids that were not identifiable morphologically to species level. Therefore, we used PCR to amplify a region of the mitochondrial cytochrome oxidase 1 gene for species identification. We identified three species of *Peristenus* from *Lygus* nymphs in our samples.

Introduction

Over 30,000 ha of alfalfa is grown for seed annually in the United States with an average production of 36 million kg of seed (Anonymous 2005). Most of this production takes place in the western United States. Alfalfa seed is considered a minor crop, but it is the foundation of the alfalfa forage industry. Forage alfalfa ranks third in planted agricultural acres in the United States at 9.3 million ha annually (Anonymous 2005). In Washington State, 5,666 ha of alfalfa seed and 180,490 hectares of alfalfa forage are planted annually (Screiber 2008). The alfalfa seed crop is worth $14 million annually, while the alfalfa forage crop is worth $267 million annually (Screiber 2008).

Lygus hesperus attacks a broad range of hosts in the Pacific Northwest including fruit, vegetable, seed, tree, and fiber crops (Kelton 1975, Scott 1977, Johnson and Lyon 1988, Mayer et al. 1998) in addition to their persistence on a number of native and weedy non-crop plants (Berry 1998, Barlow et al. 1999, Goodell 2001). Once alfalfa fields are cut for hay, *Lygus* may fly to nearby fields to feed (Stoltz and McNeal 1982, Scott 1987). *Lygus* is the primary insect pest of alfalfa seed in the Northwest United States, causing reduced seed yields if not controlled. Feeding by *Lygus* causes several types of injury to alfalfa. Feeding on flowers may cause floral bud abortion, while feeding on developing seed pods may make seeds unviable (Strong 1970). Seed-feeding constitutes the major crop loss caused by *Lygus* in the alfalfa seed production system (Mayer and Johansen 1991). Late instar nymphs feed significantly more than adults (Rosenheim et al. 2004).

In alfalfa seed crops, insecticide applications solely targeting *Lygus* are typically recommended three times per season: one prior to alfalfa bloom, one during the bloom, and the final after bloom during seed set and maturation. A normal grower practice would be to apply the insecticides with a broadcast ground sprayer at 20 gallons of water carrier per acre. Prior to bloom, applications are made during the day, but as bloom begins insecticide applications are made exclusively after sunset to avoid killing pollinating bees. Alfalfa seed producers must balance *Lygus* control with pollinator safety. This concern has led to the use of chemicals having shorter residual life and reduced efficacy.

Peristenus spp. (Hymenoptera: Braconidae) are parasitoids of *Lygus* in many regions of North America. Surveys in Idaho during 1997 and 1998 reported parasitism rates of *L. hesperus* in alfalfa as high as 81% by *P. howardi* Shaw (Day et al. 1999). Surveys were also conducted in Oregon and Washington in 1996 and 1997; no parasitoids were collected in Oregon, but parasitism rates ranged from 28 to 70% in Washington (Mayer et al. 1998). Further work in Idaho in 1999 showed parasitism rates as high as 75% (Colgrove and Bitner 1999). Intentional releases of *P. digoneutis* Loan took place in the eastern United States as early as 1979, and the parasitoid was shown to reduce some *Lygus* populations (Coulson 1987). Intentional releases of *P. digoneutis* and *P. relictus* (Ruthe) also took place in California in 1998, 1999, and 2000 and the two species were successfully established and reduced *Lygus* numbers (Pickett et al. 2007, Pickett et al. 2009). Results from a 2000 survey of *Lygus* in Idaho and Nevada

determined that only ten percent of the *Lygus* nymphs collected in Idaho and none of those collected in Nevada showed evidence of parasitism (Ball et al. 2001) in contrast to the survey conducted by Colgrove and Bitner (1999) in a similar region of Idaho. High parasitism rates, as shown by Colgrove and Bitner in Idaho in 1999, naturally led researchers to speculate on the practical application of *Peristenus* spp. as a potential biological control agent for *Lygus* in alfalfa seed. However, a potential problem is that introductions of non-native *Peristenus* spp. could displace the native *Peristenus* spp. in regions where biological control of *Lygus* is desirable (Mayer et. al 1998, Broadbent et. al 2006). A possible alternative to introduction of non-native *Peristenus* spp. into the Pacific Northwest would be to increase habitat and thus populations of native species of *Peristenus*. This might be possible in this system because *Lygus* living in weedy areas may be more heavily parasitized by *Peristenus* than *Lygus* found in alfalfa (Lim and Stewart 1976).

Previously, there was some confusion as to whether the parasitoid in Washington was *Peristenus howardi* Shaw or *Peristenus pallipes* (Curtis) (Mayer et al. 1998). Though the species of *Peristenus* cannot be discerned by morphology as larvae (Goulet and Mason 2006), molecular methods have been developed to reliably do so for *P. digoneutis, P. pallipes, and P. conradi* (Tilmon et al. 2000), *P. digoneutis, P. pallipes, P. pseudopallipes, P. howardi,* and *P. conradi* (Mowry and Barbour 2004) and for *P. howardi* and *P. stygicus* (Zhu et al. 2004). *Peristenus* spp. are similar in biology and life history. *Peristenus howardi* Shaw is a recently described species believed to be native to the Pacific Northwest (Goulet and Mason 2006). In Idaho, where it was

originally discovered, *P. howardi* produces three generations per year (Day et al. 1999). *P. howardi* is a small black wasp about 2.5 mm in length with a female-biased sex ratio (Ball et al. 2001). Adults of *P. howardi* are readily discernible from other species of adult *Peristenus* (Goulet and Mason 2006). The reproductive biology and life stages of *P. howardi* are believed to be similar to those life history traits most other *Peristenus*, although more studies are required for a more complete understanding. For our study we examined adult specimens of *P. howardi, P. carcamoni, P. broadbenti,* and *P. gillespei*. The geographic ranges for the species in the study may or may not overlap in Eastern Washington State (Goulet and Mason 2006). From Goulet and Mason's review of *Peristenus* (2006) it seems most likely that *P. howardi* is the common species in Eastern Washington State, but *P. broadbenti, P. carcamoni,* and *P. gillespei* have all been found west of the Cascade Mountains in Washington State.

The objective of this research were to determine the distribution of *Peristenus* parasitoids in southeastern Washington State and to identify the species using larval parasitoid monitoring method and a PCR based assay.

Materials and Methods

Late instar (4-5th) *Lygus* nymphs were collected by sweeping a 38 cm diameter sweep net through vegetation. *Lygus* nymphs were aspirated into 9-dram styrene tubes with a suction aspirator (Bioquip Products Inc.). Samples were placed in a cooler on ice until they could later be dissected. *Lygus* nymphs were dissected under a 0.7-30x Stereo-

Zoom® stereo-scope (Bausch & Lomb Inc.) When larval parasitoids were detected, they were placed in 95% EtOH for storage.

To identify *Peristenus* larva dissected from *Lygus* to species, adult specimens of *Peristenus* species known to be in Washington State were collected and used for mitochondrial DNA extraction and PCR amplification to act as vouchers. Adults of *P. carcamoni* Goulet*, P. gillespei* Goulet, and *P. broadbenti* Goulet were obtained from H. Goulet (Agriculture and Agri-Food Canada, K. W. Neatby Building, 960 Carling Ave., Ottowa, Ontatrio, Canada) who also identified the specimens. The specimens were critically point dried and preserved prior to being shipped. Adult specimens of *P. howardi* were obtained from J. D. Barbour (University of Idaho, Parma Research and Extension Center, 29603 U of I Lane, Parma, ID, USA) who also identified the specimens. In order to amplify a region of the cytochrome oxidase 1 gene, mitochondrial DNA (mtDNA) was first extracted from these species and then also for all larval unknown *Peristenus* species. Mitochondrial DNA was extracted using the Chelex extraction method as follows (Walsh et al. 1991). A fore, mid, and hind-leg of each adult specimen were removed and crushed using a P200 micropipette tip, in a microtube containing 40 μL of 5% Chelex resin (Instagene Matrix, Biorad, Hercules, CA, USA). Suspensions were vigorously vortexed for 10 s, incubated at 56 °C for 35 min, vortexed again for 10 s, and incubated at 95 ◦C for 15 min. Samples were then stored at -20° C. After removal from the freezer, suspensions were centrifuged at 15,300 *g* for 3 min to remove the supernatant containing the DNA from the top of the tube, with care taken to avoid removing any Chelex resin from the bottom. For the

larval samples, the isolated mtDNA was extracted using the Chelex method as described here for adults except the entire larva was used in each extraction.

A region of the mitochondrial gene cytochrome oxidase subunit 1 (CO1) was amplified from the known adult samples and the unknown larval samples. This region is used primarily to distinguish individuals within populations and also closely related species from each other (Mowry and Barbour 2004, Tillmon et al. 2000). The primers C1-1718 (GGA GGA TTT GGA AAT TGA TTA GTT CC) with C1-2191 (CCC GGT AAA ATT AAA ATA TAA ACT TC) (Sigma-Aldrich, St. Louis, MO) were utilized to amplify a 500 bp region of the CO1 gene of the parasitoids. Amplifications were done in 25 µL volume reactions as follows: 845 µL of water, 50 µL 2.5 µM dNTPs, 125 µL 10X Rapid Buffer, 62.5 µL C1-1718 4 µM forward primer, 62.5 µL C1-2191 4 µM reverse primer, 5 µL of recombinant *Taq*DNA polymerase (Sigma-Aldrich, St. Louis, MO) and 2 µL DNA. Amplifications were performed in a PTC 100 Programmable Thermal Controller (MJ Research, Inc) with the following cycling parameters: 35 cycles at 1 min., 94° C; 2 min., 50° C; and 1.5 min., 72° C. PCR products were separated by a 2% agrose gel electrophoresis containing ethidium bromide, and DNA fragments were visualized under UV light. Images were captured using a digital camera to visualize the bands.

The PCR products of the known adult specimens and unknown larvae were purified with Exo-sap-it (USB Corp., Cleveland, OH) and sent to McLab (San Francisco, CA)

for sequencing. All individuals were sequenced in both directions (forward and reverse).

To analyze the CO1 gene sequence and to identify unique haplotypes and species-specific sequences, the raw sequences for each sample were compiled in DNASTAR Lasergene 7.0 Seqman software (Madison, WI) to form consensus sequences. After directionality of the sequences was determined, DNASTAR Lasergene 7.0 MegAlign software (Madison, WI) was used to trim the ends and to align the sequences. Samples from the adult specimen sequences were aligned using Clustal W, slow and accurate to determine sequence similarities. The phylogenetic tree of knowns and unknowns was first aligned using the Clustal V pairwise method followed by a second alignment of a selected region using the slow and accurate Clustal W method. Haplotypes and species were identified based on similarity of the unknown samples to the known samples.

Voucher specimens for the adult *Peristenus* were deposited in the M. T. James Entomological Collection (Washington State University, Pullman, WA).

Results

Of the 15,197 *Lygus* nymphs collected, only 406 were parasitized for an overall parasitism rate of 2.67 %. *Lygus* were most frequently collected from alfalfa or alfalfa grown for seed, and the majority of the *Peristenus* parasitoids were obtained also from alfalfa or alfalfa seed. Parasitized *Lygus* were collected from alfalfa (*Medicago*

sativa L.), lambsquarters (*Chenipodium album* L.), black mustard (*Brassica nigra* Koch), perennial pepperweed (*Lepidium latifolium* L.), white clover (*Trifolium repens* L.), purple loosestrife (*Lythrum salicaria* L.), kochia (*Kochia scoparia* Schrad.), canola (*Brassica napus* L.), and mint (*Mentha spicata* L.). Collections began in late May and resumed until September each year coinciding with peak *Lygus* activity. The majority of the *Peristenus*, 79%, were collected in July with very few being collected during the other months for all years of the project. Table 1 shows the percentage of *Lygus* nymphs parasitized by *Peristenus* at all of the collection sites throughout Washington State with the total number of *Lygus* nymphs collected. *Lygus* from over half of the sites had no parasitism by *Peristenus*. Parasitism rates were the highest in the Naches, Touchet, and Warden areas.

Figure 1 shows a consensus alignment of *P. howardi, P. carcamoni, P. gillespei*, and *P. broadbenti* compared to two Braconid out-group species (*Euphorine* and *Apantles*). The alignment shows that the following pairs are 0% divergent from one another: *P. howardi* 73 and *P. howardi* 74, *P. howardi* 75 and *P. broadbenti* 79, *P. broadbenti* 78 and *P. broadbenti* 79, *P. broadbenti* 78 and *P. gillespei* 80, and *P. howardi* 75 and *P. gillespei* 80 (Figure 1). The following pairs were 99.1% identical in sequence to each other: *P. broadbenti* 78 and *P. howardi* 75, *P. broadbenti* 79 and *P. howardi* 75, *P. broadbenti* 79 and *P. gillespei* 80, *P. howardi* 73 and *P. howardi* 75, *P. howardi* 74 and *P. howardi* 75 (Figure 1). *P. howardi* 73 and *P. howardi* 74 were 99.3% identical in this region of the COI gene used in this alignment (Figure 1).

Figure 2 shows a tree that includes all the unknown larval parasitoid samples in a pairwise and neighbor-joining grouping with the known *Peristenus* species from Figure 1. The branching point at Figure 2 position A shows that the two known samples of *P. carcomoni* and are closely related to one another, but not to any of the other samples in the study. In the same alignment at Figure 2 position B, an individual sample from Watsonsville, CA (#106) is quite different from the rest of the samples and is the only unknown sample not from Washington State. At position C, the haplotype contains two specimens, both of which were collected from fields near La Crosse, WA (Figure 2); this site was the eastern-most part of Washington from which *Peristenus* was collected. The position D haplotype consists of 10 specimens, which most closely resemble *P. broadbenti* 79 and *P. gillespei* 80 (Figure 2). The remaining 73 samples in Figure 2 position E belong to the haplotype that represents the *P. howardi* clade. The majority of the samples in position E come from Touchet, Prosser, Naches, Moxee, Monitor, and Wapato Washington State (Figure 2). Within the position E clade, there are several samples from the same location which align with identical sequences and are denoted by color coding (Figure 2).

Discussion

The relatively low overall parasitism rate of *Lygus* by *Peristenus* in this study (2.67%) suggests that this parasitoid has relatively no impact on biological control of *Lygus* in Washington State crops. *Lygus* samples were collected from a number of different vegetation types, and the majority of the parasitized *Lygus* were found on alfalfa

forage or alfalfa seed crops, which suggests that the most potential for *Peristenus* control of *Lygus* would be in alfalfa seed. In addition, parasitized Lygus were also found on weedy species of plants which could have positive implications on biological control since many of the weeds are found near and in alfalfa seed fields.

For biological control, alternative hosts for parasitoids can be beneficial to sustain parasitoids when there primary prey is not abundant. Mirids other than *Lygus*, specifically *Closterotomus norvegicus* Gmelin, have been noted to serve as hosts for *P. relictus* in California at times early in the season when *Lygus* are not present (Pickett et al. 2009). In the system described by Picket et al. (2009), the presence of *C. norvegicus* in weedy areas adjacent to strawberries provided a positive impact by increasing and sustaining population of *P. relictus* before *Lygus* arrived and provided a more significant level of control of *Lygus* in the adjacent strawberry fields. *Closterotomus* spp. can be found in some regions in Washington State where *Lygus* are also present. Future studies could sample for this species and determine if *P. howardi* is utilizing *Closterotomus* spp. as an alternative host. This association could prove useful in biological control of *Lygus* in alfalfa seed since weedy areas that could host *Closterotomus* spp. and subsequently increased populations of *Peristenus*, as long as *Closterotomus* spp. prove not to be pests of alfalfa seed.

An important factor in biological control with parasitoids is the timing at which mortality of the pest occurs in relation to the crop the pest is attacking. The peak of parasitism occurred in July, at which time the alfalfa seed crop would have already

experienced economic damage from *Lygus* feeding. There were detections of parasitism by *Peristenus* earlier in the season, but at very low levels not likely having a significant impact on *Lygus* populations. The relatively late season reduction in *Lygus* numbers due to parasitism may have an impact on overwintering *Lygus* populations and could reduce populations in the subsequent season. Overall parasitism rates in the two alfalfa seed growing regions of Washington State were among the highest in the study; 8.10% and 8.90% for Warden and Touchet, respectively (Table 1). Parasitism rates were also high in Naches (9.20%) and Monitor (6.80%). The Touchet, Naches, and Monitor areas have numerous sites including riparian areas where weeds and wild alfalfa persist without harvest or insecticide disturbance which may benefit *Peristenus*. No *Peristenus* were detected in the Pasco, Ephrata, Quincy, Royal City, Mabton, Moses Lake, and Mattawa areas. These include significant areas of forage alfalfa and other irrigated row crops. Most of the crops in these areas receive several insecticide applications per season and the alfalfa forage crops are harvested 4-6 times per season; both of these practices would be disruptive to *Peristenus* populations.

When comparing the four known specimens of *Peristenus* to the unknown larvae collections, it is clear that *P. carcamoni* is most distantly related to the other known specimens (Figure 1) and that none of the unknown larvae are *P. carcamoni* (Figure 2). This finding is consistent with the distribution listed for *P. carcamoni* in a recent revision of *Peristenus* by Goulet and Mason (2006). Figure 1 also shows that *P. howardi* 73 and *P. howardi* 74 are genetically similar, but there are several

discrepancies with the other specimens. This is probably due to ambiguous alignment of nucleotides in the CO1 region used and will be addressed in future work in order to unambiguously determine the nucleotide at these sites within the sequence. It is unlikely the original identification of the adult specimens based on morphology were incorrectly identified since they were sent by a Braconid expert, (H. Goulet) and by another individual who has done similar work with the genus (J. Barbour) although it is conceivable that these known species were incorrectly identified. Another potential problem is that the CO1 gene region that we used for this study does not have enough informative sites to accurately separate these species and additional sequences that would provide more information are necessary to separate and identify these species from each other. These discrepancies can be addressed by examining the trace data chromatographs to clean up the sequences to unambiguously assign the nucleotide base pair at each ambiguous position or by reanalyzing the specimens using CO1 primers from Mowry and Barbour (2004).

The cladogram in Figure 2 shows how the four known species of *Peristenus* are related to the unknown larval samples. In Figure 2 at position B, there is only one specimen represented, which came from a sample of *Lygus* from Watsonville, CA. This position represents a haplotype that is different from any of the other unknown samples, but is closely related to the *P. howardi, P. gillespei*, and *P. broadbenti* known specimens; potentially representing a different genotype of the *Peristenus* species in our study or representing a different species of *Peristenus* entirely, perhaps *P. relictus* or *P. digoneutis*. The branch at Figure 2 position C is a haplotype

represented by two specimens, both of which were collected at LaCrosse, Washington State. The haplotype at the branching point of Figure 2 position C is different from any of the other specimens and the two are identical to one another indicating that a different genotype of *Peristenus* occurs in this area and possibly even a different *Peristenus* species. The fact that a unique genotype is found here raises the likely possibility that there is not gene flow between widely dispersed populations of *Peristenus* since the area is further east than where any other *Peristenus* were collected for this study. The LaCrosse, WA area has a completely different crop profile and climate than the other regions of the state where samples were collected. Figure 2 position D represents a haplotype that contains 10 specimens coming from the Touchet, Monitor, and Wapato areas of Washington. It is unclear if this could be *P. broadbenti*, *P. gillespei*, or a different species of *Peristenus.* Multiple parasitism could also be at play further complicating results.

The cluster of genotypes in Figure 2 position E is the consistent with the known *P. howardi* genotype. Within genotype E, there are ten haplotype groups; labeled 1-10, where there are at least pairs of specimens with identical molecular profiles that were collected from the same locations. This indicates that there could be some distinct populations of *Peristenus* within Washington State. Conversely, there are several individuals within the branching at Figure 2 position E where specimens collected on the same date in the same location differ from one another indicating multiple haplotypes from a single location.

In summary, *Peristenus* can be found in several areas within Washington State, but generally at very low abundance. *Peristenus* are present in alfalfa seed growing regions, but are not expected based on these low rates of parasitism to have a significant impact on reducing *Lygus* populations and *Lygus* crop damage at present. Detection of *Peristenus* larva peaked in July, therefore careful insecticide management in May and June could lead to a reduction in mortality of adult *Peristenus* and possibly an increase in parasitoid populations. The genetic results indicate that *P. howardi* is likely the predominant species in Washington State, but another species of *Peristenus* may be present as well. Additional genetic work is expected to further contribute to understanding the taxonomic composition and geographic distribution of *Peristenus* in *Lygus* populations of Washington State.

References

Anonymous 2005. United States Department of Agriculture, National Agricultural Statistics Service. Retrieved from World Wide Web on December 30, 2005: http://www.nass.usda.gov/Statistics_by_State/index.asp

Ball, J. C., C. H. Pickett, C. R. Baird, and J. Knight. 2001. Search for Native Parasitoids of *Lygus hesperus i*n Idaho and Nevada. In Biological Control Program Annual Summary, 2000, Dale M. Woods, Ed. California Department of Food and Agriculture, Plant Health and Pest Prevention Services, Sacramento, California. 78 pp. http://www.cdfa.ca.gov/phpps/ipc/biocontrol/annuals/2000annual/2000annual.pdf.

Barlow, V. M., L. D. Godfrey, and F. E. Norris. 1999. Population Dynamics *of Lygus hesperus* (Heteroptera: Miridae) on Selected Weeds in Comparison with Alfalfa. Journal of Economic Entomology 92: 846-852.

Berry, R. E. 1998. Insects and Mites of Economic Importance in the Northwest. 2nd Ed. 221 p.

Broadbent, A., S. Lachance, M. Sears, and H. Goulet. 2006. Native Braconid parasitism of the Tarnished plant bug (Hemiptera: Miridae) in Southern Ontario. Biocontrol Science & Technology, 16(7), 687-698.

Colgrove, E. and R. Bitner. 1999. Idaho *Lygus* Parasites: *Lygus* Parasitoid Progress Report for Summer 1999. International Pollination Systems. Retrieved from World Wide Web on March 30, 2005 http://www.pollination.com/PublicationsResearch/idaho*Lygus*parasites.htm.

Coulson, J. R. 1987. Studies on the biological control of plant bugs (Heteroptera: Miridae): an introduction history, 1961-1983. In Economic importance and biological control of *Lygus* and Adelphocorus. *In* R.C. Hedlund and H. M. Graham, Economic importance and biological control of *Lygus* and *Adelphocorus* in North America. U. S. Department of Agriculture, Agricultural Research Service 64. National Technical Bulletin 15: 1-12.

Day, W., C. Baird, and S. Shaw. 1999. New, native species of Peristenus (Hymenoptera: Braconidae) parasitizing *Lygus hesperus* (Heteroptera: Miridae) in Idaho: biology, importance, and description. Annals of the Entomological Society of America, 92: 370-375.

Goodell, P. B., 2001. Managing *Lygus* in the landscape. Kerney Agricultural Center Plant Protection Quarterly 11: 8-11.

Goulet, H. and P. G. Mason. 2006. Review of the Neartic Species of *Leiophron* and *Perisentus* (Hymenoptera: Braconidae) parasitizing *Lygus* (Hemiptera: Miridae: Mirini). Zootaxa 1323: 118 pp.

Hussman, S. H. 1999. Growing Alfalfa for Seed in Arizona. Cooperative Extension Bulletin AZ1129. The Univesity of Arizona College of Agriculture.

Johnson, W. T., and H. H. Lyon. 1988. Insects that feed on trees and shrubs. Cornell University Press, Ithaca, NY.

Kelton, L. A. 1975. The *Lygus* bugs (genus *Lygus* Hahn) of North America (Heteroptera: Miridae). Mem. Entomological Society of Canada. 95.

Klostermeyer, E. C. 1962. The relationship among pea aphids, Lygus bugs, and alfalfa seed yields. Journal of Economic Entomology 55: 462-465.

Lim, K. P. and R. K. Stewart. 1976. Parasitism of the tarnished plant bug, *Lygus lineolaris* (Hemiptera: Miridae) by *Peristenus pallipes* and *P. pseudopallipes* (Hymenoptera: Braconidae). Can. Entomol. 108: 601-608.

Mayer, D. F. 1991. *Lygus* Bugs and Their Predators. Cooperative Extension Bulletin EM3440. Washington State University College of Agriculture and Home Economics. Pullman, WA.

Mayer, D. F., C. R. Baird, and B. Simko. 1998. Parasitism of *Lygus* spp. (Hemiptera: Miridae) by *Peristenus* in the Pacific Northwest. Journal of the Entomological Society of British Columbia 95: 53-57.

Mowry, T. M. and J. D. Barbour. 2004. Distinguishing the Parasitic Wasp, *Peristenus howardi*, From Some of its Congeners Using Polymerase Chain Reaction and Restriction Endonuclease Digestion. 7 pp. Journal of Insect Science 4:25.

Mueller, S. 1998. Pest Management Plan for Seed Alfalfa: *Lygus*. University of California Cooperative Extension. Fresno, CA.

Pickett, C.H., R. Rodriguez, J. Brown, D. Coutinot, K. A. Hoelmer, U. Kuhlmann, H. Goulet, M. D. Schwartz, and P. D. Goodell. 2007. Establishment of *Peristenus digoneutis* and *P. relictus* (Hymenoptera: Braconidae) in California for the control of *Lygus* spp. (Heteroptera: Miridae). Biocontrol Science and Technology 17: 261–272.

Pickett, C.H., S. L. Swezey, D. J. Nieto, J. A. Bryer, M. Erlandson, H. Goulet, and M. D. Schwartz. 2009. Colonization and Establishment of *Peristenus. relictus* (Hymenoptera: Braconidae) for Control of *Lygus* spp. (Heteroptera: Miridae) in Strawberries on the California Central Coast. Biocontrol Control 49: 27-37.

Rosenheim, J. A., R. E. Gomez, and E. F. Thatcher. 2004. Omnivore or herbivore? Field observations of foraging *Lygus hesperus* (Hemiptera: Miridae). Environmental Entomology 33(5): 1362-1370.

Scott, D. R. 1977. An annotated listing of host plants of *Lygus hesperus* Knight. Bulletin of the Entomological Society of America 23: 19-22.

Scott, D. R. 1987. Biological control of *Lygus* bugs on vegetable and fruit crops, pp. 40-47. *In* R.C. Hedlund and H. M. Graham, Economic importance and biological control of *Lygus* and *Adelphocorus* in North America. U. S. Department of Agriculture, Agricultural Research Service 64. National Technical Bulletin 15: 159-169.

Schreiber, A. S. 2008. Alfalfa and Alfalfa Seed Production. In The Washington Ag. Project. Retrieved from World Wide Web on January 12, 2009: http://www.snakeriver.org/wscpr/index.cfm.

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

Stoltz, R. L., and C. D. McNeal, Jr. 1982. Assessment of Insect Emigration from Alfalfa Hay to Bean Fields. Environmental Entomology 12: 578-580.

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

Tilmon, K. J., B. N. Danforth, W. H. Day, and M. P. Hoffman. 2000. Determining Parasitoid Species Composition in a Host Population: A Molecular Approach. Annals of the Entomological Society of America 93(3): 640-647.

Walsh P.S., D. A. Metzger, and R. Higuchi. 1991. Chelex-100 as a medium for simple extraction of DNA for PCR-based typing from forensic material. Biotechniques 10: 506-513

Zhu, Y. C., E. W. Riddick, L. Williams III, D. J. Schotzko, A. L. Guillermo, and C. G. Jackson. 2004. Potential Detection and Identification of Nymphal Parasitoids (Hymenoptera: Braconidae) of *Lygus* Bugs (Heteroptera: Miridae) by Using Polymerase Chain Reaction and ITS2 Sequence Analysis Techniques. Ann. Entomol. Soc. Am. 97(4): 743-752.

Table 1. Locations where *Lygus* nymphs were collected for dissection, the percentage of those that were parasitized by *Peristenus*, and the total number collected at that location.

City	% Parasitism	No. Lygus	City	% Parasitism	No. Lygus	City	% Parasitism	No. Lygus
Beverly	0.00	37	Quincy	0.00	175	Sunnyside	1.75	741
Brewster	0.00	74	Royal City	0.00	31	LaCrosse	3.50	85
Chelan	0.00	36	Trinidad	0.00	50	Burbank	4.00	25
Ephrata	0.00	133	Twisp	0.00	168	Walla Walla	4.00	100
Grandview	0.00	12	Waschtucna	0.00	66	Othello	4.50	1315
Mabton	0.00	133	Wenatchee	0.00	479	Wapato	4.90	403
Mattawa	0.00	349	Pomeroy	0.75	142	Monitor	6.80	207
Moses Lake	0.00	147	Moxee	1.10	698	Warden	8.10	159
Orondo	0.00	112	Dryden	1.15	179	Touchet	8.90	1854
Outlook	0.00	90	Prosser	1.15	4716	Naches	9.20	314
Pasco	0.00	758	Yakima	1.50	533			

Figure 1. Phylogenetic tree of the consensus alignment of the adult *Peristenus* that were analyzed compared to *Euphorine* and *Apanteles* as outgroup species.

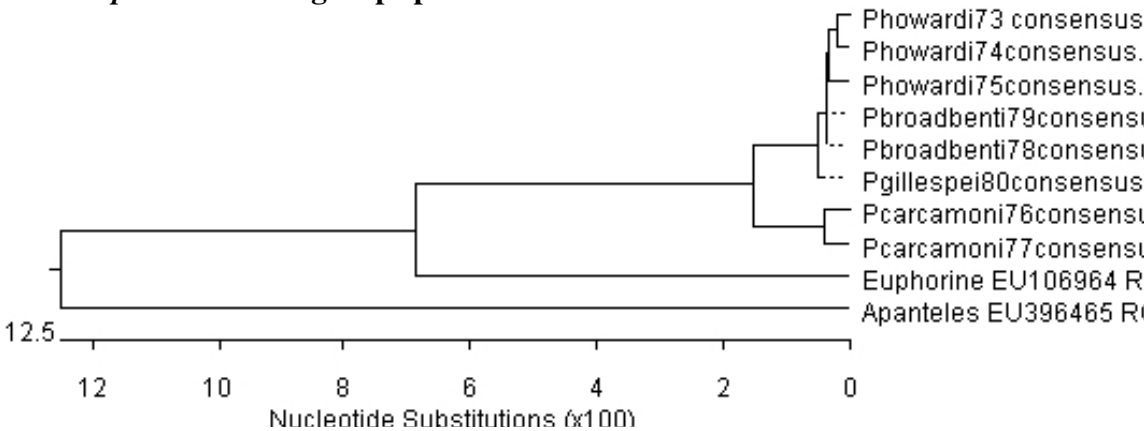


Figure 2. Phylogenetic tree of the consensus alignment of the adult *Peristenus* that were analyzed compared to the larvae collected of unknown species of *Peristenus*.