Ecology of selected European species of *Peristenus* Foerster (Hymenoptera: Braconidae) parasitoids of plant bugs (Hemiptera: Miridae), and their potential as biological control agents for native North American species of pest *Lygus* Hahn and *Adelphocoris lineolatus* (Goeze) in North America.

By

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Ecology of selected European species of *Peristenus* Foerster (Hymenoptera: Braconidae) parasitoids of plant bugs (Hemiptera: Miridae), and their potential as biological control agents for native North American species of pest *Lygus* Hahn and *Adelphocoris lineolatus* (Goeze) in North America.

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A Thesis/Practicum submitted to the Faculty of Graduate Studies of The University of Manitoba in partial fulfillment of the requirements of the degree of

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Abstract

In Germany and Switzerland observational field studies were made on species of *Peristenus* Foerster parasitoids and their mirid hosts, from 1998-2000. The intent of this research was to provide new ecological information which could be used to evaluate the potential of selected European *Peristenus* species as biological control agents for pest *Lygus* (Hahn) and *Adelphocoris lineolatus* (Goeze) in North America.

The following plant bug species, *L. rugulipennis* Poppius, *L. pratensis* (L.) and *A. lineolatus*, were collected in fields of alfalfa, red clover, mixed clover, asparagus, barley, rape seed and mustard, to determine the host's phenology and field levels of parasitism. Plant bug nymphs that were collected were used to compare the detection of parasitoids in nymphs by rearing and dissection methods. In this study, rearing was found to be more accurate in detecting parasitoids. Parasitoid cocoons obtained from the rearing of plant bug nymphs were monitored for adult parasitoid emergence. All parasitoid adults were preserved and identified. Information on the emergence patterns for *Peristenus digoneutis* Loan, *Peristenus stygicus* Loan and *Peristenus rubricollis* (Thomson) is presented. Emergence information is also presented for other *Peristenus, Leiophron*, and *Mesochorus* parasitoid species. Surveys were carried out in Germany and Switzerland in many different habitats, ranging from agricultural fields to mountain meadows, to collect mirids to rear for parasitoids to investigate the ecological host range of *Peristenus* species.

Chapter 1 General introduction

1.1 The problem

In Europe, several species of *Peristenus* Foerster attack the common mirid species *Lygus pratensis* (L.), *Lygus rugulipennis* Poppius and *Adelphocoris lineolatus* (Goeze) (Bilwicz-Pawińska 1976, 1977a, 1977b, 1982). Several of these parasitic wasp species were released in North America in an attempt to control *A. lineolatus*, an introduced pest of alfalfa, and *Lygus* spp. Hahn, a group of several native species attacking a wide range of crops (Craig & Loan 1984a, b, Day 1996, Soroka & Carl 2002). Two European species, *Peristenus digoneutis* Loan and *Peristenus conradi* Marsh, are now established in North America (Day 1996, Broadbent et al. 1999). These parasitoids are recorded to attack the target pest *Lygus* spp. in alfalfa in North America and are providing some level of control (Day 1996, Day et al. 1992). The potential impacts they might have on non-target mirids in habitats other than alfalfa are unknown. Other European species of *Peristenus*, such as *Peristenus stygicus* Loan and *Peristenus rubricollis* (Thomson), are being considered for introduction into North America. New research is needed on European *Peristenus* species, those considered for introduction and those already established in North America, to determine their potential as biological control agents of North American pest *Lygus* and *A. lineolatus* (Kuhlmann et al. 1998). We need information on field levels of parasitism in Europe in a variety of field crops, information on the host range of European parasitoids in their native habitats and other biological

information which will be useful to evaluate both the efficiency and the potential risks of these parasitoids.

Two methods commonly used to detect parasitoids in *Lygus* spp. and *A. lineolatus* are dissection and rearing. Various researchers in the field of *Lygus* biological control utilize one or sometimes both of these methods to report levels of parasitism. To provide support for the method chosen to meet the objectives of the research presented in this thesis, each technique was assessed to determine the advantages and disadvantages of each.

1.2 The objectives

Species of European *Peristenus* were studied in Germany and Switzerland to determine the nature of their relationships with their hosts in agricultural field crops and other habitats. This information will be used to assess their potential as biological control agents for pest plant bugs in North America. The specific objectives were:

1) to evaluate methods used to detect parasitism

2) to determine the temporal and spatial abundance of *L. rugulipennis* Poppius, *L. pratensis* (L.) and *A. lineolatus* adults and nymphs, and their larval endoparasitoids in various agricultural ecosystems in Europe.

3) to gather biological information on the parasitoid species, such as their number of generations and patterns of emergence.

4) to investigate the range of mirid host species the parasitoids attack and in which they successfully develop in Europe.

1.3 Thesis organization

The thesis is divided into four main sections: introduction, literature review, research and general discussion. The literature review is an introduction to information on 1.) practical considerations for implementing biological control; 2.) the target mirid pest species, including distribution, biology and pest status; and 3.) the North American and European parasitoid species, including their distribution, biology, releases in North America and an evaluation of the potential of the parasitoid species as biological control agents for North American pest *Lygus* spp. and the introduced *A. lineolatus*. The research section is organized in scientific paper format and new research covering the following topics is described: 1.) a comparison of the dissection and rearing methods traditionally used to determine percent parasitism in *Lygus* spp. and *A. lineolatus*; 2.) observational studies on the seasonal abundance of the hosts and parasitoids in various agricultural field crops in Germany, including estimates of field levels of parasitism by *Peristenus* spp.; 3.) laboratory emergence patterns of parasitoids reared from field-collected plant bug nymphs; and 4.) the ecological host range of selected European parasitoids. In the general discussion important findings of the four research papers and general conclusions on the parasitoid species' potential as biological control agents for North American pest *Lygus* spp. and the introduced *A. lineolatus* are provided.

Chapter 2 Literature review of biological control of Nearctic pest *Lygus* species and the introduced *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae) in North America using euphorine braconid wasps.

2.1 Introduction

The plant bugs, *Lygus* spp. and *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae), are pests of many agricultural crops grown in North America (Jackson et al. 1995). They are widely distributed throughout North America. They damage crops by feeding on growing tissues and, in some instances they require chemical control. Integrated pest management strategies are being considered for control of these pests in Canada and biological control by natural enemies is a preferred option because the pests can be managed permanently by established biological control agents.

Several species of euphorine parasitoids (Hymenoptera: Braconidae) attack and kill the nymphal stages of plant bugs in Africa, Europe and North America. They belong to the closely related genera *Leiophron* Nees and *Peristenus* Foerster. The taxonomy of Nearctic and Palearctic species was reviewed by Loan (1974a, b). Ecological and biological studies were carried out on Nearctic species (Clancy & Pierce 1966, Day 1999, Day et al. 1999, Lim & Stewart 1976a, b, Norton et al. 1992) and Palaearctic species (Bilwicz-Pawińska 1982, Carignan et al. 1995, Drea et al. 1973, Day 1999, Hormchan 1977).

In the early 1970s, an attempt was made to establish a native North American parasitoid for control of pest *Lygus* in southern California, but the

program was unsuccessful (Clancy & Pierce 1966). Subsequent efforts were then directed at establishing European parasitoids as control agents in North America (Craig & Loan 1984 a, b, Day et al. 1990, Hormchan 1977, Van Steenwyk & Stern 1977).

Two Palaearctic species are established in the United States of America (hereafter US) and suppression of pest *Lygus* populations is documented (Day et al. 1990, 1992). There is renewed interest in using European parasitoids to control *Lygus* pests in Canada (Broadbent et al. 2002). The main objective of this review is to evaluate the potential of plant bug parasitoid species as biological control agents, and to compare the biology of and available information on European species and North American species.

2.2 Classical and novel biological control

The intent of biological control is to enhance pest mortality through the use of natural enemies, by conserving natural enemies, augmenting natural enemy populations, and introducing new natural enemies (Anonymous, OECD 1988). The focus of this literature review is on arthropod pests and their natural enemies, specifically insect pests and their parasitoids. Several characteristics of biological control that are considered desirable and offer advantages over other control methods are permanent control by established biological control agents, unassisted dispersal of control agents after their establishment, and adaptation through natural selection of the agent in the new environment (Hopper 1998). Biological control programs may be classical or novel. These strategies require the same basic information, such as host and agent biology

and ecology. These two types of biological control are fundamentally different in their approach to solving pest problems and it is important to recognize this.

Classical biological control involves the suppression of non-native or introduced species using the existing natural enemies found in the native environment of the introduced pest species. Usually the exotic or invasive species becomes a pest because it lacks population regulating mechanisms found in its native environment, or it may have other advantages that allow it to flourish in the new environment. To select classical biological control agents, the area of origin of the pest is surveyed for natural enemies of the pest. From the natural enemies found, one or more may be selected for introduction to regulate populations of the pest species (Carl 1982).

Novel biological control is an alternative to classical biological control and the term refers to the relationship established between the control agent and the target pest (Carl 1982, Hokkanen & Pimentel 1984, Pimentel 1963, Wiedenmann & Smith 1997). Novel control, also refereed to as new association, relies on exploiting a new or novel host association of a natural enemy with the target pest species. First, existing pest/parasitoid relationships are surveyed among species which are considered similar to the pest, either phylogenetically or through sharing a similar biology, ecology or behavior. Species which share such similarities are called homologues. For example, two species may be ecological homologues if they share similar ecological characteristics. Species considered homologous to the pest are surveyed for their natural enemies and from these existing relationships, parasitoid species are selected and tested for their

acceptance of the target pest species. If a new host association can be successfully achieved, the biological control agent may be used in a novel biological control program. Novel biological control agents can be used against exotic or native pest species (Weidenmann & Smith 1997).

In a novel biological control program a new host association must be made and this requires successful parasitism. Several steps must be accomplished for the parasitoid to parasitize the potential host, including; location of the host habitat, location of the host in the habitat, acceptance of the host and successful development in the host (Vinson 1976). The first three steps can be considered the host selection process. Vet & Dicke (1992) found that the most important factors regulating host selection are chemical cues. Vinson (1976) found that cues such as host size, shape, age and sound as well as chemical cues, are important in selection of hosts. The final step in successful parasitism requires physiological compatibility of the biological control agent and host. This may involve specific adaptations by the parasitoid to avoid negative consequences of the host's immune system (Quicke 1997) and the new host must provide adequate nutritional requirements for the developing parasitoid (Wiedenmann & Smith 1997).

2.2.1 Biological control research needs

A responsible biological control program, whether classical or novel, requires similar types of background information and testing. The following types of information are discussed here as they are critical when selecting appropriate parasitoid species as biological control agents for implementing biological control

programs; taxonomy, general biology, host range and release habitat criteria.

Taxonomy

The taxonomy of both pest and parasitoid are the foundation of any biological control program, because without them biological information is meaningless (Rosen 1977). Taxonomic information is critical for both classical and novel control (Howarth 1991), but is not always available. Other taxonomic considerations relate to cryptic species and the presence of hyperparasitoids (Hopper 1998, Rosen 1977). When species are very similar in their morphology, they are considered cryptic because they are not easily seperable. A hyperparasitoid is a secondary parasite species which parasitizes the primary parasitoid of the host. If biological control is to be successful, then the identity of the host and parasitoid must be known to ensure efforts are focused on the appropriate species. The presence of hyperparasitoids may cause confusion as to which species is the appropriate biological control agent and this may confound results of biological control programs. Their presence in a biological control program may also be detrimental to establishing biological control agents, and may result in the failure of the program. In order to document the species studied, voucher specimens should be preserved to aid in species identification.

General biology

A good knowledge of the pest's and biological control agent's biology is an important starting point for any biological control program and is necessary to select the most appropriate agent for the release area. This may seem obvious; however, if there is a variety of agents from which to select, the choice may be

difficult. The selection may be complicated if the most appropriate agent for introduction is not the obvious choice based on its performance in the native habitat. In fact, it is often difficult to predict which agent will be the most effective at managing pest populations in the new environment (Wiedenmann & Smith 1997).

For novel biological control programs, the control agents need to be tested to see if they will accept the new host, and if they can successfully parasitize the new host. Other considerations pertain to the agent's ability to adapt to the release environment. The possibility that different races of the control agent may be better adapted for survival in different parts of the new environment must be considered in both classical and novel approaches. Specific climatic, physiological and geographical adaptations of races of the control agent should be considered when selecting them. Host synchrony is also important for establishing biological control agents and this may be considered a part of selecting a suitable race (Sands 1998). Information regarding pest and parasitoid biology should be evaluated to determine the parasitoid's suitability for introduction (Howarth 1991).

Host range

The biological control agent's potential effect on non-target organisms needs to be considered. The suitability of candidate agents, either classical or novel, may be determined from an evaluation of their host range. To create a successful biological control program it may be necessary to provide the control agent with access to its alternative host species or access to the alternative

host's habitat (Van Driesche & Hoddle 1997).

Generalist predators and parasitoids are considered undesirable for biological control because they may not be consistent at effectively reducing pest populations, and they may have negative impacts on non-target organisms (Howarth 1991). In general, predators are less species specific compared to parasitoids. Possible negative effects on non-target organisms are reduction in their abundance because of direct attack on non-targets, competitive displacement of non-target predator or parasitoid species and possibly non-target species extinction (Follett et al. 2000, Henneman & Memmott 2001, Howarth 1991, Nafus 1993).

Egg and pupal parasitoids are more polyphagous than adult and larval parasitoids, commonly attacking species belonging to different genera, families, and sometimes even different orders (Drost & Cardé 1992). Parasitoids which are specific to one or a few species of hosts are more suitable for controlling target pests than those with wide host ranges (Sands 1998, Van Driesche & Hoddle 1997).

There are two concepts used to describe the host range; each one based on the method used to obtain host range information. The concepts are ecological host range (or realized, natural, or effective host range) and physiological host range (or potential host range). Ecological host range includes those species which serve as hosts in a natural environment (Condit & Cate 1982). The physiological host range of an agent can be defined as "all species which may serve as hosts under experimental conditions" (Condit & Cate

1982). To predict both the effectiveness of the parasitoid against the target pest and its impact on target and non-target organisms the host range of biological control agents should be evaluated.

Several methods can be used to evaluate the host range of a parasitoid including literature surveys, examining museum specimens for host records, field studies of the natural host range and host range testing in the laboratory (Sands 1998, Van Driesche & Hoddle 1997). Literature surveys include published scientific papers and catalogues. Host associations cited in old literature should be evaluated with caution until they can be verified by taxonomists because of discrepancies and synonymy (Van Driesche & Hoddle 1997).

Natural or ecological host range surveys must be conducted in the agent's region of origin and area of establishment. Studies of this nature consist of collecting hosts from native habitats and rearing them for parasitoids. Examples are given by Van Driesche & Hoddle (1997). This may provide accurate host associations and the number of host species successfully parasitized. Prior to a survey of this nature, some method should be utilized to determine which species to include in the survey. For instance, information from literature surveys or evaluation of taxonomic relationships of the target species to non-target species will help to determine which species should be included in the host range survey (Sands 1998).

Host range testing by laboratory screening of possible hosts may add relevant information regarding species which may be successfully parasitized by biological control agents. A protocol for testing the host range of herbivorous

insects has been developed and is used widely (Wapshere 1974). However, a host range screening protocol for entomophagous insect parasitoids is not as well developed (Van Driesche & Hoddle 1997). With regard to test hosts, species may be selected as previously noted using a taxonomic or phylogenetic approach (testing related species). Alternatively an ecological approach could be taken and only those species likely to be contacted by the parasitoid screened. Another approach is to test any known endangered or beneficial insects that could potentially be at risk. Examples of laboratory host range studies are given by Van Driesche & Hoddle (1997) and they suggested the following host range tests: adult oviposition tests including no choice tests, choice tests, field tests, and larval development tests. For oviposition tests, female parasitoids are given selected species to attack. Dissections of attacked host larva can be made to determine the success of attack. Larval development can be tested to measure the physiological host range of the immatures, to determine if the potential hosts provide the necessary nutrients for development and are unable to mount immune responses such as encapsulation.

Ecological host range can be overestimated or underestimated by laboratory studies. In laboratory studies, herbivorous insects exhibit a greater physiological host range than ecological host range as determined in the field. Laboratory studies exclude the environmental and ecological context of the habitat and its effect on parasitoid foraging (Van Driesche & Hoddle 1997), therefore caution must be exercised when interpreting and evaluating laboratory host range data (Sands 1998).

Information about the parasitoid's biology, ecology and behavior should be considered and reviewed extensively before laboratory screening of potential hosts begins. It may be useful to test a wide variety of species, including even those species which are not considered likely to be encountered (Follett et al. 2001) and any species for which clarification of the host range is still needed. Studies on natural enemies considered for biological control programs should include specific testing in the case of novel or new host associations (Howarth 1991, Nafus 1993).

Selecting release habitats

Prior to releasing control agents, it is necessary to identify the most appropriate habitats for parasitoid releases, as the habitat where control is desired may not be the most suitable habitat for establishment of the agent. One guideline to follow is to identify habitats similar to those in which the control agent is naturally found (Sands 1998). Release areas need to be selected which favor parasitoid establishment, and methods to ensure suitable conditions for the introduced agent need to be considered and provided (Beirne 1985).

2.2.2 Assessing the benefits and risks of biological control programs

In recent years, concern over non-target effects has prompted the biological control community to re-evaluate the benefits and risks of this type of control (Follett et al. 2000, Harris 1990, Henneman & Memmott 2001, Howarth 1991, Nafus 1993, Philogène 1998, Solter & Maddox 1998). The benefits and risks of introducing exotic biological control agents can be assessed by several different criteria.

Field surveys, enclosure and exclosure experiments, and pre/post introduction experiments are methods which can be used to detect impact (Hopper 1998). Field surveys include collections of target and non-target hosts to measure percent parasitism, by rearing and dissection or by analysis of molecular markers. Field data can include qualitative data on host range or quantitative data and can be used to analyze the effects of natural enemies on pest and non-target population dynamics. Physical barriers are used in exclosure experiments to exclude arthropods from patches of habitat to assess the dynamics of the host in the absence of parasitoids. In such exclosures, there may be problems associated with microclimatic differences, but the method is useful to determine impacts (Hopper 1998). Pre- and post- introduction experiments, such as field surveys on host and non-target populations should be measured over a long period. It is recommended that surveys are replicated across many diverse habitats to measure non-target species abundances (Van Driesche & Hoddle 1997).

Parasitoid establishment can be evaluated by several different methods. Sex ratios of the control agent can be determined by collecting adults in the field. This information combined with information on the reproductive biology of the control agent can help determine if a stable population has established or not. For example, arrhenotokous females produce only male offspring when unmated; therefore, if field populations do not exhibit a 1:1 ratio of the sexes, a stable colonizing population may not be established and more releases may be needed. Knowledge of the sex ratio of parasitoids can be used to determine the

status of the field population and also allow estimates of potential impacts of the biological control agents to be made (Hopper 1998).

Finally, it is necessary to evaluate the risks of the project. Methods are available to determine parasitoid impact on targets and non-targets (Harris 1990, Van Driesche & Hoddle 1997). Studies incorporating these methods will provide information to assess the benefits and risks of the proposed control agent. In terms of effects on non-targets, few of the described methods have been used to evaluate impacts. The ecological relationships of the organisms involved in the biological control program could be used to predict the outcomes of biological control efforts, but there is a lack of reliable predictive theory in population, community and evolutionary ecology. However, past releases of biological control agents provide us with opportunities to test and evaluate ecological principles concerning parasitoid establishment, host range expansion and population dynamics. This body of knowledge will contribute to ecological theory and aid in the development of practical applications useful for selecting biological control agents (Howarth 1991).

Legal and ethical issues concerning biological control

The state of awareness regarding threats to our world's biodiversity is increasing. There are acts of legislation in place today to protect endangered species and their habitats, such as the US Endangered Species Act of 1973 , the National Environmental Policy Act of 1969 (NEPA), and the Environmental Protection Agency (EPA) (Solter & Maddox 1998), and in Canada, pending Bill C-441 (Mason et al. unpublished). These acts make it an offence to harm

endangered species. In the US biological control agents are regulated by the USDA Animal and Plant Health Inspection Service (APHIS) and the Plant Pest Act (Solter & Maddox 1998). Agencies such as the Species Survival Commission, The World Conservation Union (hereafter IUCN), have constructed recommendation lists regarding the protection of biodiveristy against invasive species. For example, Article 8 of the convention on biological diversity states that, "each contracting party shall, as far as possible and as appropriate prevent the introduction of, control or eradicate those alien species which threaten ecosystems, habitats or species" (Anonymous 1999). While the focus of biological control is to suppress invasive pest species, there is potential for conflict with respect to maintaining ecological diversity if the impact of an introduced agent threatens biological diversity.

An introduced agent may displace competing native parasitoids and it may be considered a biological contaminant in the new habitat, or it may adversely affect populations of non-target organisms. The potential negative environmental impacts, and the potential legal implications of implementing a biological control program require biological control practitioners to consider all the available information and weigh the benefits and risks regarding the potential control agents prior to their introduction. Only those biological control programs where the benefits outweigh the risks, according to publically legislated criteria should be initiated (Anonymous 1999). Legal and ethical implications involved with introducing exotic biological control agents should be reviewed before introductions are made.

2.3 The Mirid Pests

In North America, several species of native *Lygus* bugs and the introduced Palaearctic species, *Adelphocoris lineolatus,* are considered pests of agricultural crops (Broadbent et al. 2002, Soroka & Karl 2002). The *Lygus* species are *L. lineolaris* (Palisot de Beauvois), the eastern tarnished plant bug, *L. hesperus* Knight, the western tarnished plant bug, *L. elisus* Van Duzee, the pale plant bug, and *L. borealis* (Kelton). In Europe, the most common *Lygus* species in agricultural ecosystems is *L. rugulipennis* Poppius, the European tarnished plant bug (Bilwicz-Pawińska 1982). *Lygus rugulipennis* which also occurs naturally in North America is considered an ecological homologue of the North American species, *L. lineolaris.*

2.3.1 Pest taxonomy

The species which make up the *Lygus* complex all belong to the family Miridae of the order Hemiptera. R.T. Schuh (1995) published a catalogue on the mirids of the world. A list of the species of Hemiptera found in Canada and in continental US is given by Henry & Froeschner (1988) and Maw et al. (2000). The species of mirids which occur in the Prairies of Canada, includes native North American species and species introduced (Kelton 1980). Schwartz and Foottit (1998), revised the genus *Lygus* Hahn. They provided detailed descriptions of the species, their distribution, host plant lists, keys to the Nearctic, Holarctic and Palaearctic *Lygus* species, and a proposed phylogeny for the group.

2.3.2 Pest biology

The mirid life cycle is hemimetabolous. All of the pest species oviposit into plant stem tissue. After hatching, nymphs develop through five instars before moulting to the adult stage. *Adelphocoris lineolatus* overwinters as eggs laid in the base of plant stems (Craig 1963), whereas *Lygus* species overwinter as adults in crop debris, shelter belts or hedgerows (Fye 1982).

In North America, between 50°N and 53°N latitude, the *Lygus* and *Adelphocoris* species are univoltine. Below 50° N latitude they have two generations a year and in the southern US, they may have up to 5 generations a year (Schwartz & Foottit 1992). Stewart (1968) found *L. rugulipennis* in Scotland to have one generation a year, while in northern Germany and in southern Great Britain, two generations were produced. This variation in number of generations is a reflection of the fact that in different geographical areas heat unit accumulation is different and affects developmental times of the bugs.

2.3.3 Pest distribution

Adelphocoris lineolatus was introduced to North America in the early 1900's (Craig 1963, Craig & Loan 1984a). This species is found in the north central US, eastern Canada, Manitoba, Saskatchewan and Alberta. The following information on pest distribution was compiled from Kelton (1980), Maw et al. (2000) and Schwartz and Foottit (1992). The pest *Lygus* species are Nearctic and *L. lineolaris* is the most widespread species in North America. It occurs from Alaska to Newfoundland and as far south as southern Mexico.

Lygus hesperus is found in western North America. *Lygus elisus* is present in Alaska and in the western US as well as northern Canada, British Columbia and the prairie provinces. *Lygus borealis* occurs in Alaska, the north central and north western US, and eastern Canada. *Lygus rugulipennis* is Holarctic, being found in the Palaearctic region (Holopainen & Varis 1991), and in western North America from Alaska to northern California (Schwartz & Foottit 1998). All of the pest species described here are widely distributed in their areas of origin.

2.3.4 Pest status

The *Lygus* species complex is extremely polyphagous. They feed on agriculturally important crop species such as alfalfa, cotton, canola, and fruit and vegetable crops (Schwartz & Foottit 1992). They also utilize weed species such as chenopods and amaranths. The following information on the plant host range was compiled from Schwartz & Foottit (1992) unless otherwise noted.

The plant host range of each species varies. *Adelphocoris lineolatus* feeds on alfalfa (*Medicago sativa* L.), *Melilotus* spp., *Trifolium* spp. and many herbaceous plants (Kelton 1980). Of the Nearctic *Lygus* species, *L. lineolaris* has the greatest number of recorded host plants, over 300 host species, 130 of which are economically important. Agricultural crops attacked include alfalfa (seed and forage), canola, fruit (peaches, strawberries, apples) and vegetables. Schwartz & Foottit (1998) reported that *L. hesperus* feeds on 117 non-crop plant species as well as agriculturally important ones. Most of the plant species that *L. hesperus* attacks belong to the Asteraceae. There are over 34 recorded host

plants in 14 different families for *L. elisus*. It feeds on weedy crucifers, chenopods and composites (Fye 1982). Butts and Lamb (1991b) reported that this species has a preference for *Brassica* spp. over alfalfa. *Lygus borealis* occurs on 15 host plants in six different families. *Lygus rugulipennis* has the widest host range of all *Lygus* species, 437 species (Stewart 1968). The most important plant families for *L. rugulipennis* are the Brassicaceae, Asteraceae and Fabaceaea (Holopainen & Varis 1991). The wide host range of these pests includes many agriculturally important plant species which contributes to the pest status of these mirids.

Damage caused by these bugs is attributable to their feeding biology. Butts (1989) reviewed *Lygus* feeding biology and damage. All of these plant bugs have piercing/sucking mouthparts. They feed on growing and reproductive tissues of the host plant, utilizing flowers and buds, fruits and seeds and vegetative tissue. The insect expels salivary secretions into the host tissue. These secretions contain digestive enzymes which liquefy the plant tissue they contact and the bugs ingest the liquid. Their feeding results in five different types of injury or damage, as classified by Butts (1989): 1) localized wilting and tissue necrosis, 2) morphological deformation of fruit and seed (catfacing), 3) abscission of fruiting forms (blasting), 4) altered vegetative growth and 5) tissue malformation. Feeding by *Lygus* causes lesions on the surfaces of stems, buds, flowers and pods, and can result in early fruit and seed dehiscence and reduced seed size (Broadbent et al. 2002). Butts & Lamb (1991a, b) reported that the 5th

instar and adult stages of *Lygus* are the most damaging to canola. The damage resulting from feeding may reduce seed set, lower seed yields (Butts & Lamb 1991a) and reduce marketability of fruits and vegetables.

Lygus bugs have pest status in many different crops, such as alfalfa (forage and seed), cotton, canola and fruits and vegetables (Craig & Loan 1984b, Kelton 1982, Mason & Soroka 1998, Schwartz & Foottit 1992). In Europe, *L. rugulipennis* causes damage to alfalfa, clover, potatoes, sugar beets and cereals (Holopainen & Varis 1991). *A. lineolatus* is a pest of alfalfa in Europe and in North America (Craig 1963). Losses caused by plant bug infestations in alfalfa in Wisconsin in one year were estimated at $24 million (Day et al. 1992). *Lygus* species can reduce the yield of canola crops (Butts & Lamb 1990, 1991a, Turnock et al. 1995, Wise & Lamb 1998a). Information on economic thresholds in canola was given in Wise and Lamb (1998a). They calculated the economic threshold for plant bugs to be 15 plant bugs per 10 sweeps at the end of flowering or the beginning of pod formation. There are sampling plans for plant bugs in other crops such as cotton, strawberries, lentils and celery (Wise & Lamb 1998b).

Chemicals are used on certain crops for controlling *Lygus* species in some years (Jackson et al. 1995). However, it is desirable to suppress pest populations before they reach economic status. There is successful biological control in alfalfa for both alfalfa blotch leafminer and for alfalfa weevil, therefore chemical control in this crop could be damaging to existing biological control

agents (Guppy et al. 1984, Harcourt & Guppy 1984). An integrated pest management approach to control pest *Lygus* would be ideal (Mason & Soroka 1998) and biological control may have an integral part in such a management plan.

2.4 The Braconid Parasitoids

In the following section, information about the braconid parasitoids that attack and kill pest *Lygus* is reviewed. The parasitoids belong to the family Braconidae, subfamily Euphorinae and are found in two closely related genera, *Leiophron* and *Peristenus.* The species of *Leiophron* and *Peristenus* can be categorized according to their geographical location of origin, as Nearctic, Holarctic or Palaearctic. Throughout this review, Nearctic species may be referred to as North American species, Palaearctic species as European species, and those which are naturally distributed in both regions as Holarctic.

The Nearctic species of concern here are *Leiophron lygivora* (Loan), *Leiophron uniformis* (Gahan), *Peristenus pseudopallipes* Loan, and *Peristenus howardi* Shaw. The selected Palaearctic species are *Peristenus adelphocoridis* Loan, *Peristenus digoneutis* Loan, *Peristenus rubricollis* (Thompson), and *Peristenus stygicus* Loan. The only Holarctic species is *Peristenus pallipes* (Curtis).

Parasitoid definition

A parasitoid is an insect which acts as a true parasite for only a portion of its lifetime and one which kills its host (Quicke 1997). Vinson (1976) described

insects that are parasitic only during their immature stages as protelean parasites. The parasitoids dealt with here are protelean parasitoids, as they are typically parasitic only during their early developmental stages, after which they destroy their host and live as free adults.

2.4.1 Parasitoid taxonomy

The North American Hymenoptera were treated by Cresson (1872) and the British braconids by Marshall (1887). Muesebeck (1936) published a review of the Nearctic species of Euphorinae. Tobias (1966) reviewed the generic groupings in the subfamily Euphorinae. Loan & Bilwicz-Pawińska (1973) re-established the genus *Peristenus* as separate from *Leiophron.* Loan reviewed the North American (Loan 1974a) and European (Loan 1974b) species of *Leiophron* and *Peristenus*. In his review of the Nearctic species of *Leiophron* and *Peristenus*, Loan described each species and provided keys to species. He recognized 19 species of *Leiophron* and 24 species of *Peristenus*. Shaw described a new species of Nearctic *Peristenus*, *P. howardi* (Day et al.1999). Loan (1974 b) reviewed the Palaearctic species of *Peristenus* and *Leiophron* and described 15 species of *Peristenus* and 11 species of *Leiophron.* He described *Peristenus adelphocoridis* (Loan 1979) following his 1974 review. Loan has contributed greatly to our present understanding of the taxonomy and biology of these species. A new species considered to be Palaearctic in origin, *P. conradi* Marsh, was described in Day et al. (1992).

The larvae of these parasitoids are very difficult to identify to species

(Carignan et al. 1995). Carignan et al. (1995) studied the larval head sclerites of *P. digoneutis*, *P. pallipes* and *P. pseudopallipes* and did not find any reliable characters for species identification. Recently, a molecular analysis of the larvae of *P. conradi*, *P. digoneutis*, and *P. pallipes* was made by Tilmon et al. (2000), who were able to identify molecular markers to associate the larvae with the correct adult species.

2.4.2 Parasitoid biology

Table 2.1 contains a list of authors that have contributed to our understanding of the biology of *Leiophron* and *Peristenus* which attack *Lygus* spp. and *A. lineolatus*. Species of *Leiophron* and *Peristenus* share a similar general life cycle. The known biology of these species is summarized in Table 2.2.

Females of these parasitic wasps lay a single egg in the host nymph. Generally nymphs at the second and third instar are selected for oviposition. The embryo will develop for approximately 4-5 days, after which time the first instar larva will hatch. The first instar larva is mandibulate and caudate, as are most braconid larvae. Teratocytes form from the trophamnion layer of the egg and host fluids and serve as food for the developing larva (Bilwicz-Pawińska & Pankanin 1974, Waloff 1967). The first instar larva actively seeks out and destroys other parasitoid eggs and larvae, resulting in only one larva developing per host (Waloff 1967). The larva continues its development to the final larval instar in the host. All stages of the parasitoid larva are vermiform.

Table 2.1 Selected Holarctic, Nearctic and Palaearctic species of *Leiophron* and *Peristenus*, parasitoids of *Lygus* spp. and *Adelphocoris lineolatus*; their center of origin, their distribution, hosts and major information sources.

Species	Distribution	Hosts	Major Information Source
Holarctic			
Peristenus pallipes (Curtis)	-Canada (Ontario, Saskatchewan, Alberta, British Columbia) - U.S. (Alaska, Utah, Idaho, New Jersey)	**Nearctic** 1.*Lygus lineolaris* (1st generation) 2.*Lygus borealis* 3.*Lygus elisus* ∞ 4.*Lygus hesperus* ∞ 5.*Adelphocoris rapidus* Say 6.*Labops hirtus* Knight **Palaearctic** 7.*Lygus pratensis* 8.*Notostira erratica* **Holarctic** 9.*Lygus rugulipennis* 10.*Leptopterna dolobrata** (L.) 11.*Trigonotylus caelestialium** (Kirkaldy) 12.*Capsus ater* L. 13.*Closterotomus norvegicus* (Gmelin) 14.*Stenotus binotatus* **Introduced Palaearctic** 15.*Adelphocoris lineolatis*	Brindley 1939 Loan 1965 Lim & Stewart 1976b
Nearctic			
Leiophron lygivora (Loan)	-Canada (Ontario)	**Nearctic** 1.*Lygus lineolaris* **Introduced Palaearctic** 2.*Adelphocoris lineolatus* ∞	

Table 2.1 continued

Species	Distribution	Hosts	Major Information Source
Leiophron uniformis (Loan)	-Canada (Quebec) -U.S. (widely distributed)	**Nearctic** 1.*Lygus hesperus* 2.*Lygus elisus,* 3.*Lygus lineolaris* 4.*Halticus bractatus** (Say)	Clancy & Pierce 1966 Graham et al 1986 Day & Saunders 1990 Norton et al 1992
Peristenus howardi Shaw ▸	-U.S. (Idaho, Oregon, Washington)	**Nearctic** 1.*Lygus hesperus** 2.*Lygus lineolaris*	Day et al 1999
Peristenus pseudopallipes (Loan)	-U.S. (Georgia, New Jersey, New York, Ohio) -Canada (Ontario, Quebec)	**Nearctic** 1.*Lygus lineolaris* *(2nd generation) 2.*Lygus vanduzeei* Knight	Loan 1965 (as *P. pallipes* 2nd generation) Loan 1970 Lim & Stewart 1976b
Palaearctic			
Peristenus adelphocoridis Loan	-Europe (France, Denmark)	**Nearctic** 1.*Adelphocoris rapidus* **Introduced Palaeactic** 2.*Adelphocoris lineolatis*	
Peristenus conradi Marsh ▸	-Canada (Quebec) -U.S. (Delaware, New York, New Jersey)	**Nearctic** 1.*Lygus lineolaris* **Introduced Palaearctic** 2.*Adelphocoris lineolatis**	Day et al. 1992

Table 2.1 continued

Species	Distribution	Hosts	Major Information Source
Peristenus digoneutis Loan ▸	-Europe (Poland, Switzerland, France, Spain, Italy, Germany, Austria) -Canada (Quebec) -US (northeastern, New Jersey, New York, Pennsylvannia, Massachusetts, New Hampshire, Vermont, Connecticut)	**Holarctic** 1.*Lygus rugulipennis** **Palaearctic** 2.*Lygus pratensis* 3.*Leptoterna dolobrata* **Introduced Palaearctic** 4.*Adelphocoris lineolatis* **Nearctic** 5.*Lygus lineolaris**	Bilewicz-Pawinska 1974 Bilewicz-Pawinska & Pankanin 1974 Bilewicz-Pawinska 1982 Hormchan 1977 Carignan et al. 1995
Peristenus rubricollis (Thomson) ▸	-Europe (Germany, Switzerland, Poland)	**Holarctic** 1.*Lygus rugulipennis** **Palaearctic** 2.*Lygus pratensis** **Introduced Palaearctic** 3.*Adelphocoris lineolatus**	Bilewicz-Pawinska & Pankanin 1974 Bilewicz-Pawinska 1974 Hormchan 1977 Bilewicz-Pawinska 1982

Table 2.1 continued

Species	Distribution	Hosts	Major Information Source
Peristenus stygicus Loan	-Europe (Poland, Switzerland, France, Turkey, Spain, Germany, Greece)	**Holarctic** 1.*Lygus rugulipennis** 2.*Polymerus unifasciatus* (F.) 3.*Trigonotylus caelestialium* **Nearctic** 4.*Lygus hesperus* ∞ 5.*Lygus lineolaris* ∞ 6.*Polymerus basalis* ∞ 7.*Lindbergocapsus geminatus* (Johnston) ∞ 8.*Pseudatomoscelis seriatus* (Reuter) ∞ **Palaearctic** 9.*Lygus pratensis*	Drea et al. 1973 Butler & Wardecker 1974 Bilewicz-Pawinska 1974 Van Steenwyk & Stern 1976 Condit & Cate 1982 Bilewicz-Pawinska 1982

* preferred host, ∞ lab data only, ▸preference for target host

Table 2.2 Biology of selected Holarctic, Nearctic and Palaearctic species of *Leiophron* and *Peristenus* which attack *Lygus* spp. and *Adelphocoris lineolatus*.

Species	Sex determination	Duration from egg to emerging larva	Duration of development in the pupa	Months in diapause	Number of generations/ year
Holarctic					
Peristenus pallipes (Curtis)		1. 34-36 days (Brindley 1939) 2. 5-6 wks in field (Loan 1965) 3. 14-21 days (24°C) (Clancy & Pierce 1966) 4. 20-33 days (Lim & Stewart 1976b)		1. 10-11 months (Bilewicz-Pawinska 1982), 2. 10-11 months (Carignan et al. 1995)	1. 1 (Bilewica-Pawinska 1982) 2. 1 (Day et al. 1990)
Nearctic					
Leiophron lygivora (Loan)					
Leiophron uniformis (Gahan)		10-12 days (Clancy & Pierce 1966)		5-9 months (Clancy & Pierce 1966)	2-3 (Day et al. 1999)
Peristenus howardi Shaw ▸	thelytokous				2-3 (Day et al. 1999)
Peristenus pseudopallipes (Loan) ▸	arrhenotokous	15 days (Lim & Stewart 1976b)		6-9 months (Lim & Stewart 1976b)	1 (Day et al. 1999)

Table 2.2 continued

Species	Sex determination	Duration from egg to emerging larva	Duration of development in the pupa	Months in diapause	Number of generations/ year
Palaearctic					
Peristenus adelphocoridis Loan					
Peristenus conradi Marsh ▸	deuterotokous				1 (Day et al. 1992, 1999)
Peristenus digoneutis Loan ▸		1.35 days (21°C) egg to adult emergence (Bilewicz-Pawinska 1977b) 2.17 days (21°C) (Carignan et al. 1995)		1. 8 months (Bilewicz-Pawinska 1974, 1982) 2. 8 months (Carignan et al. 1995)	1. 2 (Bilewicz-Pawinska 1982) 2. 2 (Carignan et al. 1995) 3. 2-3 (Day et al. 1999)
Peristenus rubricollis (Thomson) ▸	arrhenotokous	47 days (21°C) (Bilewicz-Pawinska 1982)		1. 10 months (Bilewicz-Pawinska 1974) 2. 10 months (Bilewicz-Pawinska 1982)	1. 1 (Loan & Bilewicz-Pawinksa 1973) 2. 1 (Bilewicz-Pawinska 1982)

Table 2.2 continued

Species	Sex determination	Duration from egg to emerging larva	Duration of development in the pupa	Months in diapause	Number of generations/ year
Peristenus stygicus Loan	arrhenotokous	1.<11 days (27°C) (Butler & Wardecker 1974) 2.16 day (20°C), 12 day (25°C), 9 day (30°C) (Hormchan 1977)	1.14 days (12-18) (Drea et al. 1973) 2.17 day (20°C), 13 day (25°C), 12 day (30°C) (Hormchan 1977)	1. 5 months (Drea et al. 1973) 2. 8 months (Bilewicz-Pawinska 1982)	1. 4 (Drea et al. 1973) 2. 2 (Bilewicz-Pawinska 1982)

▸ preference for a target host species

Developmental times from egg to final larval instar are summarized for each species in Table 2.2. A comparison of the developmental time with the number of generations per year suggests that multivoltine species have a shorter developmental time compared with univoltine species. There is variation in the developmental time reported from egg to emerging larva for most species and this is probably related to different temperature conditions, or to host species and host size (Lim & Stewart 1976b). When the final instar matures, it emerges from the host by rupturing the side of the host's abdominal wall. The newly emerged larva then actively seeks a pupation site. When given moist vermiculite, it will burrow downwards then spin a silk cocoon and pupate. The number of months of cold required to break diapause varies among species, see Table 2.2.

2.4.3 Parasitoid distribution

Distribution data for these parasitoids are compiled from Loan (1974a, b) and Day et al. (1998, 1999), and are summarized in Table 2.1. It should be noted that the distribution of *P. digoneutis*, a Palaearctic species, includes regions of North America. This species was introduced to North America and has become established (Day et al. 1990). It is presently found in northeastern US and has expanded its range northward into southern Quebec (Broadbent et al. 1999). The information on geographical range is sparse and more information is needed to determine the species' distributional limits (Day et al. 1998). This is true for all the species of *Leiophron* and *Peristenus.*

The geographic range described for the economically important parasitoid species may be wider than for the other species because it is better known as a result of greater research efforts.

2.4.4 Mirid host range of the parasitoids

Efforts to document the host range of these parasitoids were made using ecological analysis methods and laboratory host range testing. A list of known host associations for the species of *Peristenus* and *Leiophron* under consideration is presented in Table 2.1. Host species in which successful parasitism was demonstrated, either in the field or laboratory are included.

The ecological host range of exotic and native parasitoid species was assessed by Bilewicz-Pawińska (1982), Day & Saunders (1990), Day (1996, 1999) and Loan (1980). Loan (1980) surveyed plant bugs and their parasitoids in Ontario. He reared 14 species of *Peristenus* and four of *Leiophron* from 28 species of plant bugs. In another 24 species, he found immature euphorine larvae which could not be identified. Based on his work, most of the parasitoid species are known to parasitize only one or a few plant bug species. Loan (1980) concluded that there are probably many more unknown euphorine species of *Peristenus* and *Leiophron* waiting to be discovered.

Bilewicz-Pawińska (1982) studied the ecology of plant bugs infesting cereal crops in Poland. She reported their pest status and population dynamics, including parasitism in *Trigonotylus caelestialium* (Kirkaldy), *Notostira erratica* (Geoffroy) *Leptopterna dolobrata* (L.) and *Stenodema virens* (L.).

Day and Saunders (1990) examined the native parasitoids attacking the mirid species, *Halticus bracticus* (Say), in alfalfa in New Jersey. They discovered that up to 49% of the population of *H. bracticus* were parasitized by *Leiophron uniformis* and they considered this to be the preferred host of *L. uniformis.*

Day (1996) surveyed the host range of newly established *P. digoneutis* in North America. He found *P. digoneutis* in two field-collected *Leptopterna dolobrata* out of 1,888. In this same survey, no parasitoids were reared from *Trigonotylus caelestialium* (285 reared), *Stenotus binotatus* (Fabr.) (126 reared), or *Megaloceroea recticornis* (Goeffroy) (52 reared). Day (1999), reported on the results of an eight year ecological host range study of mirids collected in alfalfa in New Jersey and Delaware. Ecological host range analysis has contributed to our knowledge of the parasitoids' use of hosts which is important to determine impacts on non-target mirids.

Laboratory studies on the host range of some euphorine wasps were conducted by Condit & Cate (1982) and Waloff (1967). Waloff (1967) studied the laboratory host range of several Palaearctic *Leiophron* spp. and *Peristenus pallipes* (as *L. pallipes*). Nymphs of *Leptopterna dolobrata* and *Stenotus binotatus* were successfully parasitized, and one specimen of *P. pallipes* was reared from a field-collected *Orthotylus virescens* (Douglas and Scott). Female wasps were offered nymphs of *O. virescens* and *Orthotylus adenocarpi* (Perris) in the lab, and these were attacked and killed by oviposition. Condit & Cate (1982) used laboratory experiments to study the host range of *Peristenus*

stygicus. They offered female wasps a range of mirid species, representing four subfamilies, and also the lygaeid predator, *Geocoris* sp. The lygaeid was selected because it belongs to Hemiptera and it occurs in the same habitats as the target mirids, where it is a predator on many insect species, including the target mirid. Therefore, it was important to test the biological control agent's response to the beneficial insect. They found that females attacked and successfully developed in the Mirinae species *L. hesperus*, *L. lineolaris*, and *Polymerus basalis* (Reuter). They attacked but did not emerge from *Dichrooscytus* sp. and they did not attack *Taedia johnstoni* (Knight). Females parasitized and developed in mirids of the subfamilies Orthotylinae (*Lindobergocapsus geminata* (Johnston)) and Phylinae (*Pseudatomoscelis seriatus* (Reuter)). The parasitoids did not accept species of Bryocorinae and completely rejected the lygaeid species offered.

All of the parasitoid species listed in Table 2.1 are recorded only from members of the family Miridae. *Peristenus pallipes* appears to have the greatest host range with 15 species. Half of the parasitoid species presented in Table 2.1 are recorded from two hosts. The majority of these parasitoid species may be specific to only a few species of mirids. However, while there is host information for many of these species, most of the Palaearctic parasitoid species considered for release in North America have not had their host range analyzed, either from host range surveys or laboratory host range testing.

2.5 Evaluation of European Parasitoids

In the past, species of *Leiophron* and *Peristenus* were studied to determine their potential as biological control agents for pest *Lygus* species in North America. This included studies to determine their effectiveness at parasitizing the target species. In this section, the efforts to establish native and exotic parasitoid species for biological control in North America are documented; this includes information on the release of North American and European parasitoids and a review of studies presently available in which these introductions are evaluated.

2.5.1 Selection of biological control agents

Biological parameters considered to limit native North American parasitoids' capabilities for regulating pest *Lygus* populations include the number of parasitoid species which attack the target pests, their host preferences, the number of generations they produce and the level of parasitism (Carl 1979, Day & Saunders 1990). European species are considered to be superior in some of these characteristics.

From Table 2.1 we can determine that there are five Nearctic species (including *P. pallipes*) which attack a target pest in nature or in the laboratory. There are three species which have a host preference for the target pests, *L. lygivorus*, *P. pseudopallipes* and *P. howardi*. There are six Palaearctic species (including *P. pallipes*) known to attack the target species, and three species have a host preference for the North American target pest species and/or *A.*

lineolatus, *P. conradi*, *P. digoneutis*, and *P. rubricollis*.

Day (1996) hypothesized that the low levels of parasitism by our native parasitoids could be attributed to their preference for host species other than the pest *Lygus* species. Using the host preference information in Table 2.1 and the number of generations listed in Table 2.2, we can determine that to date only one Nearctic species, *P. howardi*, has a host preference for the target pest species and is also multivoltine. Only one Palaearctic species, *P. digoneutis*, has a host preference for the target pest species and is also multivoltine.

Information on parasitism levels available for each species is presented in Table 2.3. Parasitism data are not available for all species and for most species not many references concerned with levels of parasitism are published. From those available references, up to six were selected for each species, to present information on parasitism levels. The methods used to collect the samples and for determining parasitism levels are not always clearly indicated, making the results difficult to compare. In a recent study by Braun et al. (2001), parasitism levels for North American *P. pallipes* attacking *Lygus* spp. nymphs were reported to be up to 80% in June and 70% in July in alfalfa and up to 20% in early July in canola. However, it should be noted these percentages were determined based on small sample sizes. In Europe Bilewicz-Pawińska (1977a) found that in rye, first generation *L. rugulipennis* were parasitized to varying degrees in different years, 1-18%, 30%, 66%, and levels in potatoes ranged from 2-25%. The levels of parasitism vary within and among species. The levels of parasitism reported

Table 2.3 Levels of parasitism reported and comments on selected studies of selected Holarctic, Nearctic and Palaearctic species of *Leiophron* and *Peristenus*.

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Holarctic					
Peristenus pallipes (Curtis)	10	Loan 1980	Field sample, May-June Dissection	*A. lineolatus*-49% (n=100) *A. rapidus*-42% (n=100) *C. ater*-30.9% (n=84) *L. hirtus*-21.4% (n=98) *L. dolobrata*-42% (n=100) *L. lineolaris*-62% (n=100)	1. Indicated stage of parasitoid found in host stage.
		Lim & Stewart 1976a	Field sample, May-Oct. Dissection; 10 adults, 10 nymphs of each instar Rearing; surplus nymphs were reared	Dissection mean weekly rates ranged from; 0-8% in weeds, 0-19% in alfalfa	1. Using all instars in calculation may underestimate parasitism because eggs are not usually found in instars 1-3 and adults normally do not have parasitoids 2. Found 1 parasitoid egg in 1407 nymphs dissected. 3. Gave % parasitism per developmental stage of nymphs
		Loan 1965	Field sample, May-Sept Dissection and rearing	45-60% in *A. lineolatus* and *L. lineolaris* *L. hirtus* 16% (n=98)	

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Peristenus pallipes (Curtis)		Day et al. 1992	Field sample, May-June Dissection; 15 nymphs Rearing; 20 nymphs + any extra	1.1988-1990 rearing weighted mean 5% 2. Avg.peak parasitism of Generation I *A. lineolatus* was 8%. Dissection 1986-12% 1987-28% 1988-18% 1989-10% Rearing 1986-8% 1987-15% 1988-3% 1989-10%	1. Parasitism determined by number of adults emerging, cocoons counted after overwintering, checked for emergence holes and added to parasitism data.
		Braun et al. 2001	Field sample, April-Sept. Dissections	Alfalfa 1998-June 60% 1998-July 10% 1999-June 80% 1999-July 70% Canola 1999-June-July 20%	1. Gave % parasitism for early (1-3) instars and late (4-5) instars. 2. Gave number of nymphs collected and number dissected.

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Peristenus pallipes (Curtis)		Clancy & Pierce 1966	Field sample, May-Oct. Reared 50-150 nymphs in cardboard cylinders. Dissected 3-4-5th instars b/c larvae were only found in these instars	Average monthly parasitism in 10 alfalfa fields was 0.3-11% *Chenopodium* 25-50%	1. Dissected to determine % parasitism. 2. Dissected only 3-4-5th instars. 3. Details of rearing method provided
Nearctic					
Leiophron lygivora (Loan)	0				
Leiophron uniformis (Gahan)	5	Day & Saunders 1990	Field samples, May-July Dissection and rearing	Reared 1986-27% (n=807) 1987-37% (n=62) Dissected 1985-100% (n=5) 1986-45% (n=38) 1987-48% (n=63)	1. Samples for dissection were frozen -20°C and processed later. 2. Small sample size, in 1985, 1 sample with 100% parasitism in 5 individuals. 3. Stated high mortality of parasitized nymphs in rearings but no method to determine this is given.

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Leiophron uniformis (Gahan)		Norton et al. 1992	Cage experiments, Dissections, experimental nymphs were 2nd and 3rd instars 300 nymph-100 parasitoids 150 nymph-100 parasitoids	 ← 55% ← 97%	1. Calculated % parasitism by no. of dissected parasitized / total no. recovered. 2. Number of nymphs recovered much lower than released, mean recovered 15%. 3. Parasitism increased with increase parasitoid release rate.
		Graham et al. 1986	Field samples, Jan-Dec. Mean of % parasitism per month among years	Alfalfa max. mean monthly 10.6% Alfalfa 60-70% *Chenopodium* max. mean monthly 28.6%	1. Used method referenced in Debolt (1981). 2. Gave mean number of nymphs collected.
		Clancy & Pierce 1966	Field sample, May-Oct. Reared 50-150 nymphs in cardboard cylinders. Dissected 3-4-5th instars b/c larvae were only found in these instars	Average monthly parasitism in 10 alfalfa fields was 0.3-11% *Chenopodium* 25-50%	1. Dissected only 3-4-5th instars. 2. Details of rearing method provided.

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Peristenus howardi Shaw▸	1	Day et al. 1999	Field sample, all season, Dissected and reared	Dissection results 1997 Generation I, 44% (n=80) Generation II, 17% (n=7) 1998 Generation I-81% (n=83) Generation II-51% (n=80) Generation III-5% (n=98)	1. No information given on stage of nymphs dissected, could bias results.
Peristenus pseudopallipes (Loan) ▸	3	Lim & Stewart 1976a	Field sample, May-Oct. Dissected 10 adults and 10 nymphs. Reared to confirm dissection results	Dissection mean for weekly samples ranged 3-15% in weeds 0-7% in alfalfa	1. Using all instars in calculation may underestimate parasitism because eggs are not usually found in instars 1-3 and adults normally do not have parasitoids. 2. Small sample size dissected.
		Loan 1980	Field sample, Aug, 22, 100 nymphs dissected (3-4 instars)	*L. lineolaris* 8%	1. Calculated parasitism in only those stages on only one date.

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Palaearctic					
Peristenus adelphocoridis Loan	0				
Peristenus conradi Marsh▸	2	Day et al. 1992	Field sample, same method as for *P. pallipes*	Peak % parasitism by rearing weekly samples for Generation I of *A. lineolatus* were 1988-33% 1989-11% 1990-6%	

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Peristenus digoneutis Loan▸	5	Bilewicz-Pawinska 1977a	Lab experiment Field sample, parasitism per 10 day sample	← 39% *S. virens* (0-6%) *A. lineolatus* 1973-June 10%, July 10% *L. rugulipennis* 1972-Aug-15%, 8%, 25% *L. rugulipennis* 1973-Aug-6%, 13%, 10% *L. rugulipennis* 1974-Aug-0%, 4%, 13% *L. rugulipennis* 1975-Aug-0%, 0%, 4%	1. Total number of experimental replications made are not given and results of experiments are pooled. 2. Field parasitism includes contribution from three species, *P. digoneutis* (dominant), *P. stygicus* and *P. rubricollis*.
		Bilewicz-Pawinska 1982	Field sample, May-Aug. Dissections	*L. rugulipennis* highest % parasitism in samples from 1976-1979 were in rye 13%, in wheat 49%, in barley 13%	1. Parasitism rate includes contribution from three species, *P. digoneutis* (dominant), *P. stygicus* and *P. rubricollis*.

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Peristenus digoneutis Loan►		Day et al. 1990	Field sample, May-Oct. Dissection Rearing	Dissection 1987-88 Generation 1 36% (n=47) 1987-88 Generation 2 29% (n=65) Rearing 1987-88 Generation 1 20% (n=817) 1987-88 Generation 2 9.6% (n=188)	1. Parasitism rate is from *P. digoneutis*, *P. pallipes* and *L. uniformis*. 2. Numbers of individuals reared is 2x's the number dissected. 3. Parasitism determined by number of adults emerging, cocoons counted after overwintering, checked for emergence holes and added to parasitism data.
		Day 1996	Field sample, 1982-1994. Dissection and rearing	1986-92 40–50%	1. Samples averaged over 3 fields to obtain index of abundance and parasitism. 2. Includes parasitism by *P. pallipes* and *L. uniformis* (88% *P. digoneutis*, 12% other two).
Peristenus rubricollis (Thomson) ►	2	Bilewicz-Pawinska 1977a	Lab experiments 1.♀& ♂ 2.♀only 3.other experiment	← 53% Dissection ← 18% Dissection ← 82%	1. Nymphs were cultured and presented at stages 1-4 2. Number of experimental replicates are not given.

Table 2.3 continued

Species	No. of references with parasitism data	Author	Samples & methods	Mirid host &/or % parasitism	Comments/problems
Peristenus rubricollis (Thomson) ▸		Bilewicz-Pawinska 1982	Field sample	see rates in *P. digoneutis* + June 1977, rye 2-10%, barley 6%, wheat 2.5-15.5%, oats 16.7%	
Peristenus stygicus Loan	2	Bilewicz-Pawinska 1982	Field sample Dissection	Rye 13%, barley 13%, wheat 49%, oats 23%	1. Parasitism combined with that by two other species, see *P. digoneutis*.
		VanSteenwyk & Stern 1977	Field sample, May-Sept. Surveyed in release sites, Dissection; 3-5 instars	Mean for 1973 was 0.4% % parasitism in 1974 was (0-1.6%)	

for Nearctic and Palaearctic parasitoid species appear to be variable. The levels of parasitism are not consistently reported as being higher for either the Nearctic or Palaearctic species.

It is advantageous for biological control agents to attack pest species on a number of the pest's host plants. European species were found to parasitize plant bugs in a variety of crops, not just alfalfa (Bilewicz-Pawińska 1977a, Day 1996). Bilewicz-Pawińska (1976, 1982) reported that the parasitoids, *P. digoneutis*, *P. rubricollis* and *P. stygicus* were reared from hosts found in rye, wheat, oats, barley, potato, alfalfa and clover crops, although not all species were found in all crops. It is unknown if North American parasitoids are present in crops other than alfalfa and canola.

2.5.2 Historical use of *Peristenus* species

The first use of a *Peristenus* species in a biological control program occurred in the United States in 1968, when *P. pallipes* from central US was redistributed to California in an attempt to control pest *Lygus*; it did not become established (Clancy & Pierce 1966). Native parasitoids at this time were not seen to be significant in controlling populations of pest *Lygus* and *Adelphocoris lineolatus* and attempts to release and establish exotic *Peristenus* species in North America were started in the 1970's (Day 1996). From 1978-97, *Peristenus* species from Europe were liberated in Canada (Broadbent et al. 2002, Craig & Loan 1984a, b, Soroka & Karl 2002). Releases were made in alfalfa fields containing breeding *Lygus* and *Adelphocoris lineolatus* populations. *Peristenus*

adelphocoridis and *P. rubricollis* were released for *A. lineolatus* and *P. digoneutis* and *P. stygicus* were released for *Lygus* spp. (Craig & Loan 1984a, b). In follow up surveys, only the native species, *P. pallipes* was collected.

The main goal of biological control efforts for plant bug pests in the US was to establish one or more species of European parasitoids (Carl 1979, Day 1996). The first field releases of *P. stygicus* were from Turkey. Releases of *P. stygicus*, *P. digoneutis* and *P. rubricollis* from Europe were made in the western US in 1970, but they did not establish (Carl 1979). Van Steenwyk and Stern (1977) documented the release of *P. stygicus* in 1973, into alfalfa hay fields in California, where a total of 16,000 parasitic wasps were released. Hormchan (1977) released *P. stygicus* in Mississippi. In 1978, releases of *P. rubricollis* and *P. adelphocoridis* were made against *A. lineolatus* in New Jersey, none of the species released were recovered and efforts were discontinued in 1980 (Day et al. 1992). In those same fields, *P. adelphocoridis* was released again in 1984. In 1989, an undescribed species of *Peristenus* was collected from those fields where exotic parasitoids were released, and was described as a new Palaearctic species, *P. conradi* (Day et al. 1992). It was thought that the newly discovered species was a cryptic species from Europe, and so was described as a Palaearctic species. The exotic, *P. digoneutis*, was released in New Jersey from 1979-1987, and in 1998 it was discovered to be established in alfalfa fields in the region (Day et al. 1990).

2.5.3 Evaluations of introductions

Van Steenwyk and Stern (1977) found parasitoids present for two seasons after the initial release in 1973 in California. They recorded four peaks of parasitism which decreased in numbers of individuals parasitized. Parasitism calculated over the whole season was low, 0.4%. Hormchan (1977) did not recover *P. stygicus* after the initial season of release in Mississippi. It was concluded in both studies that *P. stygicus* did not establish and was therefore considered not suitable for biological control in North America.

Day et al. (1990) and Day (1996) documented the field releases and establishment of *P. digoneutis*. Levels of parasitism reported during the release period from, 1983-1986, were 12% in the first generation *L. lineolaris* and 8% in the second generation. Average levels of parasitism for 1987-1988 in the first generation were 36% and 29% in the second generation (Day et al. 1990). From 1989 -1994, *P. digoneutis* was the dominant parasitoid and accounted for 88% of all parasitism (Day 1996). The exotic parasitoid was thought to prefer *L. lineolaris* over *A. lineolatus,* based on calculated parasitism levels which are 3.6 times higher in *L. lineolaris* than *A. lineolatus* (Day 1996). After *P. digoneutis* became established, parasitism levels were three times the baseline data, accounting for a 75% decrease in *L. lineolaris* abundance (Day 1996).

Day (1996) surveyed the exotic parasitoids' sex ratio, temporal synchronization with its host, recovery of diapausing and non diapausing individuals and dispersion. From measurements of these parameters, it is clear

that *P. digoneutis* has successfully adapted to its new host, *L. lineolaris,* and to its new home in North America, where it is suppressing pest populations. To date, two species of European origin, *P. digoneutis* and *P. conradi*, are successfully established in the US and in Canada (Broadbent et al. 1999).

2.6 Discussion

2.6.1 Parasitoid potential

To assess the potential of these species as biological control agents for North American pest *Lygus,* several issues need to be explored, such as the potential impact they may have on target and non-target species, and their potential for establishment. After reviewing the information available a decision must be made as to whether exotic *Peristenus* species should be released as biological control agents for North American pest *Lygus* sp.

The native *Lygus* pest species, *L. lineolaris*, *L. elisus*, *L. borealis*, and *L. hesperus* can be considered ecological homologues of the European tarnished plant bug, *L. rugulipennis*. They share similar characteristics; they all attack a wide range of plants, they are found in agricultural habitats and they share a similar life cycle, including variability in the number of generations produced. In laboratory experiments, *P. digoneutis*, *P. stygicus* and *P. rubricollis* parasitized the pest species (Carignan et al. 1995, Condit & Cate 1982, Hormchan 1977) (Table 2.2). Field data from Day et al. (1992), Day (1996), Hormchan (1977) and Van Steenwyk & Stern (1976), also support the idea that the exotic species can and will attack native North American *Lygus* pest species successfully.

From this information, we can determine that several exotic parasitoids

can parasitize and complete their development in our pest *Lygus* species in the laboratory and under field conditions, and at present, two species of European origin are established in North America (*P. digoneutis* and *P. conradi*). However, several other European species, such as *P. rubricollis, P. adelphocoridis*, and *P. stygicus*, have failed to establish, raising the question of whether they are actually suitable or whether their failure to establish was a reflection of inadequate release programs. Of course these successes and failures are the result of many complex interactions and the parasitoids themselves are subject to extrinsic and intrinsic factors which will affect their establishment.

Extrinsic factors may play a significant role in the success or failure of an introduced parasitoid to establish (Beirne 1984). The number of individuals released must be adequate to provide sufficient genetic variability for the founder population. Successive releases may provide needed genetic variability for establishing parasitoids. Another consideration is the density of suitable hosts; the timing of releases needs to be synchronous with appropriate stages of the host population. Related to the timing of release is the availability of food resources for foraging adult parasitoids and possibly access to alternative hosts. Searching ability and longevity are greater in parasitoids which have access to food sources (Bilewicz-Pawińska 1977a, Lewis et al. 1998). Another consideration is the temporal stability of the release habitat. Hormchan (1977) revealed that his release fields were plowed under in the same season that the releases were made, obviously this could have affected the survival of these parasitoids which burrow into the soil to form their cocoons. It is critical that

release sites are chosen where control over the inputs and stability of the site can be regulated or monitored. Release sites need to be chosen that are similar to the parasitoids' native habitats. Parasitoids may have greater searching efficiency in habitats they are adapted to, and this could affect their establishment. Most of the *Peristenus* introductions made in North America were made in alfalfa, a crop in which the parasitoids are commonly collected in Europe.

Intrinsic factors of the parasitoid can determine the suitability of that particular species as a biological control agent. One of these factors is the phenology of the race of proposed control agent, which will determine its compatibility with the host. For example, *P. stygicus* and *P. rubricollis* failed to establish after releases. It is possible that in the cases where introductions of *Peristenus* sp. failed, inappropriate host races were released and the released parasitoids were not phenologically adapted to the new hosts (Hormchan 1977). The number of generations a parasitoid can produce will also affect its suitability in the release environment as the generations need to be synchronous with the hosts generations. Both *P. digoneutis* and *P. stygicus* are multivoltine, whereas *P. rubricollis* is univoltine. Multivoltine species may have an advantage for establishing in the new environment because their generation time is less, allowing more generations to be produced and selection of well adapted genotypes for the new environment may occur in fewer seasons. To increase the efficiency of introductions, many factors affecting colonization need to be considered when designing a release program as any one factor or a

combination of factors could prevent parasitoid establishment (Beirne 1985, Hopper et al. 1993).

It needs to be determined if the Nearctic parasitoids species and *P. pallipes* found in North America are effective parasitoids of the target pests in North America. This can be evaluated by looking at their host preferences and levels of parasitism. One Nearctic species has a preference for the pest species, *P. howardi* (Day et al 1999). Most of the native parasitoids prefer other host species. For example, *Leiophron uniformis* prefers *Halticus bractatus* and *P. pallipes* prefers *Leptopterna dolobrata* and *Trigonotylus caelestialium* over the pest species (Day 1999). It is likely that species which have host preferences for non-target species may not be effective biological control agents.

Percent parasitism is one criterion used to determine a parasitoid's effectiveness at controlling the desired pest species; however, this parameter is difficult to evaluate for several reasons. Parasitism levels in any given field or habitat will vary temporally and spatially. Another problem with this type of data is that it is measured by different methods, ie. rearing vs. dissection, and it is calculated in many different ways; some workers dissect only 4^{th} and 5^{th} instars while others dissect all nymphal stages and adults (Table 2.3 and references therein). Because of the variability in how percent parasitism is determined, it is difficult to evaluate the available information to determine the effectiveness of the parasitoids reviewed here. It may be useful to create some guidelines either for evaluating parasitism or for presenting the data in scientific publications.

In Canada, the Plant Protection Act provides guidelines for regulating the

release of exotic biological control agents. However, parasitic wasps which attack arthropod hosts are not adequately dealt with in this legislation. A draft document produced by the Agriculture and Agri-Food Canada Biological Control Working Group, provides some criteria to be met prior to release of biological control agents (Anonymous 1994) including taxonomic status, relevant biological information, host specificity, possible impact on non-targets and potential for competition with resident biological control agents. Kuhlmann et al. (1998) reviewed the status of European *Peristenus* as biological control agents for North American pest *Lygus*, and they presented a list of information required for proper assessment of these species. Some of the information suggested is available for the exotic parasitoids. However, our knowledge is still not adequate to provide a good assessment of the proposed agents. Until the information is available and analyzed, it is premature to release exotic agents.

2.6.2 Research needs

Several aspects of the parasitoids' taxonomy require clarification. We lack a rigorous taxonomic review of the North American and European parasitoids and there are some problems with the available keys to species. Loan (1974b) in his key to the European species, used characters which do not consistently separate the species of *Leiophron* and *Peristenus,* and several new species have been described since the key was developed. Dr. C. van Achterberg, Nationaal Natuurhistorisch Museum, Leiden, Netherlands (pers. comm.) is currently working on new keys for the Palaearctic *Peristenus* species.

There is also a problem with ambiguous species. A new Palaearctic species of *Peristenus, P. conradi* Marsh, which was recovered from North American release sites of *P. adelphocoridis* and *P. rubricollis*, was described in Day et al. (1992). There are no specimens of *P. conradi* in any of the release voucher collections, and no collections of *P. conradi* have been made in Europe. This creates some confusion as to where this species originated, and if it is a real biological species. Day et al. (1992) suggested that *P. conradi* and *P. rubricollis* look very similar. Could *P. conradi* could be a form of *P. rubricollis*? Clarification of this will have implications for evaluating Nearctic and Palaearctic species as biological control agents.

Peristenus pallipes is another ambiguous species. In Europe, it appears to be a complex of at least 4-5 species (Dr. H. Goulet, Canadian National Collection, Ottawa, Canada, pers. comm. and Dr. C. van Achterberg pers. comm.) and in North America another 10 species. *Peristenus pallipes* is

recorded from 15 species of hosts (Bilewicz-Pawińska 1982, Condit & Cate 1982, Drea et al. 1973, Loan & Bilewicz-Pawińska 1973, Van Steenwyk & Stern 1973) and has the largest host range of any of the species reported on in Table 2.1. In fact, the host range of most of the species presented in that table is on average 2-3 species of mirids. It could be hypothesized that the species of *Peristenus* have a limited host range and *P. pallipes* is an exception. Or, if *P. pallipes* is actually a complex of several species, we could predict that the species which form the *P. pallipes* complex would have a more limited host range, similar to that of the other *Peristenus* species. It will be possible to test this hypothesis when the taxonomic status of *P. pallipes* is clarified. There are more taxonomic questions regarding the species status of both Nearctic and Palaearctic parasitoids, waiting to be answered. Dr. H. Goulet is revising the Nearctic and Holarctic species of *Peristenus* and Dr. C. van Achterberg is revising keys to the Palaearctic species of *Peristenus*. Several authors agree that the taxonomy of the parasitoid species needs clarification (Day et al. 1992, Drea et al. 1973, Kuhlmann et al. 1998, Loan 1980).

The mirid host range records for all species of *Leiophron* and *Peristenus* are sparse, in part because field collected adult wasps are not associated with their hosts and host reared data are lacking. Some reasons for the lack of reared data include: it is a time consuming venture, it requires knowledge of the proper conditions required for the parasitoids to develop and it requires proper identification of the mirid hosts and parasitoids. Perhaps most importantly it also requires the development of adequate rearing techniques for the host mirids. In

many cases, these techniques are not yet available. A key to the nymphs of North American families of Heteroptera was provided by Slater & Baranowski (1978), but keys to species are lacking. A key to the nymphal instars of pest *Lygus* of the prairie provinces in Canada was developed by Schwartz & Foottit (1992). There is a lack of keys to identify nymphs of other mirid species. The problem of nymphal identifications could be circumvented by associating the nymphs with an adult of the species. This requires that enough nymphs are collected in a sample to ensure that some may be reared to adulthood. The larvae of most species of *Peristenus* can not be identified to the species level, and must be reared to adults for accurate identification. This requires parasitoids to be reared successfully from their hosts, through pupation and finally to adult emergence.

Any combination of problems associated with the above procedures could account for our current lack of detailed studies on the host range of *Peristenus* parasitoids. It is clear that more detailed studies on the host ranges of these taxa are necessary to evaluate properly the range of mirids they attack (Kuhlmann et al. 1998). Ideally this would include laboratory studies coupled with ecological studies. This knowledge would facilitate predicting possible impacts on non-targets and evaluating the exotic agents' potential as biological control agents.

Information is needed regarding host range, distribution and general biology of most of the parasitoid species. Basic biological information regarding our native and non-native parasitoids is not available to properly assess their

ecological role and relationships to their hosts.

2.6.3 Conclusions

There is a lack of appropriate biological and ecological information regarding both our native and non-native parasitoid species of *Peristenus* and *Leiophron* which is necessary for determining their potential as biological control agents. Specific information is needed including taxonomy, relevant biological information such as host range, and host synchronization with native species. Any decisions to release these exotic agents should include a proper assessment of the potential risks and benefits of the biological control program. The biological control of pest *Lygus* species by native and exotic parasitoids provides multiple opportunities to examine parasitoid/host relationships and will contribute valuable information on parasitoid biology and ecology.

2.6.4 Future studies

The taxonomy of *Leiophron* and *Peristenus* species needs to be clarified and new keys constructed. This requires a large number of specimens to be available for taxonomic study. Field research is needed on both North American and European parasitoids, in their native habitats associated with their native hosts, especially regarding host range and effects on pest abundance. Laboratory and experimental studies on the biology of these species are needed as well as studies on the impact of the parasitoids on target and non-target hosts and on parasitoid competition. More studies on native parasitoids found in North America are needed to determine their role in controlling *Lygus* pest species' populations in different habitats. There are studies of this nature being made in

Canada (J.Uddin, University of Manitoba, Winnipeg, Manitoba, pers. comm, Dr. H. Goulet pers. comm.) Recent studies by Braun et al. (2001) and Day et al. (1999) provide evidence that Nearctic species of parasitoids have the capability to affect pest *Lygus* sp. populations. At this point, it is difficult to say if our native parasitoids could be used to control pest *Lygus* populations. This is mostly due to lack of evidence based on comprehensive biological studies, but more research on Nearctic parasitoids will hopefully provide the necessary information to assess this.

Chapter 3 Analysis of the rearing and dissection methods commonly used to detect parasitism in *Lygus* spp. Hahn and *Adelphocoris lineolatus* (Goeze) (Hemiptera: Miridae) by larval endoparasitoids, *Peristenus* Foerster (Hymenoptera: Braconidae) species.

3.1 Abstract

In 1998, five fields of alfalfa and one field of red clover were sampled to collect plant bugs, *Lygus pratensis* (L.), *Lygus rugulipennis* Poppius and *Adelphocoris lineolatus* (Goeze), to rear and dissect for their larval endoparasitoids. All fields were located in the Rhine Valley of Germany. Numbers of individual plant bugs and parasitoids collected by the rearing method were compared to the numbers collected by the dissection method. More nymphs and parasitoids were collected per sweep in samples to be reared perhaps because fewer sweeps were made in a sample collection which resulted in fewer individuals being lost during the collection process. Percent parasitism was calculated by dividing the total number of parasitoid cocoons or larvae detected by the total number of nymphs reared or dissected. Statistically significant differences between percent parasitism calculated by the rearing and dissection methods were detected. Rearing produced higher percent parasitism values probably because all of the stages of the parasitoid were included, some of which were missed by dissection, such as eggs. The dissection method could be used to determine the amount of parasitism for individual host species and individual host instars. Parasitism was greatest in 3rd, 4th, and 5th instars as compared to that in 1st and 2nd instars. The many advantages and

disadvantages of each method are summarized.

3.2 Introduction

There are several methods available to detect parasitism in insects, including rearing of hosts, dissection of hosts and detection by molecular analysis. In the literature concerned with biological control of pest plant bugs, *Lygus* and *Adelphocoris* spp., rearing and dissection are the most commonly used methods to detect parasitism by larval endoparasitoids (Bilewicz-Pawińska 1977a, Day et al. 1999, Van Steenwyk & Stern 1977). Depending on circumstances, one of these methods may be considered superior to the other in some regard (Day 1994). For instance, to determine the identity of the parasitoid species, rearing may be necessary to obtain adult specimens for identification. Alternatively, dissections may be required to assess development of the parasitoid. For many different reasons, one or both of these methods may be used to determine percent parasitism in field-collected samples, or in laboratory experiments.

There are a variety of ways these methods can be performed and the data analyzed and there is no generally accepted protocol used consistently by researchers, which makes it difficult to compare percent parasitism as determined in different studies. For example, percent parasitism calculated from rearing or dissecting of only certain stages (Clancy & Pierce 1966) versus rearing or dissecting of all stages (Lim & Stewart 1976a) have been reported. It is especially difficult to make comparisons confidently between studies when the exact details of the methods used are not reported. For example, Day (1996),

Day et al. (1999) Day et al. (1992) and Day & Saunders (1990), did not give specific information as to the host stages dissected, and it must be assumed that all stages were dissected. Data analysis methods are also not consistent among studies. Some researchers prefer to report percent parasitism based on a week's sample, while others will present percent parasitism values after pooling data over an entire month and possibly over an entire season.

In a few studies, the accuracy of rearing and dissection methods in determining percent parasitism in plant bugs by larval endoparasitoids has been compared, and they consistently conclude that dissection is a more accurate method (Day 1994, Day et al.1999, Day et al. 1990, Day et al. 1992, Day & Saunders 1990). However, it is possible that these studies are biased in their design which may have an impact on the conclusions. For example, Day (1994) consistently used a greater number of nymphs for rearing than for dissection, 91% of the sample for the former and 19% of the sample for the latter. The work described here (Chapter 4) shows percent parasitism determined from small samples of nymphs can greatly inflate the value of percent parasitism and caution should be exercised when using small samples to estimate this parameter. Also, the rearing methods in many studies of *Lygus* spp. and *A. lineolatus* (Goeze) were not described in enough detail to determine the density at which the nymphs were reared or what the survival was. The density at which nymphs are reared can significantly affect the mortality of both parasitized and nonparasitized nymphs, thereby possibly affecting percent parasitism determined by rearing.

The objective of this chapter is to investigate thoroughly the advantages and disadvantages of the dissection and rearing methods, using as a model the plant bugs, *Lygus* and *Adelphocoris* species, and their larval endoparasitoids, *Peristenus* species. The specific objectives of this chapter are to determine the types of data which are collected by each method, to compare the differences between the rearing and dissection methods, and to assess the efficiency of these methods. Further, new evidence for researchers will be provided, to assist them in selecting the most appropriate method for their studies. It is also meant to provide support for the method chosen for the majority of the work described in chapters 4, 5 and 6.

3.3 Materials & Methods

3.3.1 Study sites

Field collections of *L. pratensis* (L.), *L. rugulipennis* Poppius and *A. lineolatus* were made for this study during the summer months of 1998. All study sites were located in the Rhine Valley of Germany, approximately 30 kilometers North of Basel, Switzerland. Six fields were sampled. They consisted of five alfalfa fields (Ma5, St1, Hu1, Hu2, Hu3) and one field of red clover (Ma1). Fields were coded using the first two letters of the name of the nearest village. Details of each field's location and size are listed in Appendix 1. Sampling began on 24 May and continued until 23 September, 1998.

3.3.2 Samples to be reared

Sweep nets, 37.5 cm in diameter, were used to collect plant bugs in the fields. Each week that the vegetation was at least 30 cm high, a total of eight

samples were taken from each field to obtain plant bug nymphs for rearing. A single sample consisted of 20 sweeps, where one sweep equaled a 180° arc with the sweep net. The eight samples were taken in two transects, parallel to each other, in which the sampler started at the field edge and moved in toward the center of the field. Transect 1 consisted of sample #'s 1, 2, 3, 4 and transect 2, sample #'s 5, 6, 7, 8. For this study, the data from all eight samples are combined into one weekly sample.

From each sample all adult *L. pratensis*, *L. rugulipennis* and *A. lineolatus* were counted and their numbers recorded. It is difficult to distinguish between the two *Lygus* species without a microscope, so the number of individuals was recorded only as *Lygus* spp. adults per sample. The nymphs of all species were removed from the net, placed in empty rearing containers, given a sprig of alfalfa and brought back to the laboratory for rearing. The nymphs were held in a 2°C walk-in chamber overnight.

The following day the numbers of nymphs of each instar of *Lygus* spp. and *A. lineolatus* were counted in the laboratory. This information was recorded for each sample. Adults of *Lygus* spp. and *A. lineolatus* were not reared because they are usually not found to be parasitized by European *Peristenus* species (Bilewicz-Pawińska & Pankanin 1974). The nymphs of all three species were placed together in rearing containers and fed store-bought green beans until they reached adulthood, a parasitoid emerged, or the nymph died. Nymphs of the plant bug species collected had to be reared together because there were not enough rearing containers to separate the species. The number of parasitoid

larvae to emerge and spin cocoons was recorded per sample, but not per species in each sample.

The rearing containers were made from two plastic, tapered, urine sample containers, 6 cm in diameter and 8.5 cm high, and nested one within the other. The bottom of the inner container was cut off and replaced by a piece of mesh (8x8 squares per cm^2). The outer container was filled with fine, moistened vermiculite to a depth of about 1 cm. This served as pupation medium for the emerging parasitoid larvae, and it provided moisture for both nymphs and parasitoid cocoons. Small pieces of crumpled paper towel were added to the inner container to increase the structural diversity for the plant bug nymphs, to reduce predation by providing hiding places for molting nymphs. Air circulation in the container was improved by cutting a hole in the lid, 2 cm in diameter, which was screened over with a very finely woven material.

On average, 10-15 nymphs were placed together in one rearing container. During peaks of the plant bug nymphal populations, a maximum of 20 nymphs would be reared together in one container. All rearing containers with plant bug nymphs were monitored on average every 3-5 days, when beans were removed and replaced with fresh beans and any emerged adult plant bugs were also removed. When all the plant bug nymphs had died or completed development, the outer container and the vermiculite were inspected for parasitoid cocoons. The number of cocoons was recorded and the lid was replaced. The total number of parasitoids to spin cocoons and the total number of nymphs reared were used to calculate percent parasitism per sample.

To determine the amount of rearing mortality a small study was undertaken in 2002. Ten colony reared *Lygus* spp. nymphs were placed in rearing containers and subject to the same conditions as nymphs reared for parasitoids. This was replicated 15 times. The stage of nymphs at the beginning of the experiment were recorded and the number of nymphs reaching adulthood was also recorded.

3.3.3 Samples to be dissected

Samples of plant bugs to be dissected were taken in the same fields as the samples for rearing, and they were usually taken on the same day as the samples taken for rearing. Immediately after taking the samples for rearing, two 50-sweep samples were made from the center of the field out toward the field edge. The sweeps were made in vegetation that was not swept for rearing samples. The adults and nymphs of *Lygus* spp. and *A. lineolatus* were removed from these samples directly in the field, stored in containers with a sprig of alfalfa and brought back to the laboratory alive. They were killed by freezing and then stored in 70% alcohol until they were dissected.

Dissections were made using a Wild 40X binocular stereo microscope. Every specimen collected, including adults, was dissected in water using fine forceps. The stage and species of each plant bug was recorded as was the stage of any parasitoid larva found in the host. The stages of development of the parasitoids were noted as; (a) egg, (b) first instar larva, (c) second instar larva, or (d) third instar larva (Carignan et al. 1995).

3.3.4 Comparison of rearing and dissection samples

To examine how effective each method is at estimating percent parasitism, it first has to be established if the samples taken were statistically different because of differences in the sampling methods. The reared samples were taken in 20-sweep samples, eight per field each week, in total 160 sweeps per week. The samples for dissection were made in two, 50-sweep samples, in total 100 sweeps per week. The samples for rearing were converted from numbers per 160 sweeps to numbers per 100 sweeps and then samples from both methods were analyzed. ANOVA was used to assess the total numbers of individuals collected per category each week in samples for each method. As the samples were taken from the same fields on the same day, the sampling is a paired design and a paired t-test was used to test for significant differences in the weekly means and percent parasitism.

3.3.5 Comparison of rearing and dissection methods

In total, 50 samples were taken for rearing and 43 samples were taken for dissection. Within this data set, there were 38 cases of samples being taken from the same field, on the same day, for rearing and for dissection. These are the samples used to compare the two methods. The fields and dates on which the paired samples were taken are shown in Table 3.1.

3.3.6 Comparison of parasitism in *Lygus* spp. nymphs and *A. lineolatus* nymphs

In this study, it is possible to determine host species percent parasitism using the dissection data. In these samples, the species of each plant bug dissected was recorded, and its stage of development and the stage of any

Table 3.1 The dates paired samples of plant bug nymphs were taken in 1998 in six forage fields in Germany to compare % parasitism detected by rearing and dissection methods.

Sampling date	Fields					
	Hu1	Hu2	Hu3	Ma1	Ma5	St1
30.VI.98		X		X	X	X
9.VII.98		X		X	X	X
13.VII.98						
22.VII.98	X			X		X
28.VII.98			X	X	X	
5.VIII.98	X		X	X	X	
11.VIII.98	X		X	X	X	X
19.VIII.98	X		X	X	X	X
26.VIII.98	X		X	X	X	X
1.IX.98				X	X	X
23.IX.98	X			X		

parasitoid found was noted. Using this information, it is possible to determine the level of parasitism in the *Lygus* spp. nymphs versus *A. lineolatus* nymphs. It is also possible to determine parasitism in the different instars of each species, and in combinations of instars. In the analysis two combinations, 3rd, 4th and 5th instars and 4th and 5th instars were used because some studies (Clancy & Pierce 1966, Loan 1980, Van Steenwyk & Stern 1977) used these different combinations of stages to determine percent parasitism. Total percent parasitism is the proportion of parasitoids occurring in a given instar stage determined by dividing the total number of parasitoids found in dissections by the total number of individuals collected per instar in the 43 samples pooled. Mean percent parasitism is the percent parasitism found in a particular instar based on the mean of 43 samples.

3.4 Results

The number of samples collected and the total numbers of all individuals collected in 1998 are listed by category in Tables 3.2 and 3.3, for rearing and dissection respectively. No parasitoid eggs were found in any of the plant bugs dissected in 1998. Of 764 adults dissected in 26 samples, only two contained a parasitoid larva. The number of parasitoid larvae found and the total number of nymphs dissected were used to calculate percent parasitism. From the rearing mortality experiment it was found that 52% of 1st and 2nd instar Lygus spp. nymphs reached adulthood, ranging from 0-90% survival, and 88% of 3rd and 4th instars reached adulthood, ranging from 70-100% survival.

3.4.1 Comparison of rearing and dissection samples

Table 3.2 Samples collected in forage fields in 1998 in Germany for *Lygus* spp. and *Adelphocoris lineolatus* for rearing to estimate parasitism by *Peristenus* spp.

Field code	Number of samples	Total # of *Lygus* spp. adults	Total # of *A. lineolatus* adults	Total # of *Lygus* spp. nymphs	Total # of *A. lineolatus* nymphs	Total # of nymphs combined	Total # of parasitoid cocoons	Total % parasitism
St1	9	241	81	294	108	402	60	14.8
Hu1	9	263	135	266	363	629	60	9.5
Hu2	3	73	14	66	12	78	13	16.6
Hu3	8	251	228	735	325	1060	163	15.3
Ma1	12	330	93	803	46	849	161	18.9
Ma5	9	231	159	788	200	988	212	21.4

Table 3.3 Samples collected in forage fields in 1998 in Germany for *Lygus* spp. and *Adelphocoris lineolatus* for dissection to estimate parasitism by *Peristenus* spp.

Field code	Number of samples	Total # of *Lygus* spp. adults	Total # of *A. lineolatus* adults	Total # of *Lygus* spp. nymphs	Total # of *A. lineolatus* nymphs	Total # of nymphs combined	Total # of parasitoid larvae	Total % parasitism
St1	7	89	50	183	70	253	27	10.6
Hu1	6	118	83	178	288	466	5	1.0
Hu2	2	30	7	37	5	42	2	4.7
Hu3	7	149	144	469	443	912	178	19.5
Ma1	11	266	69	444	32	476	48	10.0
Ma5	10	199	168	627	170	797	186	23.3

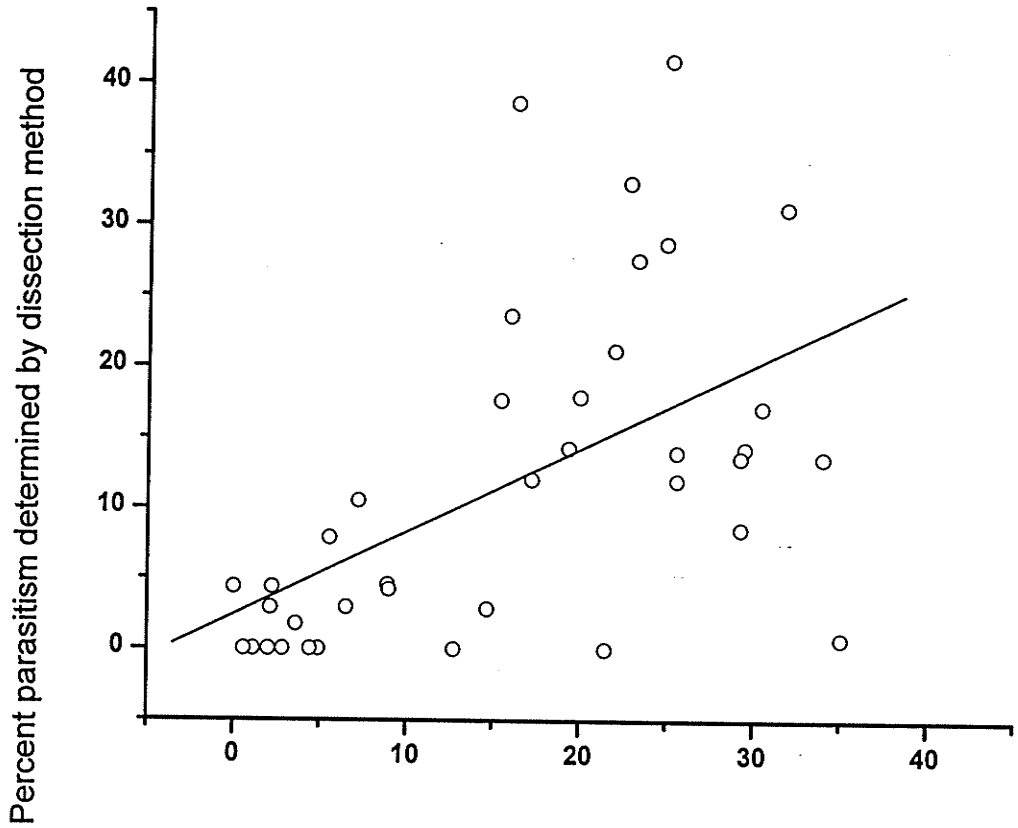
Table 3.4 Means, standard errors and paired t-test results for the number of individual plant bugs and parasitoids collected in 38 paired, 100-sweep samples taken for rearing and dissection in six forage fields in Germany in 1998.

Parameter	Rearing		Dissection		Paired t-test
	Mean	Standard error	Mean	Standard error	Significance
Lygus spp. adults	29.9	±2.52	20.5	±2.51	P≤ 0.01
A. lineolatus adults	15.6	±2.04	13.7	±2.23	N.S.
Lygus spp. nymphs	73.9	±10.28	46.2	±7.98	P≤ 0.01
A. lineolatus nymphs	25.4	±5.64	22.7	±5.36	N.S.
Nymphs combined	99.3	±12.17	68.9	±9.95	P≤ 0.01
Parasitoids collected	16.6	±3.00	10.4	±2.89	P≤ 0.05
% parasitism	15.8	±1.78	12.1	±1.88	P≤ 0.05

The samples of plant bugs which were collected for rearing and for dissections were compared for statistical differences in the number of nymphs collected. The mean number of plant bugs collected per week was higher in all categories for the samples reared compared with the samples dissected (Table 3.4). This difference was significant for the mean number of *Lygus* spp. adults and nymphs, combined nymphs, number of parasitoids collected and percent parasitism. The standard errors are largest for the number of *Lygus* spp. nymphs collected and the total nymphs combined. The number of *Lygus* spp. nymphs collected was about twice the number of *A. lineolatus* nymphs collected.

3.4.2 Comparison of percent parasitism determined by rearing and dissection methods

Regression analysis was performed on the % parasitism data calculated from the results of each method and is shown in Figure 3.1. There were significant differences between the % parasitism determined by rearing and dissection: parasitism was higher in reared samples. Note the many zero values (7 out of 38 samples) visible on the x axis which were obtained by the dissection method, for which the paired reared sample had a non-zero % parasitism value. The % parasitism values range from 0 - 40% for both methods. In all cases the mean number of insects were also greater in the samples for rearing. The slope of the regression line is 0.5893, and is significantly different from zero ($P = 0.0003$). The slope is significantly different from one, which means the correlation between percent parasitism calculated by rearing and by dissection ($t = 0.0399$) is not perfect, and the two methods give different estimates of %



Slope = .5893
R^2 = .3077
Prob. F = .0003
Intercept P = .3215
t-test = .0399
DF= 37

Figure 3.1 Regression analysis of percent parasitism of *Lygus* spp. and *Adelphocoris lineolatus* nymphs by *Peristenus* spp. determined by rearing and dissection methods for samples collected in six forage fields in Germany in 1998.

parasitism with the dissection method underestimating the parameter compared with the rearing method. The two measures of percent parasitism are not well correlated ($R^2 = 0.3077$). The percent parasitism, as determined by the dissection method, accounts for approximately 58% of the variation seen in the percent parasitism determined by the rearing method.

3.4.3 Comparison of parasitism in *Lygus* spp. and *A. lineolatus* nymphs

In order to assess if parasitism was more prevalent in one of the host species, correlation matrices were produced based on only the rearing data, only the dissection data and both data sets combined. These are presented in Tables 3.5a, 3.5b, and 3.5c respectively. In this analysis, there will be some auto correlation because the number of parasitoids is directly related to the presence of nymphs. However, correlation is used here to determine the relationship between the parasitoids and species of hosts. In all three correlation matrices, the strongest correlation was between the parasitoids and the *Lygus* spp. nymphs, next were the correlation between the parasitoids and the *A. lineolatus* nymphs and then the *Lygus* spp. nymphs and *A. lineolatus* nymphs.

In the dissection samples it is possible to assign parasitism to a particular plant bug species or stage and make direct comparisons. Percent parasitism values are given for *Lygus* nymphs in Table 3.6, for *A. lineolatus* nymphs in Table 3.7, and for both species combined in Table 3.8. In the *Lygus* spp. the total percent parasitism was greatest in the 5th instars (24.4%), and in the 4th and 5th instar combination (21.5%). The total percent parasitism for all instars of *Lygus* spp. combined was 18.5%, the mean was 14.2%. Total percent

Table 3.5 Correlations between the number of *Lygus* spp. nymphs, *Adelphocoris lineolatus* nymphs and parasitoids collected in 38 samples taken for rearing and dissection in six forage fields in Germany in 1998.

a) samples for rearing

Variable	*Lygus* spp. nymphs	*A. lineolatus* nymphs	Parasitoids
Lygus spp. nymphs	1.0000	0.0907	0.7866
A. lineolatus nymphs	0.0907	1.0000	0.3231
Parasitoids	0.7866	0.3231	1.0000

b) samples for dissection

Variable	*Lygus* spp. nymphs	*A. lineolatus* nymphs	Parasitoids
Lygus spp. nymphs	1.0000	0.0772	0.7973
A. lineolatus nymphs	0.0772	1.0000	0.3161
Parasitoids	0.7973	0.3161	1.0000

c) samples for rearing and dissection combined

Variable	*Lygus* spp. nymphs	*A. lineolatus* nymphs	Parasitoids
Lygus spp. nymphs	1.0000	0.0915	0.7932
A. lineolatus nymphs	0.0915	1.0000	0.3216
Parasitoids	0.7932	0.3216	1.0000

Table 3.6 The total numbers and the mean numbers of *Lygus* spp. nymphs of various stages collected in 43, 100-sweep samples, which were dissected, and the total and mean percent parasitism and standard errors calculated from samples collected in 1998 in six forage fields in Germany.

Category	Number of nymphs			% Parasitism		
	Total in 43 samples	Mean #s per sample	Standard error	Total of 43 samples	Mean % parasitism of 43 samples	Standard error
Lygus spp. instar 1	26	0.6	± 0.23	3.9	0.6	± 0.58
Lygus spp. instar 2	155	3.6	± 0.96	7.7	3.4	± 2.39
Lygus spp. instar 3	475	11.0	± 2.73	14.7	16.0	± 3.74
Lygus spp. instar 4	568	13.2	± 2.59	17.8	16.8	± 4.05
Lygus spp. instar 5	714	16.6	± 3.68	24.4	13.7	± 3.27
Lygus spp. instars 3, 4, 5	1757	40.9	± 7.47	19.6	15.7	± 2.56
Lygus spp. instars 4, 5	1282	29.8	± 6.10	21.5	13.9	± 3.00
Lygus spp. instars 1, 2, 3, 4, 5	1938	45.1	± 7.80	18.5	14.2	± 2.30

Table 3.7 The total numbers and the mean numbers of *Adelphocoris lineolatus* nymphs of various stages collected in 43, 100-sweep samples, which were dissected, and the total and mean percent parasitism and standard errors calculated from samples collected in 1998 in six forage fields in Germany.

Category	Number of nymphs			% Parasitism		
	Total in 43 samples	Mean #s per sample	Standard error	Total of 43 samples	Mean % parasitism of 43 samples	Standard error
A. lineolatus instar 1	3	0.1	± 0.05	0	0	0
A. lineolatus instar 2	57	1.3	± 0.49	3.5	0.8	± 0.54
A. lineolatus instar 3	214	5.0	± 2.12	11.7	7.3	± 2.92
A. lineolatus instar 4	336	7.8	± 2.75	9.2	5.2	± 1.74
A. lineolatus instar 5	398	9.3	± 2.59	7.5	2.7	± 1.00
A. lineolatus instars 3, 4, 5	948	22.0	± 5.42	9.1	7.7	± 1.98
A. lineolatus instars 4, 5	734	17.1	± 4.50	8.3	5.1	± 1.47
A. lineolatus instars 1, 2, 3, 4, 5	1008	23.4	± 5.50	8.7	6.4	± 1.49

Table 3.8 The total numbers and mean numbers of *Lygus* spp. and *Adelphocoris lineolatus* nymphs of various stages collected in 43, 100-sweep samples, which were dissected, and the total and mean percent parasitism and standard errors calculated from samples collected in 1998 in six forage fields in Germany.

Category	Number of nymphs			% Parasitism		
	Total in 43 samples	Mean #s per sample	Standard error	Total of 43 samples	Mean % parasitism of 43 samples	Standard error
Lygus spp. and *A. lineolatus* instar 1	29	0.7	± 0.24	3.5	0.7	± 0.47
Lygus spp. and *A. lineolatus* instar 2	212	4.9	± 1.30	6.6	3.2	± 1.86
Lygus spp. and *A. lineolatus* instar 3	689	16.0	± 4.64	13.8	12.3	± 2.44
Lygus spp. and *A. lineolatus* instar 4	904	21.0	± 3.93	14.6	11.5	± 2.21
Lygus spp. and *A. lineolatus* instar 5	1112	25.9	± 5.09	18.4	10.7	± 2.80
Lygus spp. and *A. lineolatus* instars 3, 4, 5	2705	62.9	± 10.31	15.9	12.8	± 1.88
Lygus spp. and *A. lineolatus* instars 4, 5	2016	46.9	± 8.18	16.6	10.7	± 2.08
Lygus spp. and *A. lineolatus* instars 1, 2, 3, 4, 5	2946	68.5	± 10.66	15.1	11.5	± 1.72

parasitism in *A. lineolatus* nymphs was highest in the 3rd instars (11.7%), and in 4th and 5th instars combined was 9.2%. The total percent parasitism for all instars of *A. lineolatus* combined was 8.7%, the mean was 6.4%. The data for each species was combined to give percent parasitism for both *Lygus* spp. and *A. lineolatus* nymphs. Total percent parasitism was highest in the 5th instars (18.4%), and in the 4th and 5th instars combined was 16.6%. The total percent parasitism for all instars combined was 15.1% and the mean was 11.5% for both *Lygus* spp. and *A. lineolatus* combined.

3.5 Discussion

3.5.1 Comparison of rearing and dissection samples

Most likely the larger numbers of plant bugs collected by the rearing method are due to the sampling method. The weekly samples to be reared were collected in eight samples of 20-sweeps each, as compared to two samples of 50-sweeps each for dissection. Fewer individuals may have been lost during the sweeping process when the number of sweeps per sample was less.

There were significant differences in the mean numbers of *Lygus* spp. adults and nymphs collected by the two methods (Paired t-test). Significant differences were also found between the number of combined nymphs collected by each method and the number of parasitoids and % parasitism detected by the two methods. It was found that the *Lygus* spp. nymphs and parasitoids were correlated (Tables 3.5a-c). As the number of *Lygus* spp. nymphs is statistically different, this will have an effect on the statistical difference for both the

combined nymphs and the number of parasitoids collected in samples for rearing.

In general, the numbers of *Lygus* spp. were greater than the number of *A. lineolatus*, for both adults and nymphs in the reared samples. The greater number of *Lygus* spp. nymphs collected will have a greater influence on the total number of combined nymphs collected and the number of parasitoids collected is clearly correlated with the *Lygus* spp. nymphs. From dissection samples, parasitism was greater in *Lygus* spp. compared to *A. lineolatus* nymphs. The results of the correlation analysis indicate that there should be more parasitoids collected in larger samples of *Lygus* spp. nymphs. As there were more *Lygus* spp. nymphs collected in the samples for rearing, more parasitoids are expected to be collected from the samples which were reared.

Theoretically, percent parasitism should not be affected by the method used for sample collection because it is calculated from the number of parasitoids collected divided by the total number of nymphs collected. This transformation creates a proportion which removes the effect of large numbers of individuals in a sample. Therefore, any statistical differences in mean percent parasitism should be an indication of differences due to the method of parasitoid detection, and not due to the sampling methods used here.

There were no statistical differences in the mean number of adults and nymphs of *A. lineolatus* collected in samples for rearing or for dissection. This species is larger than the *Lygus* spp., both as adults and as nymphs. One

possible explanation for the lack of difference in the number collected in 20-sweeps versus the number collected in 50-sweeps, is that *A. lineolatus* may be better than *Lygus* spp. at detecting the sweeping disturbance in the field, and detecting this earlier they could have dispersed faster, or farther, resulting in fewer individuals being collected. More *Lygus* spp. individuals may have escaped during the sweep netting when 50-sweep samples were taken, versus 20-sweeps. Alternatively, it is also possible that *A. lineolatus* is less efficient at escaping from the sweep net during sweeping or during collection, resulting in similar numbers collected in samples for each method.

3.5.2 Abundance of *Lygus* spp. nymphs and *A. lineolatus* nymphs in samples

Both genera were more numerous in reared samples, although *A. lineolatus* not significantly so. More *Lygus* spp. nymphs were collected than *A. lineolatus* nymphs. This could be a reflection of this species being more abundant than *A. lineolatus*. It is important to note how abundant each host species is in the environment, and to note the levels of parasitism in them to try and determine if % parasitism is a reflection of how often the host is encountered by a parasitoid or how preferred it is as a host. From a practical perspective, the relative level of parasitism in each species is important to know because both species were reared together in most of the research presented in the remaining chapters of this thesis.

3.5.3 Comparison of percent parasitism determined by rearing and

dissection methods

There was a significant difference between the % parasitism calculated by dissection and rearing methods. In this study, percent parasitism was usually higher when determined by the rearing method. There was not a very strong correlation of percent parasitism determined by dissection with that determined by rearing (Figure 3.1).

It is known that the eggs of parasitic Hymenoptera are often transparent and tiny. In this study, eggs were not found in any of the plant bugs dissected. Clancy & Pierce (1966) dissected 12,000 field-collected *Lygus hesperus* Knight and reported finding only parasitoid larvae in the hosts, not eggs. Several researchers have reported detecting the eggs of *Peristenus* species in dissections, including Bilewicz-Pawińska (1977b), Bilewicz-Pawińska & Pankanin (1974), Day (1994) and Lim & Stewart (1976b). Day (1994) noted that the detection of eggs, using the dissection method, is 26% lower than for detecting larvae. It is also possible that doing dissections on freshly killed nymphs may increase the chances of finding eggs in the host, as opposed to dissecting hosts that were stored in alcohol. It is found here that dissection underestimated the incidence of parasitism compared with rearing because the egg stage was not detected during dissection.

The results of this study support the conclusion that the rearing method is better at detecting parasitism and estimating % parasitism by *Peristenus* spp., than the dissection method. The rearing technique is more inclusive of all stages

of the parasitoids and therefore gives a more complete assessment of parasitism, aside from parasitoid mortality in the host. In this study, all stages of nymphs collected were reared which gives a better indication of field parasitism levels than does dissection. In order to determine which method, rearing or dissection, is more accurate at estimating percent parasitism, an experiment with controls needs to be performed.

3.5.4 Parasitism in host species and nymphal instars

Using the dissection method, it was found that parasitism in *Lygus* spp. nymphs was much higher than in *A. lineolatus* nymphs. Parasitoids had a greater association with *Lygus* spp. nymphs than with *A. lineolatus* nymphs. Therefore, even though *Lygus* spp. nymphs and *A. lineolatus* nymphs were reared together, the % parasitism values obtained by rearing are mostly reflecting parasitism in *Lygus* spp. Parasitism based on dissecting different instars has been reported in several studies, so there is also interest in determining how much of the total percent parasitism is detectable when dissecting groups of certain instars. All instars of *Lygus* spp. and *A. lineolatus* can be parasitized by *Peristenus stygicus* Loan, but earlier instars are more susceptible (Drea et al. 1973). The very low mean percent parasitism found in *Lygus* spp. and *A. lineolatus* 1st and 2nd instars (<4%) is an indication that parasitoids were not readily detectable in these stages. The mean percent parasitism of *Lygus* spp. 3rd, 4th and 5th instars was 14.2%. Mature parasitoid larvae probably emerge from all three of these instars. Mean percent parasitism

in *A. lineolatus* 3rd, 4th and 5th instars, was 6.4%. Parasitism in the 5th instars was noticeably lower than in 3rd and 4th instars, perhaps because the parasitoids are exiting before the host reaches the 5th instar. In both genera percent parasitism was highest in the 3rd, 4th and 5th instars compared with the 1st and 2nd instars. It is felt that mean percent parasitism values should be higher in the 1st-3rd instars for both genera, as these are the stages in which parasitoids are likely to be in the egg stage. Not many 1st and 2nd instars were collected, this combined with the eggs being missed by the dissection method probably explains the very low parasitism seen in these stages. While dissecting all stages is the only way to detect total percent parasitism, it may be more time efficient to dissect only a sub-sample of the instars and still have a good indication of % parasitism.

3.5.5 Advantages and disadvantages of each method

Several advantages and disadvantages for each method are listed below. The advantages of rearing include: 1. all stages of parasitoids, including eggs and young larvae, are included 2. adult specimens of parasitoids and hyperparasitoids are collected which allows for species level identification (Day 1994), 3. host association information is guaranteed, 4. a more realistic impact of parasitism on the host population is determined because mortality factors are included, 5. biological and behavioral effects of parasitism, such as biological development of infected versus non-infected hosts, host feeding behavior and host behavior are monitored, 6. information on the stage attacked is available, 7. stinging and attack mortality are accounted for (Day 1994), 8. host feeding mortality is determined (Day 1994), 9. mortality of hosts at different

developmental stages is detected (Day 1994), 10. diapause of host/parasite is detected (Day 1994), 11. disease is detected (Day 1994).

There are also disadvantages of rearing: 1. rearing is time and labor intensive, 2. rearing requires special equipment and special food, 3. partial development of the parasitoid in the host is not detected.

The advantages of dissection include: 1. prompt results are obtained, 2. information on parasitoid development in the host, such as location, number of parasitoids, hyperparasitism, parasitoid larval development, general biology is obtained, 3. combinations of superparasitism, multipleparasitism and parasite-host disease interactions are detected (Day 1994), 4. host stages attacked are determined, 5. rearing mortality due to artificial conditions, such as crowding, food quality and stress in caged studies is avoided (Day 1994), 6. sterilization of host is detected (Day 1994).

The disadvantages of dissection include: 1. not all stages of parasitoids, i.e., eggs, are readily detectable and therefore parasitism is underestimated, 2. the effect of parasitism on the host population is underestimated because natural mortality factors are avoided, 3. immature hosts and immature stages of parasitoids and hyperparasitoids are difficult to identify to species.

3.6 Conclusions

1.There is a statistical difference between % parasitism determined by rearing and dissection methods.

2. Mean % parasitism was significantly higher in the reared samples.

3. Not all stages of the parasitoid may be readily detected in dissections,

particularly eggs, thereby underestimating % parasitism.

4. Numbers of individuals may be higher in samples taken with fewer sweeps, possibly explaining why there were more individuals collected in samples to be reared.

5. More *Lygus* spp. nymphs were collected than *A. lineolatus* nymphs.

6. Percent parasitism was significantly higher in *Lygus* spp. than in *A. lineolatus* nymphs.

7. It may be less time consuming to dissect only specific instars of nymphs to determine % parasitism.

8. Each method has advantages and disadvantages, which should be weighed carefully before selecting a method to use.

3.7 Acknowledgments

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Chapter 4 Seasonality and abundance of *Lygus* spp. (Hahn) and *Adelphocoris lineolatus* (Goeze), and their natural enemies, *Peristenus* species, in annual and perennial crops in Southern Germany.

4.1 Abstract

From 1998 - 2000, several field crops were monitored for the presence of *Lygus pratensis* (L.), *Lygus rugulipennis* Poppius and *Adelphocoris lineolatus* (Goeze). These included asparagus, barley, rape seed, mustard, mixed clover, red clover and alfalfa crops. The seasonal abundance of *Lygus* spp. and *A. lineolatus* and their parasitoids was quantified by taking samples weekly with a sweep net to collect the hosts to rear for parasitoids. In the region of the Rhine Valley in Germany *Lygus* spp. and *A. lineolatus* were found to have two generations. Percent parasitism was determined by dividing the total number of parasitoid cocoons formed by the number of plant bug nymphs reared. On a weekly basis parasitism fluctuated greatly in all fields sampled, ranging from 0% to 80% over the season. Percent parasitism varied depending on the month and crop sampled; monthly percent parasitism ranged from 0% to 49% in forage crops. Total seasonal percent parasitism was calculated for all fields sampled and ranged from 0% to 36%. The stage of each host collected was recorded, and the temporal distribution of *Lygus* spp. nymphal instars are determined for each sampling week. A typical collection sample consisted mostly of later instar nymphs, this is felt to be an artifact of the sweep sampling method. The seasonal levels of plant bug nymphs are related to seasonal percent parasitism.

Data from six fields of alfalfa were selected on which to perform analysis of variance to determine if any patterns in the spatial distribution of plant bug adults, nymphs, parasitoid cocoons and percent parasitism could be detected, and no patterns were detected. Finally, all parasitoid cocoons reared were held until adults emerged, and then these were identified. In total 7 parasitoid species were reared. The most common species were *Peristenus digoneutis* Loan, *Peristenus stygicus* Loan, *Peristenus rubricollis* (Thomson) and an ichneumonid hyperparasitoid, *Mesochorus* sp. Typically, *P. digoneutis* and *P. stygicus* were the dominant parasitoids reared in this study. Parasitoid species composition for each field sampled is presented.

4.2 Introduction

Several species of Nearctic *Lygus* (Hahn), and the alfalfa plant bug, *Adelphocoris lineolatus* (Goeze), which was accidentally introduced to North America in the early 1900s, are considered pests of agricultural crops in North America. During the 1970s Bilewicz-Pawińska studied plant bugs in agricultural crops in Poland and discovered several species of parasitoids attacking them (Bilewicz-Pawińska 1974, 1976, 1977a, b, 1982). This research lead to taxonomic efforts by Loan and Bilewicz-Pawińska (1973) to identify and describe the new species of parasitoids, which belong in the genus *Peristenus* Foerster. Since this time, efforts have been made to introduce several of the Palaearctic species of *Peristenus* into North America, for control of Nearctic pest *Lygus* and the introduced *A. lineolatus* (Broadbent et al. 2002, Craig & Loan 1984a, b, Day

1996, Day et al. 1990, 1992, 1998, Hormchan 1977, Soroka & Carl 2002, Van Steenwyk & Stern 1977). Two of the introduced species of *Peristenus* are established in North America (Day et al. 1990, 1992, Broadbent et al. 1999). Kuhlmann et al. (1998) describe the current status of biological control of *Lygus* spp. in North America using Palaearctic *Peristenus* species and they list several topics which require more information in order to properly assess the potential of these parasitoid species as biological control agents.

Recent efforts have been made to evaluate these parasitoids and their potential for use as biological control agents in Canada against Nearctic pest *Lygus* spp. To make an assessment of range of hosts attacked by these parasitoids, relationships with their natural hosts in agricultural systems in Europe were studied. The objectives of the research presented here are to document the phenology of the hosts and parasitoids in agricultural crops, to determine if spatial patterns for either the hosts or parasitoids exist, to document the parasitoid community structure in several agricultural habitats and to determine the impact of the parasitoids on the hosts.

4.3 Materials & Methods

4.3.1 Study sites

Field collections of *Lygus pratensis* (L.), *Lygus rugulipennis* Poppius and *A. lineolatus* were made for this study during the summer months of 1998, 1999 and 2000. All study sites were located in the Rhine Valley of Germany, approximately 30 km North of Basel, Switzerland. A total of seven crops were

sampled. They consisted of *Asparagus officinalis* (L.) (asparagus), *Hordeum vulgare* L. (barley), *Brassica napus* L. (rape seed), *Sinapis alba* (L.) (white mustard), a *Trifolium* spp. mixture (clover mixture), *Trifolium pratense* L. (red clover) and *Medicago sativa* L. (alfalfa). Fields were coded using the first two letters of the name of the nearest village or the first two letters of the crop and a field number. All of the fields sampled are listed in Appendix 1, and details of each field's location, size and years sampled are also listed. The sampling period each year was slightly different but in general, sampling began in May and continued for some time into September.

A total of 23 fields were sampled during this three year study. Some fields were sampled each year while others were sampled for only a short period of one season. Only a few weeks of sampling were performed in asparagus, barley and rape crops. No parasitoids were found in the asparagus or the rape but parasitoids were found in all the other crops sampled. The number of plant bugs and parasitoids collected in the asparagus, barley and rape seed crops in 1999 was low, so these crops were not sampled in 2000, instead efforts were concentrated on the mustard and legume crops. Based on the number of fields sampled and the number of years each field was sampled, it would be possible to present 29 seasonal abundance graphs. This is not feasible for the thesis, so representative fields of each crop were selected for presentation. To present data which are representative of the patterns found in the different crops, fields were selected based on several criteria such as crop type, field size, and the time of season samples were taken. In each figure the total number of *Lygus*

spp. and *A. lineolatus*, adults and nymphs collected in a 100-sweep sample, the number of nymphs reared, and total weekly percent parasitism based on calculations from samples collected weekly are graphed. The *Lygus* spp. and *A. lineolatus*, adults and nymphs were actually collected in eight, 20-sweep samples, totaling 160 sweeps per field each sampling day, these numbers were converted to numbers per 100 sweeps for presentation, as this is the historical method of reporting parasitism in these hosts.

Abundance of *Lygus* spp. and *A. lineolatus* is shown for selected fields of mustard, mixed clover, red clover and alfalfa crops. One mustard field was planted in late spring, while another mustard field was planted the previous fall. The mixed clover fields were only sampled in the 2000 field season, and sampling in this crop started in July and continued through August. Several fields of red clover were sampled over the years. The first red clover field presented in this study, Ma1, is classified as a small field. Another field of red clover, Nu2, was selected because it was sampled over the course of a full season and this field was also classified as a small field. Data from fields of alfalfa which are representative of large alfalfa fields (the field width is greater than 50m and the length is greater than 100m) and small alfalfa fields is presented. As the numbers of plant bugs and parasitoids were low in the asparagus, barley and rape crops therefore, no graphs for these crops were generated.

4.3.2 Sampling procedures

Sweep nets, 37.5 cm in diameter, were used to collect plant bugs in the fields. Each week that the vegetation was at least 30 cm high, a total of eight samples were taken from each field to obtain plant bug nymphs for rearing. A single sample consisted of 20-sweeps, with one sweep being a 180° arc. The eight samples were taken in two parallel transects, in which the sampler started at the field edge and moved in toward the center of the field. Transect 1 consisted of sample #'s 1, 2, 3, 4 and transect 2, sample #'s 5, 6, 7, 8. Samples 1 and 5 were located at the edge of the field and samples 4 and 8 were at the center of the field. A total of 160 sweeps was taken at each field each week.

From each sample, all adult *L. pratensis*, *L. rugulipennis* and *A. lineolatus* were counted and their numbers recorded. It is difficult to distinguish between the two *Lygus* species without a microscope, so the number of individuals was recorded only as *Lygus* spp. adults per sample. The nymphs of all species were removed from the net, placed in empty rearing containers, and given a sprig of alfalfa and taken back to the laboratory for rearing.

In the laboratory, the numbers of nymphs of each instar of *Lygus* spp. and *A. lineolatus* were counted. This information was recorded for each sample. Adults of *Lygus* spp. and *A. lineolatus* were not reared because they are usually not found to be parasitized by European *Peristenus* species (Bilewicz-Pawińska & Pankanin 1974). The nymphs of all three species were placed together in rearing containers and fed store-bought green beans until they reached

adulthood, a parasitoid emerged, or the nymph died. Nymphs of the plant bug species collected had to be reared together because there were not enough rearing containers to rear the species separately. The number of parasitoid larvae that emerged and spun cocoons was recorded for each sample, but not for each host species in each sample.

The rearing containers were made from two plastic, tapered, urine sample containers, 6 cm in diameter and 8.5 cm high, and nested one within the other. The bottom of the inner container was cut off and replaced by a piece of mesh (8x8 squares per cm^2). The outer container was filled with fine, moistened vermiculite to a depth of about 1 cm. This served as pupation medium for the emerging parasitoid larvae, and it provided moisture for both nymphs and parasitoid cocoons. Small pieces of crumpled paper towel were added to the inner container to increase the structural diversity for the plant bug nymphs, and to reduce predation by providing hiding places for molting nymphs. Air circulation in the container was improved by cutting a hole in the lid, 2 cm in diameter, which was screened over with a very finely woven material.

On average 10-15 nymphs were placed together in one rearing container. During peaks in numbers of plant bug nymphs, a maximum of 20 nymphs would be reared together in one container. During the peak of nymphs of the second generation, if there were more than 30 nymphs collected in a 20-sweep sample the total number of nymphs collected was recorded and then a subsample of 30 nymphs was selected to be reared. The subsample was chosen to be a representative sample of the instars of *Lygus* spp. and *A. lineolatus* collected in

the sample. To obtain parasitoids, the nymphs were reared in the laboratory under natural light supplemented by a 16L:8D artificial light regime. All rearing containers with plant bug nymphs were monitored on average every 3-5 days, when beans were removed and replaced with fresh beans and any emerged adult plant bugs were also removed. When all the plant bug nymphs had died or completed development the outer container and the vermiculite were inspected for parasitoid cocoons. The number of cocoons was recorded and the lid was replaced. The total number of parasitoids to spin cocoons and the total number of nymphs collected were used to calculate percent parasitism per sample.

The parasitoid cocoons were monitored almost daily for adult emergence. All of the cocoons which did not produce adults the year that they were formed were overwintered in an outdoor insectary and brought back inside the following spring. When adults emerged, the date was recorded, and they were killed in 70% alcohol. Most specimens were shipped to Dr. Henri Goulet at the Eastern Cereal and Oilseed Research Center in Ottawa, Canada, where they were prepared using a critical point drying technique, sexed and identified.

4.3.3 Spatial distribution

The following alfalfa fields were used to determine if there were any detectable differences in the spatial distribution of the plant bugs, adults and nymphs, the number of parasitoid cocoons collected and percent parasitism: Hu3 (1998), St1 (2000), Air2 (2000), Ma5 (1998), Nu1 (2000) and Air1 (2000). Each field was analyzed for differences for each sampling week. Samples were averaged according to their distance from the field edge. Samples 1 & 5 were

closest to the edge (outer edge), were paired and samples 2 & 6, which were the next samples in from the edge (inner edge), were paired and samples 3 & 7, which were further infield (middle), were paired and samples 4 & 8, which were located in the center of the field (center), were paired. These combinations give two replications of the distance sampled from the edge of the field to the center of the field. Percent parasitism was transformed (% arcsin) to remove the effect of proportions on the analysis. Analysis of variance was then performed to determine if, on any sampling date, the abundance of host adults and nymphs, parasitoids and percent parasitism were influenced by the distance from the edge of the field. Fields were classified as small or large to determine if there were any trends related to field size. Fields greater than 50m wide and greater than 100m in length were considered large. Fields less that 50m wide, regardless of their length, were considered small. Small fields of alfalfa were; Hu3 (1998), St1 (2000), Air 2 (2000), and large fields of alfalfa were; Ma5 (1998), Nu1 (2000) and Air 1 (2000). A total of 756 tests were performed on the large alfalfa fields and 651 tests were performed on small alfalfa fields. The difference in the number of tests made was dependant on the number of sampling weeks available for the different fields.

4.4 Results

4.4.1 Seasonal abundance summary statistics

The total number of *Lygus* spp. and *A. lineolatus* adults and nymphs collected during the entire season, the number of parasitoids reared from the nymphs and percent parasitism based on the total number of nymphs reared and parasitoid cocoons formed are given in Appendices 2 and 3. Appendix 2 contains information for fields of asparagus, barley, rape, mustard, mixed clover and red clover and Appendix 3 contains information for each alfalfa field sampled.

4.4.2 Abundance and parasitism in selected fields

Mustard

Data from two sampling seasons are presented because the sampling periods each year were quite different. In 1999, summer mustard was sampled from July to August, and in 2000, winter mustard was sampled from May to June. The total number of nymphs collected was higher towards the latter part of the season (Figure 4.1) as compared to the early season generation (Figure 4.2). Weekly parasitism of mirids in the mustard fluctuated from 0% to 25%. In both years *Lygus* spp. nymphs were dominant over *A. lineolatus* nymphs. In early June there was a steep decline in percent parasitism (Figure 4.2).

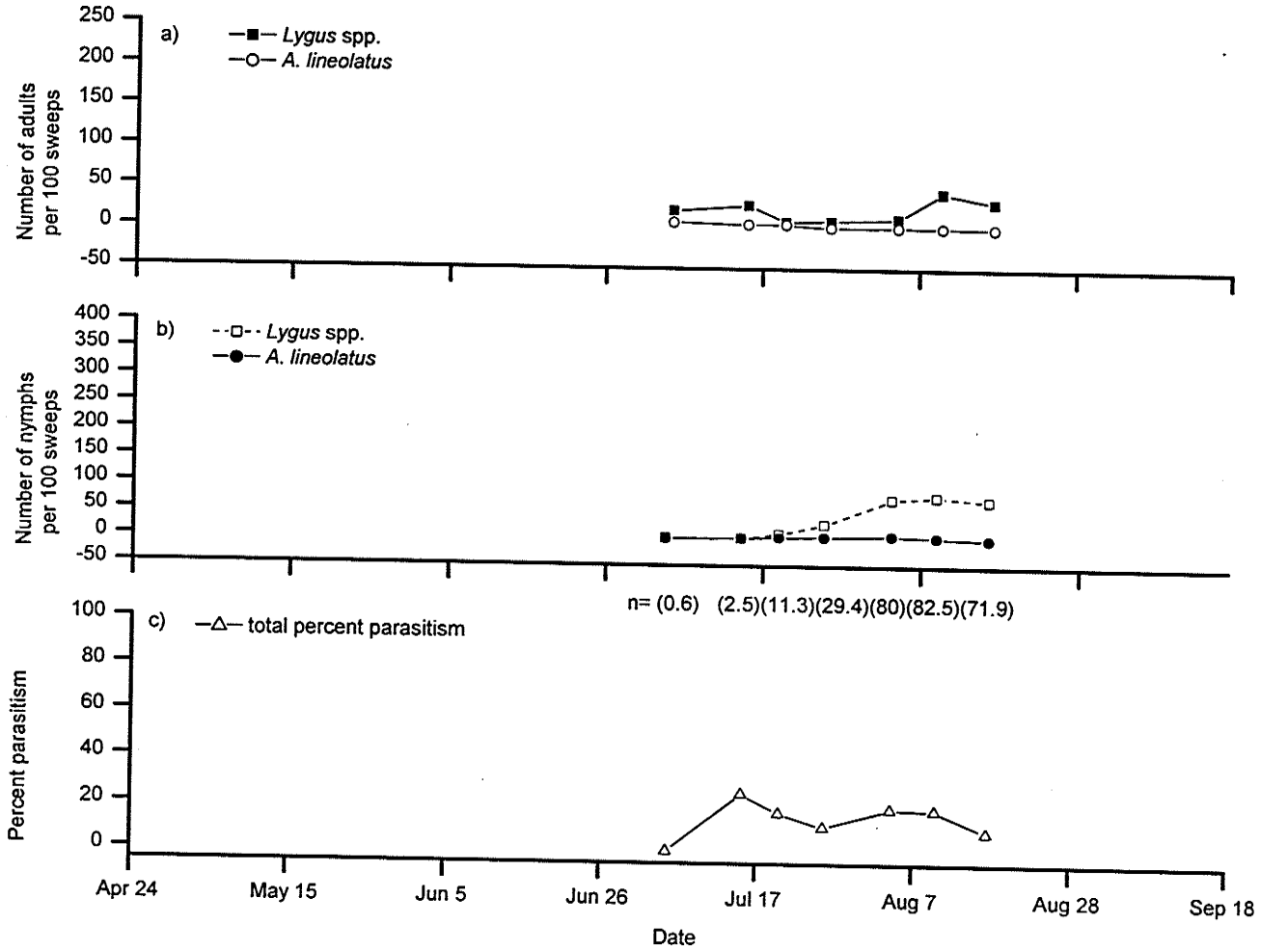

Figure 4.1 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a mustard field, Mu1, in Germany in 1999.

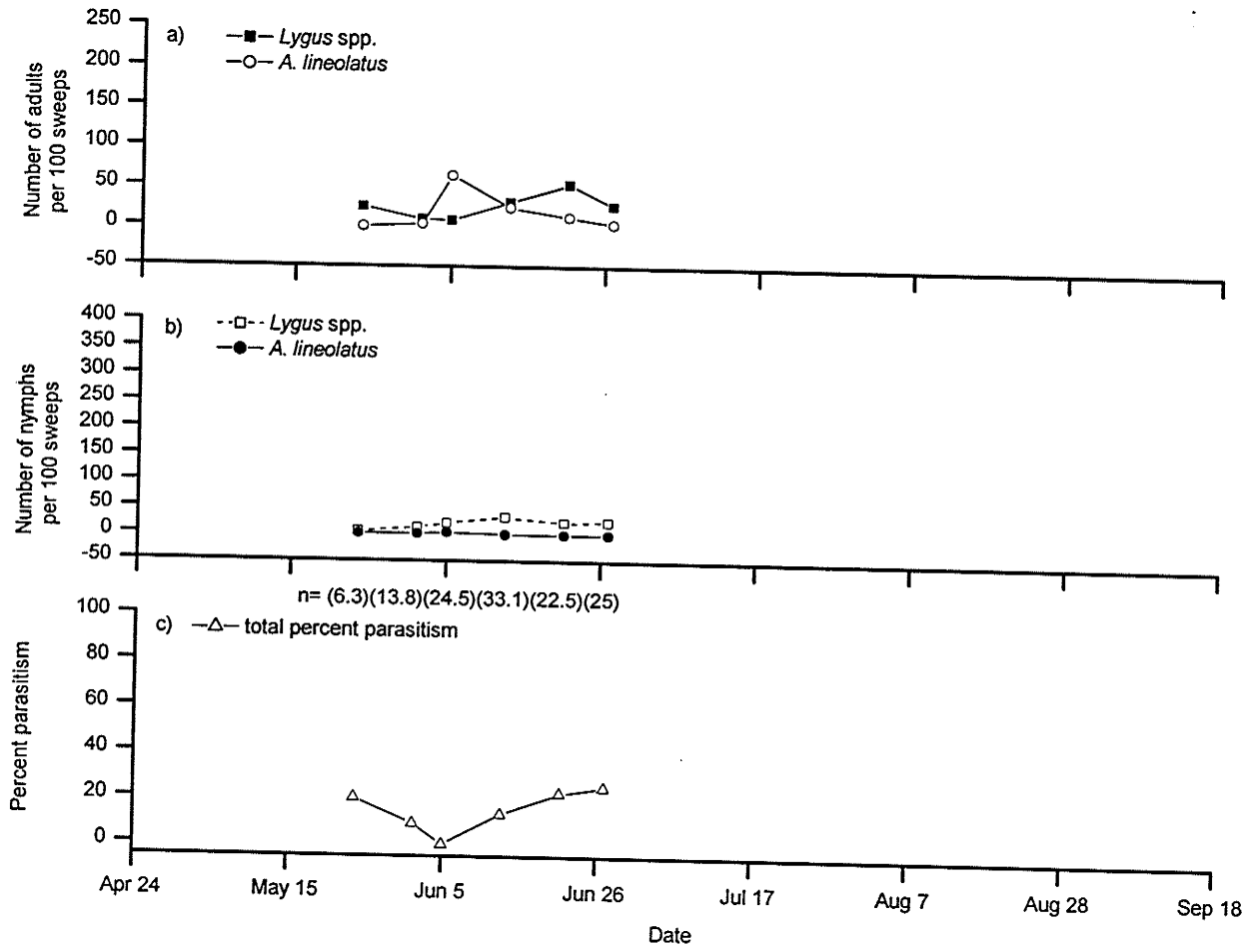

Figure 4.2 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a mustard field, Mu4, in Germany in 2000.

Mixed clover

In 2000, two fields of a mixed clover crop were sampled from mid July until early September. One of these fields is represented in Figure 4.3. *Lygus* spp. nymphs were dominant over *A. lineolatus* nymphs. Numbers of plant bug nymphs were high until mid August. Plant bug nymphs were heavily parasitized in this crop. Parasitism ranged from 15% to 65% in weekly samples.

Red clover

Red clover fields were sampled in 1998, 1999 and 2000. Data from a field, Ma1, sampled in 1998 from 24 June to 23 September, are presented in Figure 4.4. The number of *Lygus* spp. nymphs was greater than *A. lineolatus* nymphs. Plant bug nymphal abundance in the first generation was high but declined at the beginning of the sampling period. The second generation of *Lygus* spp. nymphs peaked on 14 August. The weekly % parasitism ranged from 0% to 30%. In 2000, in field Nu2, sampling began on 24 May and continued until 6 September (Figure 4.5). The first generation of *Lygus* spp. nymphs peaked in early June and the second generation peaked in early August. The first generation of *A. lineolatus* peaked in mid to late June and the second generation peaked in late August. The weekly percent parasitism increases as do numbers of nymphs. In late May, the weekly percent parasitism was around 10%. It rose in late June and early July, to over 20%, following an increase of *A. lineolatus* nymphs, and then peaked in early August at 30%, which corresponds

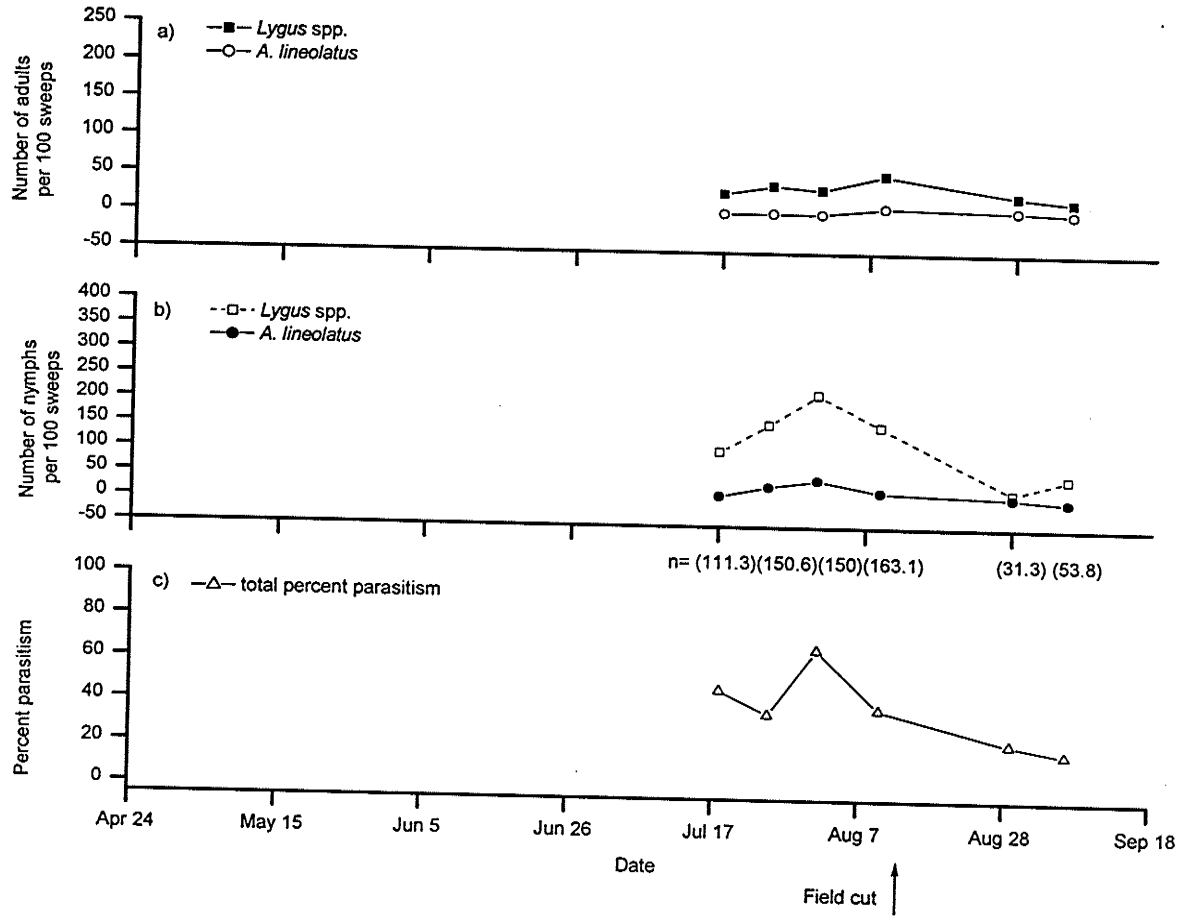

Figure 4.3 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a mixed clover field, Pt1, in Germany in 2000.

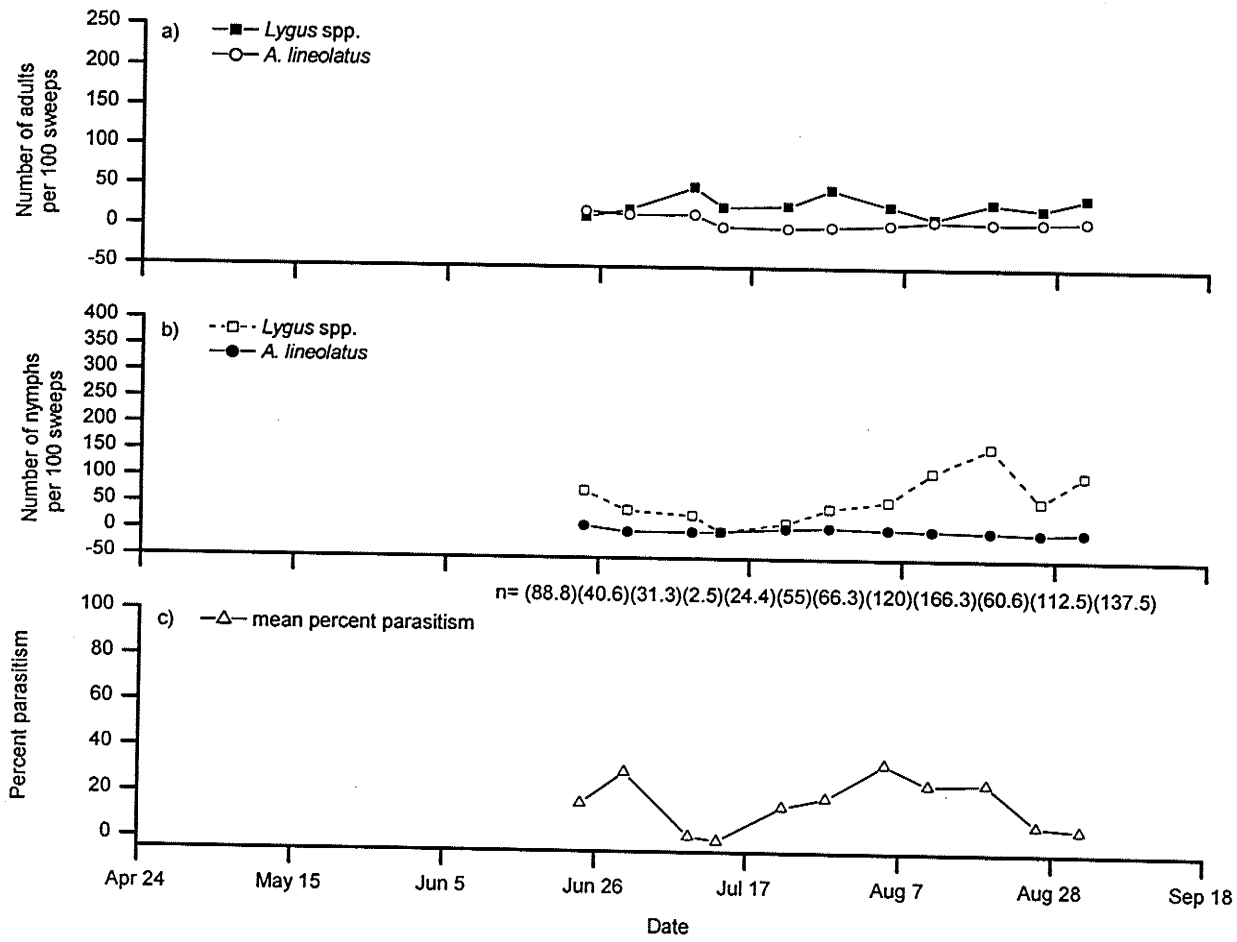

Figure 4.4 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus:* a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a field of red clover, Ma1, in Germany in 1998.

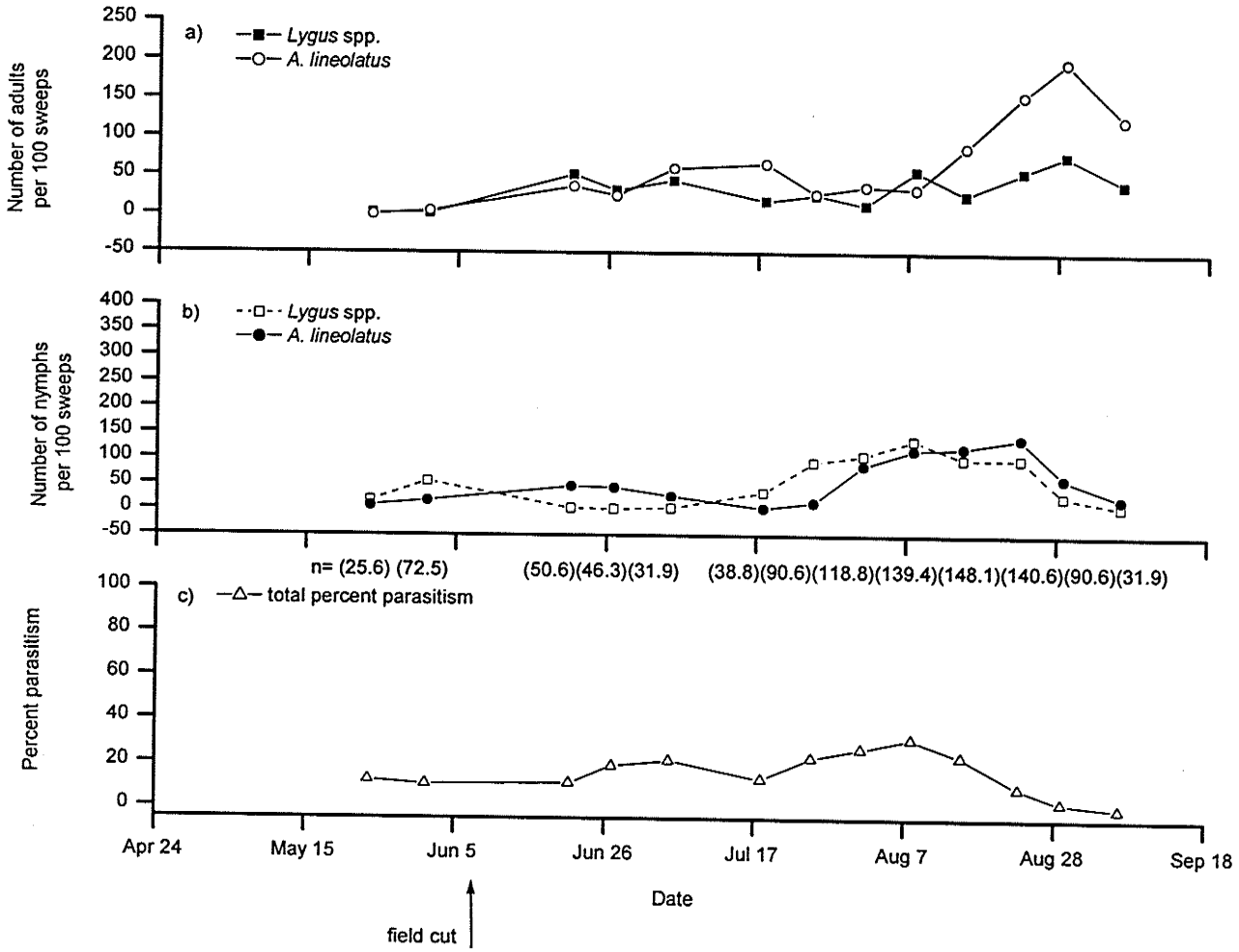

Figure 4.5 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a field of red clover, Nu2, in Germany in 2000.

to the peak of *Lygus* spp. nymphs. As the nymphal population declined, so did mean percent parasitism.

Alfalfa

The plant bug abundance and percent parasitism in five fields of alfalfa are presented in Figures 4.6 - 4.10. The populations of plant bugs and parasitoids in 1999 were very low, so results from this year are not presented. Figures 4.6 and 4.7 are for fields sampled in 1998. In 1998, field cutting in mid July, in the large field, Ma5 (Figure 4.6), may have delayed an increase in the population of nymphs. Weekly percent parasitism in the Hu3 field (Figure 4.7), in first generation nymphs was about 20% in late June. The peak in percent parasitism of 80% is not considered accurate because it is determined from an extremely small sample size. The number of *Lygus* spp. nymphs and *A. lineolatus* nymphs collected was quite similar until early August when the second generation of *Lygus* spp. nymphs peaked in abundance. Hu3 was cut twice during the season, in late June and late July. The second cutting did not appear to suppress the increase in the nymph population, although the increase might have been greater had there been no cutting. It is possible that the cutting had an effect on weekly percent parasitism, slightly reducing the peak.

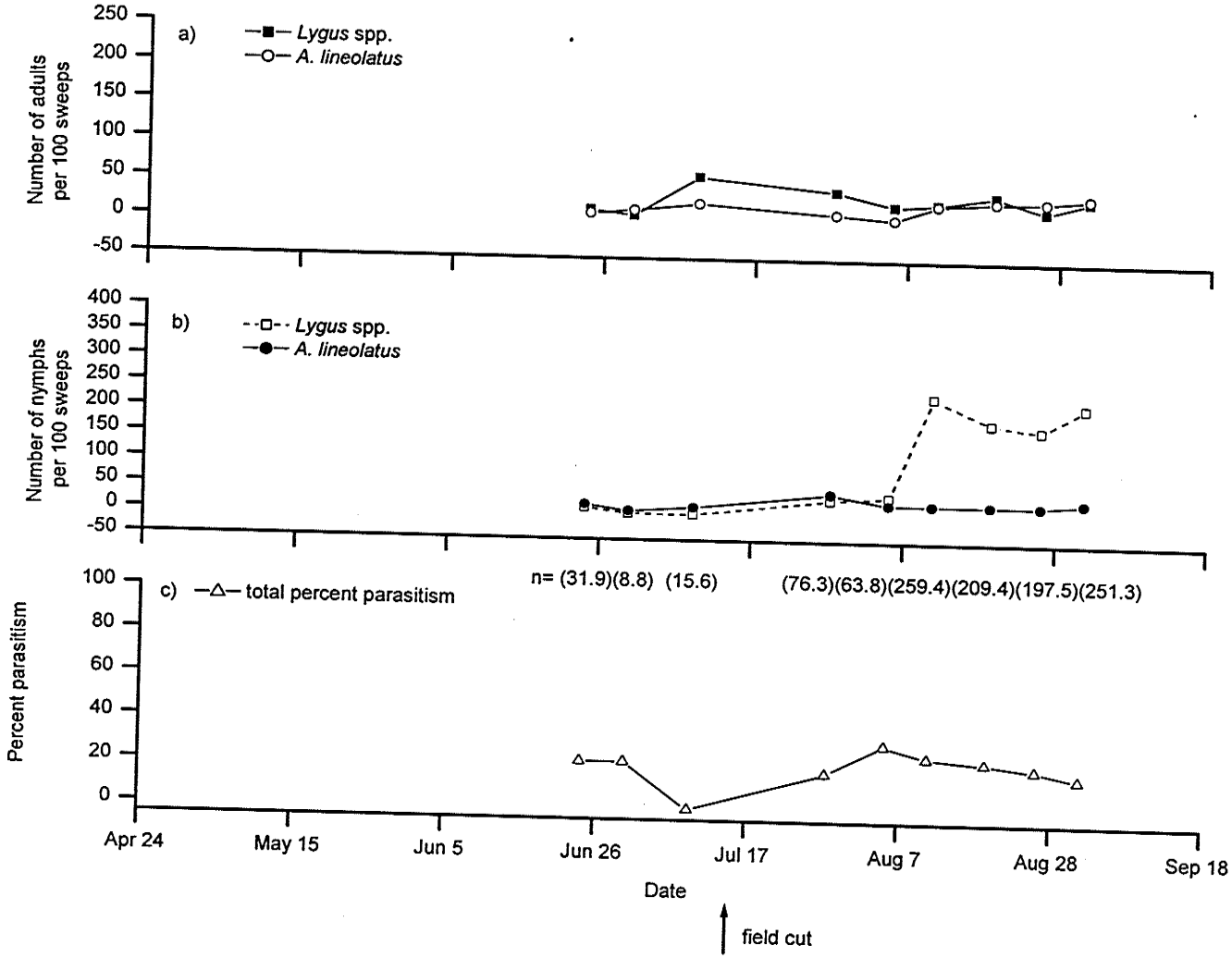

Figure 4.6 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a field of alfalfa, Ma5, in Germany in 1998.

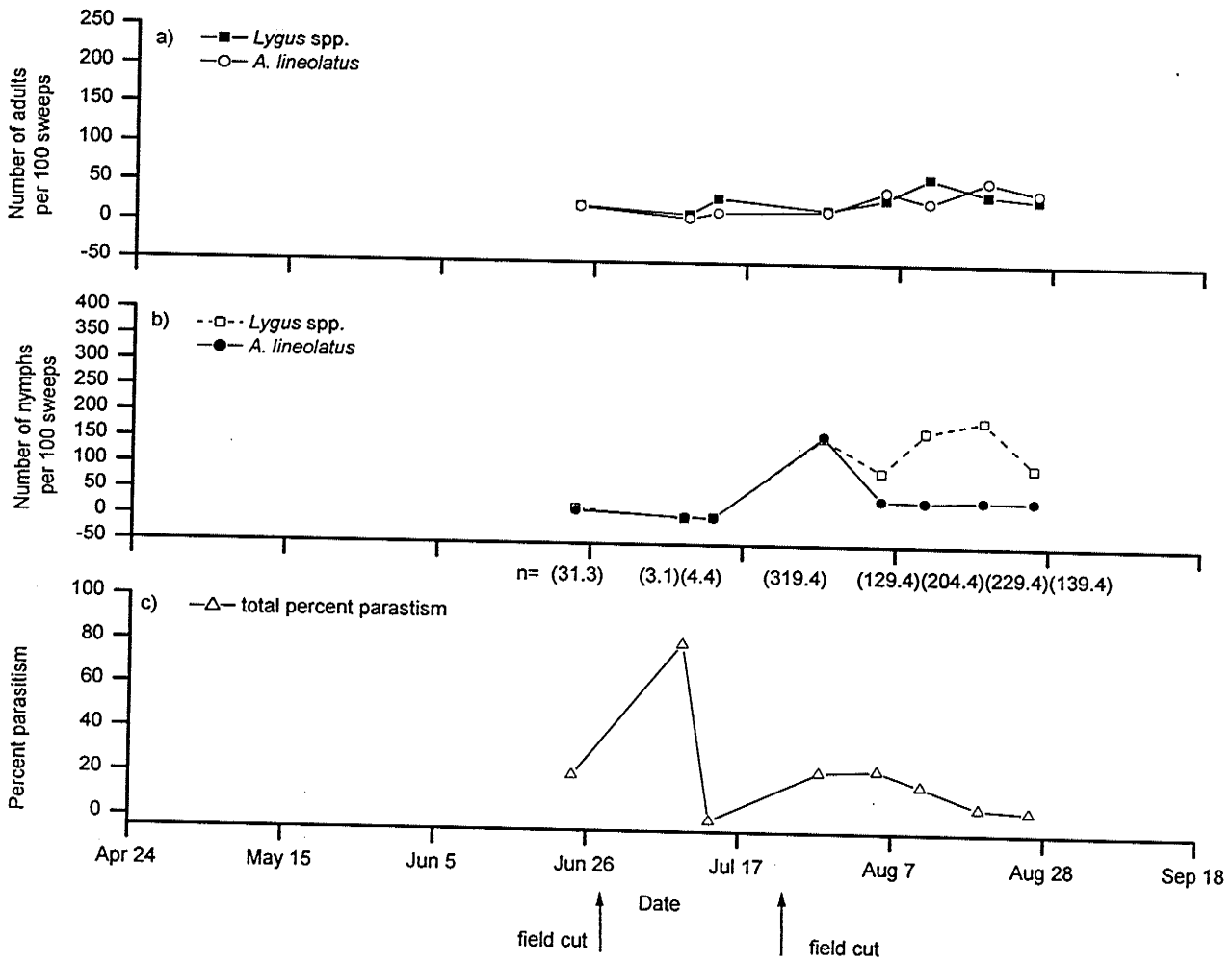

Figure 4.7 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus:* a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a field of alfalfa, Hu3, in Germany in 1998.

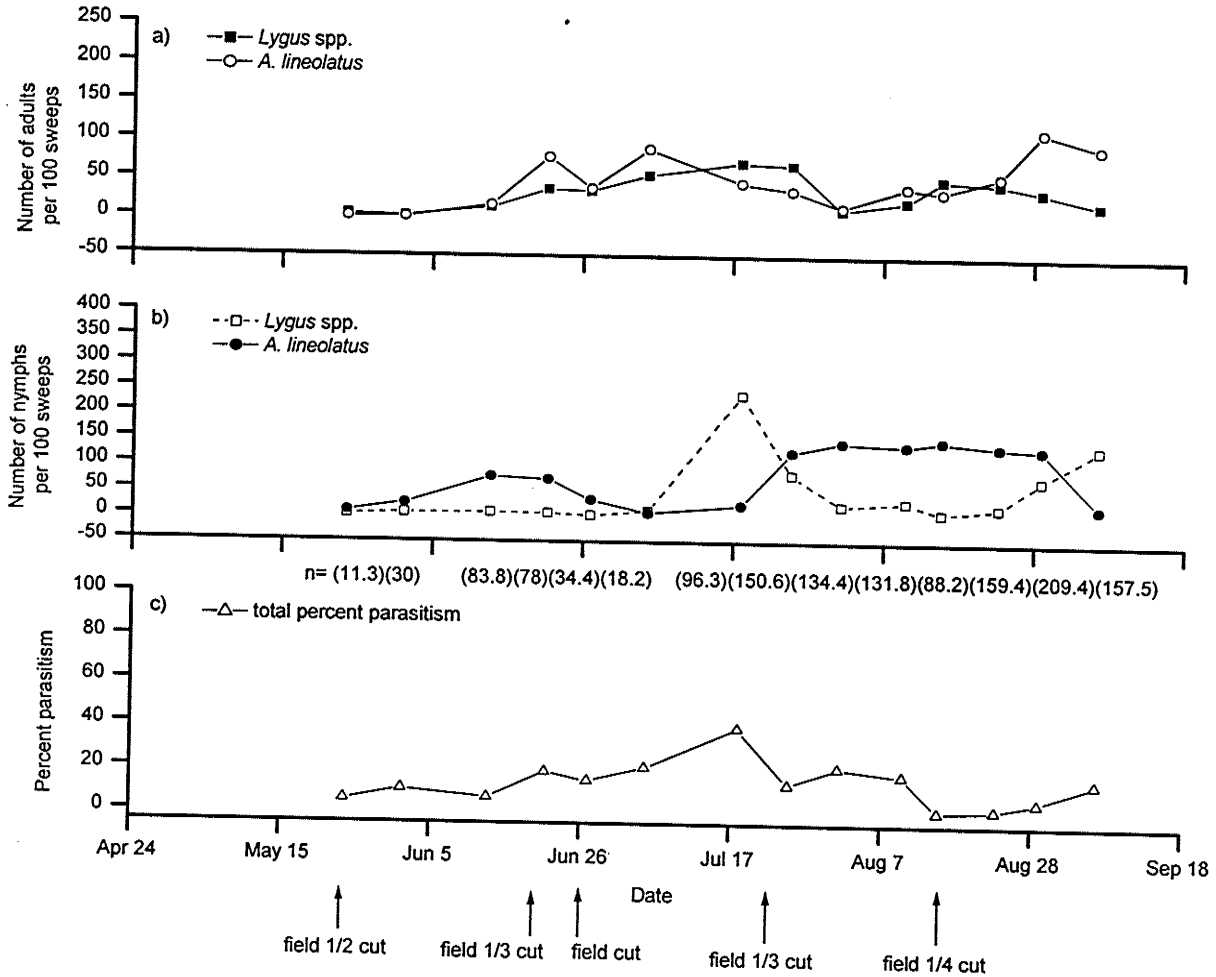

Figure 4.8 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a field of alfalfa, Air1, in Germany in 2000.

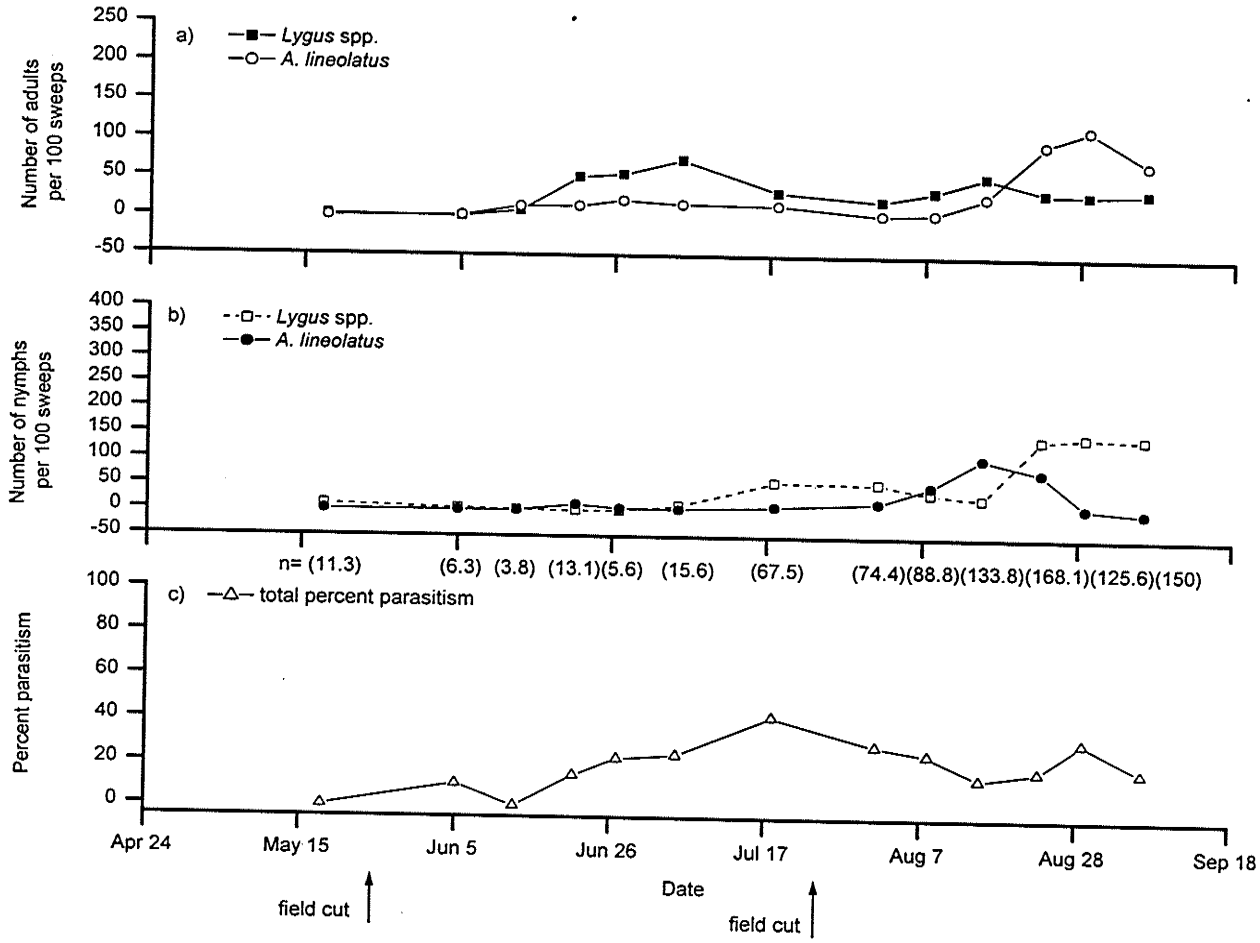

Figure 4.9 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in an alfalfa field, St1, in Germany in 2000.

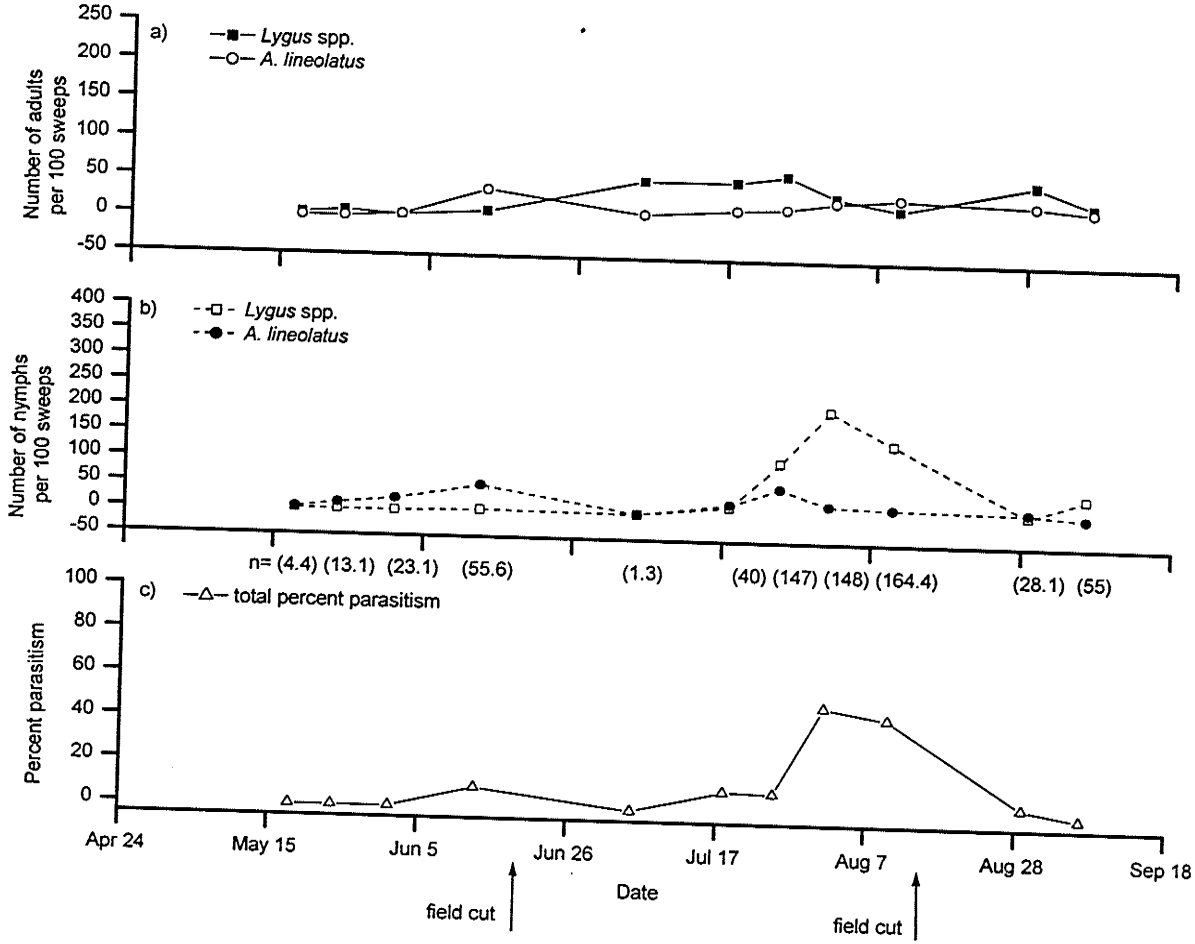

Figure 4.10 Seasonal abundance of *Lygus* spp. and *Adelphocoris lineolatus*: a) adults collected in 100 sweeps, b) nymphs collected in 100 sweeps, with the total number of nymphs reared indicated in brackets, and c) total weekly percent parasitism in a field of alfalfa, Air2, in Germany in 2000.

Data from fields sampled in 2000 are shown in Figures 4.8 - 4.10. The large alfalfa field, Air 1 (Figure 4.8), was cut many times throughout the season, but only once during the season was the whole field cut. It is difficult to know when the first generation of *Lygus* spp. nymphs peaked because of the many field cuttings, however, there was a peak on 18th July. The population of *A. lineolatus* nymphs was quite low in June but peaked in early August, and remained high and stable until late August. Total weekly percent parasitism peaked in mid to late June and again in late July. Had the field not been cut near the end of July (4th cut on graph) both the nymphal population and the percent parasitism are expected to have been higher. In field St1 (Figure 4.9), the nymphal population remained low until mid July. The second generation of *Lygus* spp. peaked in late August, and *A. lineolatus* peaked mid to late August. Total weekly percent parasitism peaked mid July at 40%, prior to the second field cutting. After this cut, parasitism declined until late August, when it peaked again, following the peak in second generation *Lygus* spp. nymphs. In field Air2, (Figure 4.10) nymphal abundance was low in the first generation, and only *A. lineolatus* peaked in early June, which was followed by a cutting. The second generation of *Lygus* spp. and *A. lineolatus* peaked in early August. Total weekly percent parasitism was low until the second generation peak of nymphs and peaked at around 50%.

4.4.3 Total monthly parasitism

For each field, sampled the total monthly percent parasitism was

calculated. This value was obtained by dividing the total number of parasitoid cocoons reared each month by the number of nymphs reared each month. Monthly percent parasitism for nymphs in fields of asparagus, barley, rape, mustard, mixed clover and red clover are shown in Table 4.1a. Monthly percent parasitism for nymphs in fields of alfalfa are shown in Table 4.1b. It should be noted that the high monthly parasitism in nymphs in barley are affected by very small sample sizes. Parasitoids were present in all mustard fields sampled in 1999 and 2000. Monthly percent parasitism in the fields of mixed clover was very high, reaching almost 50% in mid season. In the red clover, parasitoids were collected from May to September, with percent parasitism at its highest in either July or August. In alfalfa, monthly percent parasitism was lowest during the 1999 field season. Parasitism was notably low in alfalfa in May, although not all fields were sampled in this month. Parasitoids were reared from nymphs collected from May to September. A mean monthly percent parasitism was calculated for the alfalfa fields. July had the highest monthly percent parasitism, followed by June, August, September and May.

Table 4.1a Monthly total percent parasitism, based on the total number of plant bug nymphs reared, indicated in brackets, and parasitoid cocoons reared from asparagus, barley, rape, mustard, mixed clover and red clover fields in Germany from 1998 - 2000.

Crop	Field Code	Year	Total monthly percent parasitism				
			May	June	July	August	September
Asparagus	Asp1	1999	-	-	-	0% (29)	-
	Asp2	1999	-	-	-	0% (2)	-
Barley	Ba1	1999	-	53.8% (13)	100% (1)	-	-
	Ba2	1999	-	23.3% (30)	-	-	-
Rape seed	Ca1	1999	0% (0)	0% (3)	-	-	-
	Ca2	1999	0% (0)	0% (11)	-	-	-
Mustard	Mu1	1999	-	-	12.9% (70)	15.5% (375)	-
	Mu2	1999	-	-	13.5% (96)	13.7% (395)	-
	Mu3	2000	17.3% (29)	9.3% (241)	-	-	-
	Mu4	2000	20% (10)	14.2% (190)	-	-	-
Mixed clover spp.	Pt1	2000	-	-	35.3% (475)	36% (747)	17.4% (86)
	Pt2	2000	-		49.6% (123)	26.5% (548)	13% (139)
Red clover	Ma1	1998	-	20.3% (207)	13.3% (181)	24.2% (661)	9.5% (400)
	Nu2	1999	-	-	22.7% (295)	10.8% (1609)	-
	Nu2	2000	12.2% (41)	11.7% (299)	20.2% (258)	19.7% (1020)	0% (51)
	RC2	2000	-	1.6% (123)	28.4% (183)	44.5% (665)	-

Table 4.1b Monthly total percent parasitism, based on the total number of plant bug nymphs reared indicated in brackets, and parasitoid cocoons reared from alfalfa fields in Germany from 1998 - 2000.

Crop	Field Code	Year	Total monthly percent parasitism				
			May	June	July	August	September
Alfalfa	Hu1	1998	-	0% (20)	33.5% (242)	2.6% (582)	0.6% (163)
	Hu1	1999	-	5% (20)	9.1% (22)	5% (1281)	-
	Hu2	1998	-	12.4% (283)	29.4% (17)	-	-
	Hu3	1998	-	20% (50)	22.2% (523)	12.1% (1124)	-
	Hu3	1999	0% (1)	17.4% (23)	0% (2)	-	-
	Ma5	1998	-	21.5% (65)	14.2% (147)	23.3% (1168)	15.9% (402)
	St1	1998	-	27.5% (120)	25.5% (153)	5.6% (283)	8.9% (178)
	St1	1999	-	25% (4)	20% (5)	4% (175)	-
	St1	2000	0% (18)	13% (46)	38.3% (133)	20.6% (1001)	17% (240)
	Nu1	1999	-	-	1%	3.3%	-
	Nu1	2000	0% (40)	12.1% (173)	10.7% (159)	20.3% (617)	12% (25)
	Air1	2000	5.6% (18)	12.5% (361)	23.1% (424)	9.5% (1157)	14.7% (252)
	Air2	2000	0% (28)	6.4% (126)	9% (301)	43.2% (544)	0% (88)
Average per month			1.1%	14.4%	18.2%	13.5%	9.8%

4.4.4 Temporal evaluation of *Lygus* spp. nymphs

In this study, the stage of each nymph collected in each sample was recorded. This information allows examination of the phenology of the plant bugs. Five fields of alfalfa were selected for presentation and they are the same fields that were chosen to assess seasonal abundance and percent parasitism.

Figures 4.11 and 4.12 are from fields sampled in 1998 and they are similar in their nymphal instar seasonal composition. In Figure 4.11 the peak of second and third instars is on 11 August, and within one week of this peak in nymphal instars, there is also a peak in total weekly percent parasitism shown in Figure 4.6. In the last three sampling weeks, there is a decrease in the proportion of second and third instars and an increase in the proportion of fourth and fifth instars collected. Figure 4.12 is of field Hu3, an alfalfa field. There is a consistent number of second and third instars over a four week period, mostly in August. Again, as the number of second and third instar nymphs decreased there was an increase in the number of fourth and fifth instars. The total weekly percent parasitism is consistent across most of the month of August, but decreased near the end of the month.

Figures 4.13 - 4.15 were generated from fields sampled in 2000. Figure 4.13 is of a large field, Air1. The *Lygus* spp. nymphal population peaked in mid July. This peak was dominated by first, second and third instar nymphs. This peak of instars was associated with the greatest peak in total weekly percent parasitism, about 35%. In Figure 4.13, a pattern similar to that in Figures 4.11

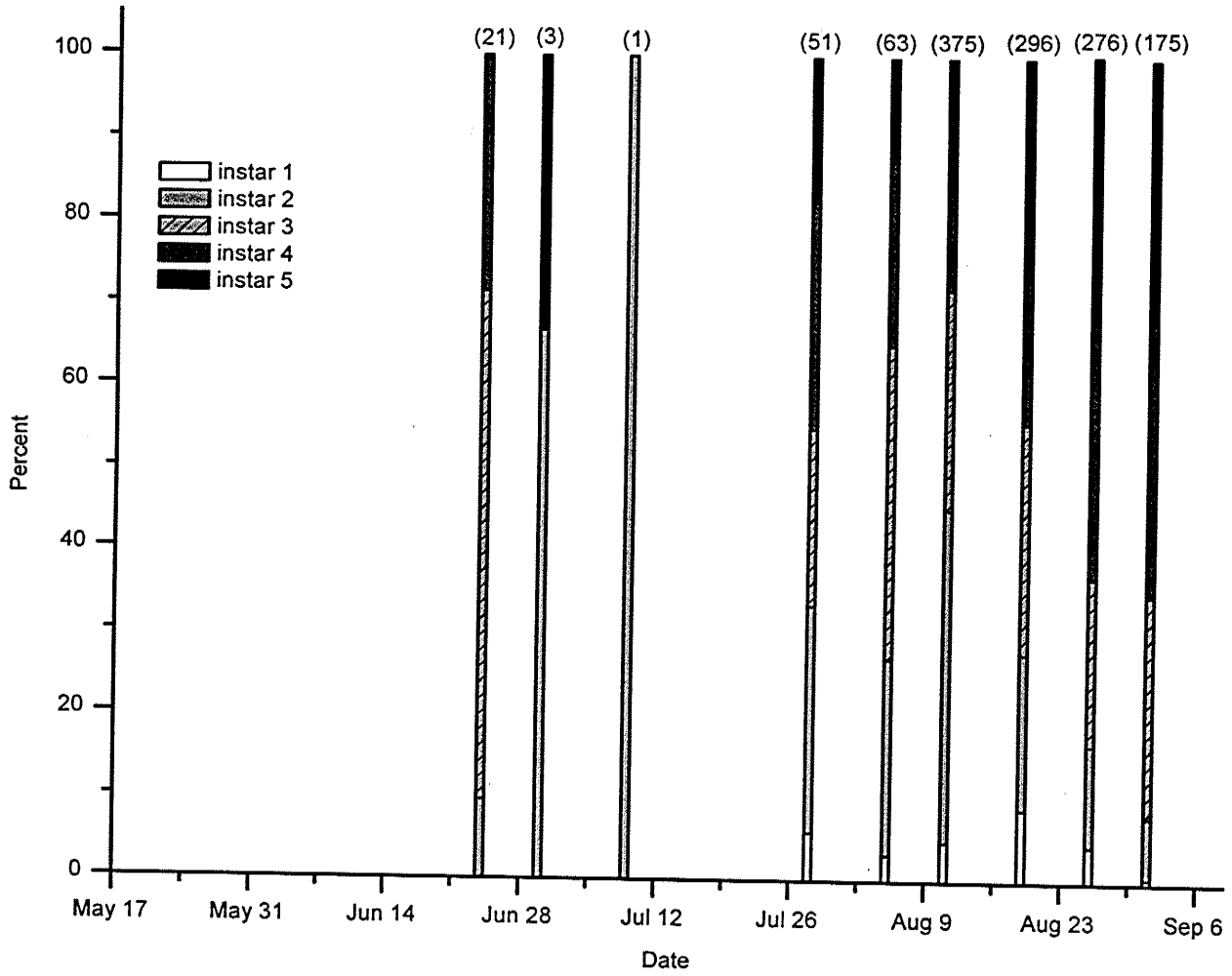

Figure 4.11 Proportion of each instar of *Lygus* spp. nymphs, with the total number of nymphs indicated in brackets, that were collected in 160-sweeps, each sampling week in a field of alfalfa, Ma5, in Germany in 1998.

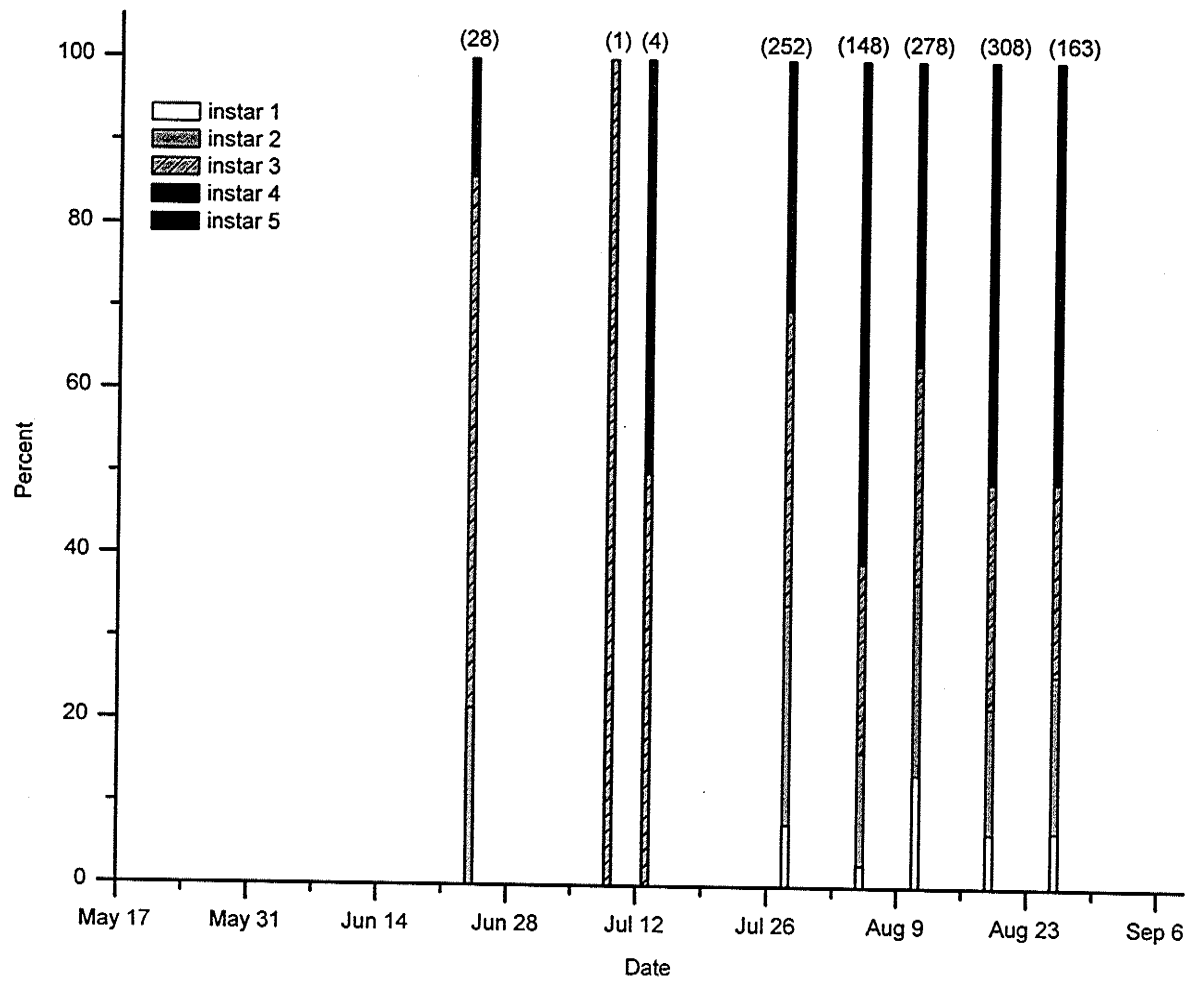

Figure 4.12 Proportion of each instar of *Lygus* spp. nymphs, with the total number of nymphs indicated in brackets, that were collected in 160-sweeps each sampling week in an alfalfa field, Hu3, in Germany in 1998.

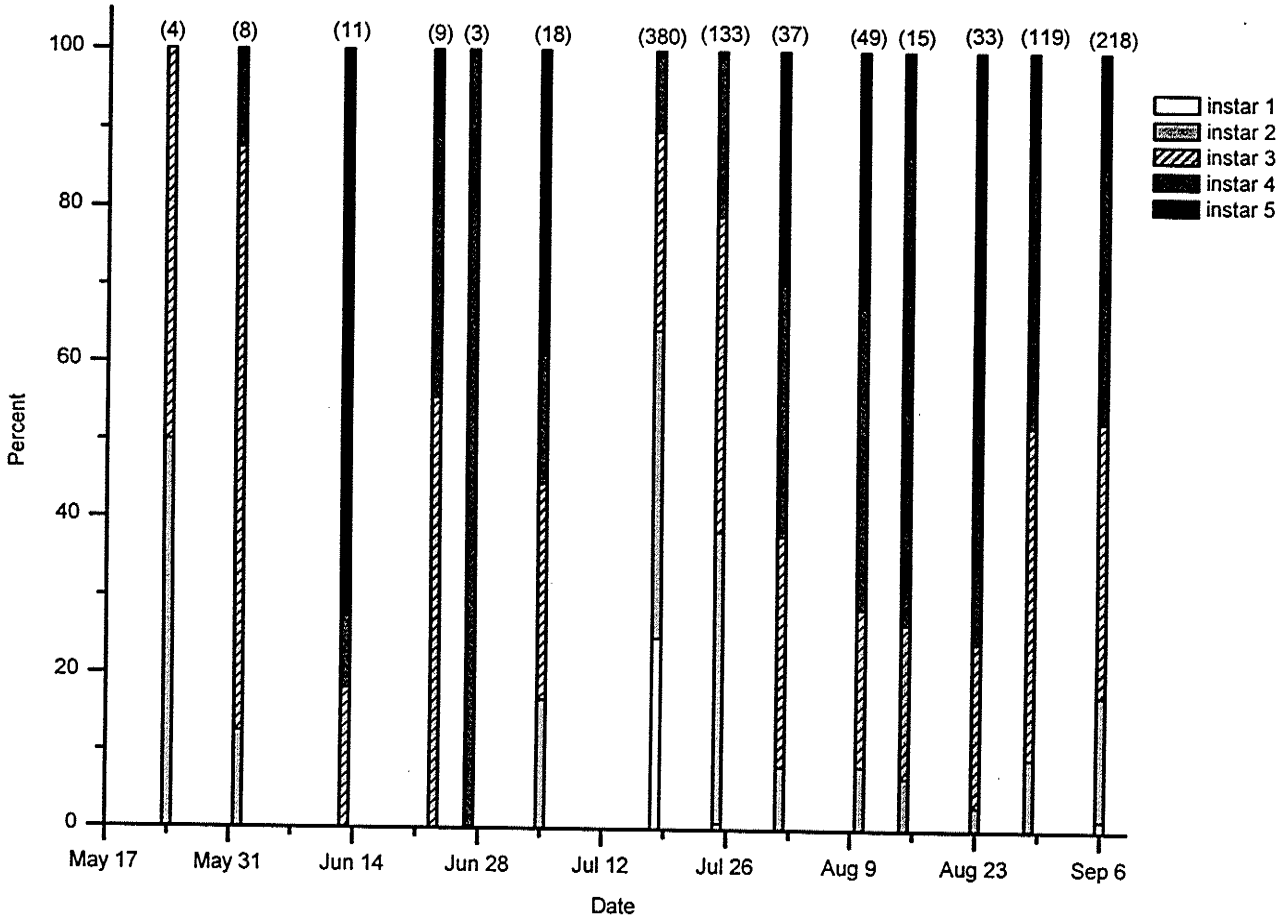

Figure 4.13 Proportion of each instar of *Lygus* spp. nymphs, with the total number of nymphs indicated in brackets, that were collected in 160-sweeps each sampling week in an alfalfa field, Air1, in Germany in 2000.

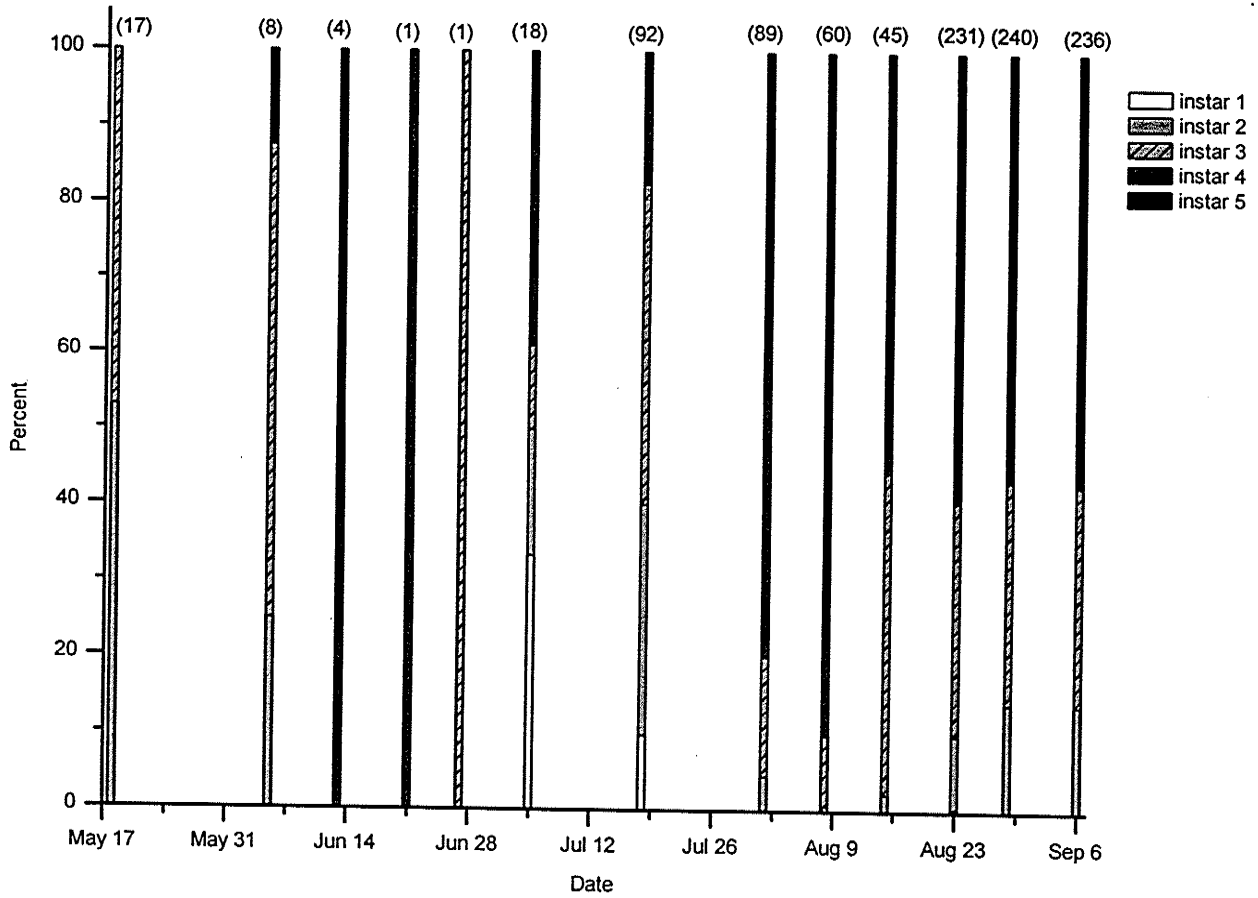

Figure 4.14 Proportion of each instar of *Lygus* spp. nymphs, with the total number of nymphs indicated in brackets, that were collected in 160-sweeps, each sampling week in a field of alfalfa, St1, in Germany in 2000.

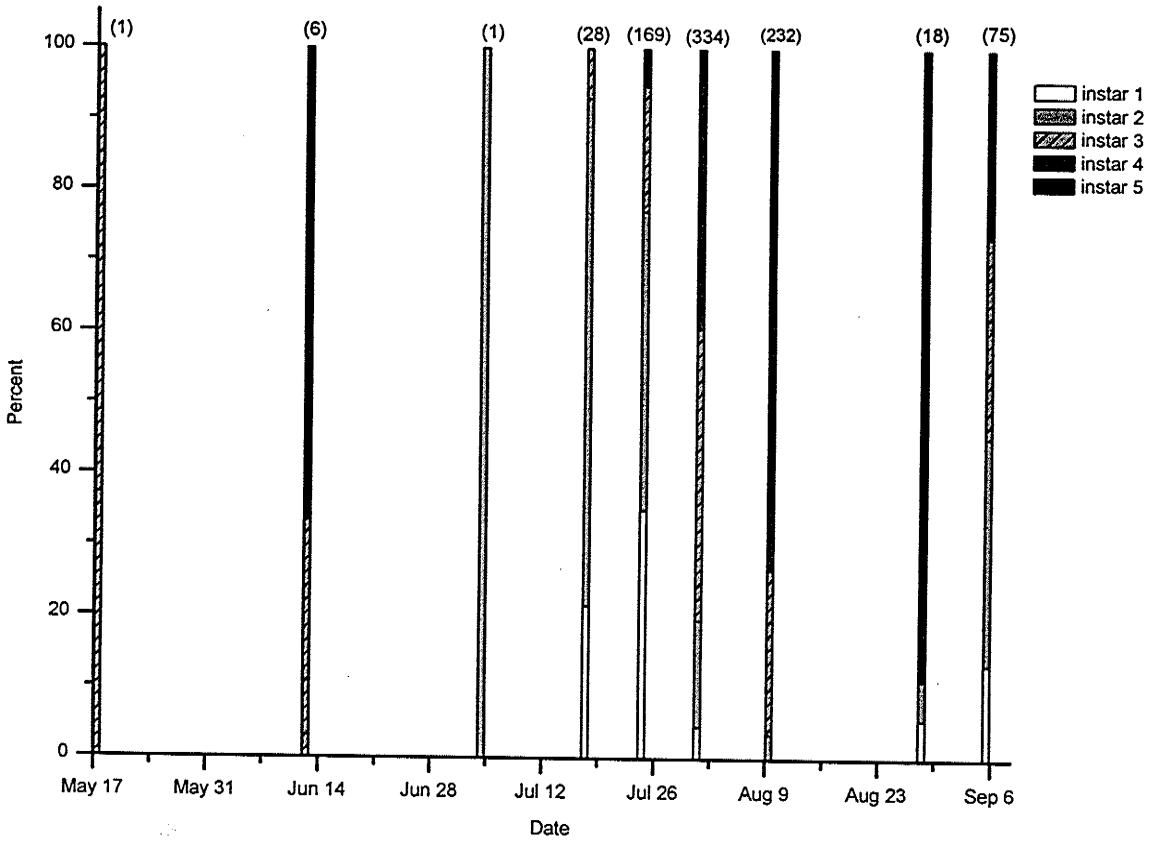

Figure 4.15 Proportion of each instar of *Lygus* spp. nymphs, with the total number of nymphs indicated in brackets, that were collected in 160-sweeps, each sampling week in a field of alfalfa, Air2, in Germany in 2000.

and 4.12 is detectable, as the number of second and third instars decreased there was an increase in the number of fourth and fifth instar nymphs. In the late season, August into September, there was a gradual increase in the proportion of second and third instar nymphs and an increase in total weekly percent parasitism (Figure 4.8). In the St1 field (Figure 4.14), there was a peak of early instar nymphs on 19 July, and the greatest peak in total weekly percent parasitism occurred this same week (Figure 4.9). The number of fourth and fifth instars increased after this date for approximately 1 month. Later in the season, as the number of second, third and fourth instars rose there was another rise in weekly percent parasitism. In the Air2 field (Figure 4.15), the peak of third instar nymphs was at the very end of July; this peak was associated with the greatest peak in total weekly percent parasitism (Figure 4.10). There was a decrease in percent parasitism as the numbers of early instar *Lygus* spp. collected decreased.

4.4.5 Spatial distribution

There are no trends in spatial distribution of the bugs or their parasitoids among fields sampled the same year in any of the parameters measured, for large or small fields. Significant differences are reported for large fields in Table 4.2 and for small fields in Table 4.3. There were significant differences found in percent parasitism only in large fields of alfalfa. However, there is no consistency in this difference in terms of distances from the field edge for the different fields on different dates. Using an α level of 0.05 it is expected that 38

Table 4.2 Results which indicate significant differences from 13 of 756 tests on the spatial distribution of the number *Lygus* spp. and *Adelphocoris lineolatus*, nymphs and adults collected, the number of parasitoid cocoons reared and percent parasitism calculated each week from selected large fields of alfalfa sampled in Germany.

Field	Date	Parameter	ANOVA significance	Mean per 20 sweeps			
				Outer edge (1)	Inner edge (2)	Middle (3)	Center (4)
Air 1	24.V.2000	Total nymphs	P≤.005	1.5	1.5	5.0	1.0
Air1	13.VI.2000	Total nymphs	P≤.05	9.5	18.5	21.5	17.2
Air1	5.VIII.2000	*A. lineolatus* nymphs	P≤.05	0.0	1.5	3.5	0.5
Air1	15.VIII.2000	*Lygus* spp. adults	P≤.05	7.5	14.0	6.5	12.0
Air1	29.VIII.2000	% Parasitism	P≤.001	14.2%	8.3%	0.0%	4.7%
Ma5	28.VII.1998	Parasitoid cocoons	P≤.05	5.0	1.5	1.0	3.0
Ma5	19.VIII.1998	Parasitoid cocoons	P≤.05	9.0	8.0	13.5	7.5
Nu1	21.VI.2000	% Parasitism	P≤.05	25.0%	0.0%	7.1%	12.0%
Nu1	27.VI.2000	*A. lineolatus* nymphs	P≤.05	2.0	3.5	9.5	16.5
Nu1	27.VI.2000	Total nymphs	P≤.01	2.5	4.0	10.0	16.5
Nu1	25.VII.2000	% Parasitism	P≤.05	10.6%	13.0%	3.0%	0.0%
Nu1	1.VIII.2000	*Lygus* spp. adults	P≤.05	14.0	6.5	19.0	15.0
Nu1	6.IX.2000	*A. lineolatus* adults	P≤.05	0.0	0.5	1.5	2.0

Table 4.3 Results which indicate significant differences from 12 of 651 tests on the spatial distribution of the number *Lygus* spp. and *Adelphocoris lineolatus*, nymphs and adults collected, the number of parasitoid cocoons reared and percent parasitism calculated each week from selected small fields of alfalfa sampled in Germany.

Field	Date	Parameter	ANOVA significance	Mean per 20 sweeps			
				Outer edge (1)	Inner edge (2)	Middle (3)	Center (4)
Hu3	28.VII.98	*Lygus* spp. nymphs	P≤.05	49.0	52.0	20.5	4.5
Hu3	28.VII.98	*A. lineolatus* nymphs	P≤.05	49.5	41.5	27.5	11.0
Hu3	28.VII.98	Total nymphs	P≤.001	98.5	93.5	48.0	15.5
Hu3	26.VIII.98	*Lygus* spp. adults	P≤.001	10.0	11.0	4.5	1.5
Air2	18.V.2000	*A. lineolatus* nymphs	P≤.05	0.0	0.5	2.5	0.0
Air2	18.V.2000	Total nymphs	P≤.01	0.0	0.5	3.0	0.0
Air2	5.VII.2000	*Lygus* spp. adults	P≤.01	13.0	9.0	7.5	11.5
Air2	25.VII.2000	Parasitoid cocoons	P≤.05	2.5	3.5	3.5	1.0
Air2	1.VIII.2000	*A. lineolatus* adults	P≤.05	13.5	3.0	4.5	1.5
St1	18.V.2000	*Lygus* spp. adults	P≤.05	0.0	1.5	0.0	0.0
St1	1.VIII.2000	*A. lineolatus* nymphs	P≤.05	2.5	2.5	8.0	2.0
St1	29.VIII.2000	*Lygus* spp. adults	P≤.005	12.0	7.5	2.5	3.5

tests out of 756 and 33 tests out of 651 would be significant on the basis of random chance when there are no significant spatial patterns. The results reported here are less than these predictions. The significant differences reported here are thought to be due to individual field conditions at the time of sampling or random variation. There are no major trends in differences due to field size or to sampling distance within the field.

4.4.6 Parasitoid species composition

The proportion of each parasitoid species found to emerge from cocoons reared from each field, each year were calculated and are presented in Tables 4.4 and 4.5. Parasitoid species composition for barley, mustard, mixed clover and red clover are found in Table 4.4 and for alfalfa in Table 4.5. Data from parasitoids emerging from overwintered cocoons during the 2001 field season are not included in these results.

Barley

Few parasitoids were reared from nymphs collected in barley fields, and they were identified as *Peristenus digoneutis* Loan, and *Mesochorus* spp. The *Mesochorus* spp. is thought to be an ichneumonid hyperparasitoid of euphorine braconid wasps (Brindley 1939).

Table 4.4 Parasitoid species composition of adult parasitoids reared from *Lygus* spp. and *Adelphocoris lineolatus* nymphs collected in barley, mustard, mixed clover and red clover fields in Germany, sampled during 1998 - 2000.

Crop	Field code	Collection year	Emergence year	Total number of parasitoids	Percent				
					Peristenus digoneutis	*Peristenus rubricollis*	*Peristenus stygicus*	*Mesochorus* sp.	*Leiophron* n. sp.
Barley	Ba2	1999	1999	3	33.0	0	0	66.0	0
Mustard	Mu1	1999	1999	19	31.6	0	68.4	0	0
	Mu1	1999	2000	39	77.0	0	7.7	2.6	12.8
	Mu2	1999	1999	13	38.5	0	46.2	15.4	0
	Mu2	1999	2000	42	85.7	0	9.5	2.4	2.4
	Mu3	2000	2000	21	66.7	0	33.3	0	0
	Mu4	2000	2000	23	17.4	0	78.3	4.3	0
Mixed clover	Pt1	2000	2000	132	32.6	0	13.6	53.8	0
	Pt2	2000	2000	46	41.3	0	19.6	39.1	0
Red clover	Ma1	1998	1998	47	42.6	0	12.8	44.7	0
	Ma1	1998	1999	148	89.9	2.7	7.4	0	0
	Nu2	1999	1999	35	57.1	0	22.9	20.0	0
	Nu2	1999	2000	168	97.0	0	3.0	0	0
	Nu2	2000	2000	56	64.3	0	17.9	8.9	0
	RC2	2000	2000	50	28.0	0	42.0	30.0	0

Table 4.5 Parasitoid species composition of adult parasitoids reared from *Lygus* spp. and *Adelphocoris lineolatus* nymphs collected in alfalfa fields in Germany, sampled during 1998 - 2000.

Crop	Field code	Collection year	Emergence year	Total number of parasitoids	Percent					
					Peristenus conradi	*Peristenus digoneutis*	*Peristenus pallipes*	*Peristenus rubricollis*	*Peristenus stygicus*	*Mesochorus sp.*
Alfalfa	Air1	2000	2000	33	0	78.8	0	0	15.2	6.1
	Air 2	2000	2000	31	0	80.6	0	0	16.1	3.2
	Hu1	1998	1998	12	0	91.7	0	0	8.3	0
	Hu1	1998	1999	42	0	90.5	0	7.2	2.4	0
	Hu1	1999	1999	8	0	62.5	0	0	25.0	12.5
	Hu1	1999	2000	33	0	97.0	0	0	0	3.4
	Hu2	1998	1999	7	0	0	0	85.7	14.3	0
	Hu3	1998	1998	36	0	77.8	0	0	5.6	16.7
	Hu3	1998	1999	197	0	83.8	0	15.2	1.0	0
	Hu3	1999	1999	2	0	100.0	0	0	0	0
	Hu3	1999	2000	1	0	0	0	100.0	0	0
	Ma5	1998	1998	42	0	64.3	0	0	16.7	19.0
	Ma5	1998	1999	218	0.5	87.2	0.5	1.8	10.1	0
	Nu1	1999	1999	7	0	57.1	0	0	28.6	14.3
	Nu1	1999	2000	4	0	100.0	0	0	0	0
	Nu1	2000	2000	21	0	66.7	0	0	23.8	4.8
	St1	1998	1998	6	0	50.0	0	0	16.7	33.3
	St1	1998	1999	74	0	41.9	37.8	14.9	5.4	0
	St1	1999	2000	8	0	62.5	12.5	25.0	0	0
	St1	2000	2000	83	0	15.7	0	0	41.0	43.4

Mustard

Four parasitoid species were reared from nymphs collected in mustard: *P. digoneutis*, *P. stygicus* Loan, *Mesochorus* spp. and a new species of *Leiophron*. The new species of *Leiophron* was reared only from nymphs collected in mustard fields sampled in 1999. In general, during the season when cocoons were formed, many *P. stygicus* emerged, and this was the dominant parasitoid that season. The year following cocoon formation *P. digoneutis* was the active, dominant parasitoid.

Mixed clover

This crop was sampled only in 2000. Three species of parasitoids were reared from nymphs collected in mixed clover fields: *P. digoneutis*, *P. stygicus*, and *Mesochorus* spp. In 2000, *P. digoneutis* and *Mesochorus* spp. emerged in similar proportions. *Peristenus stygicus* also emerged in the year of cocoon formation.

Red clover

Four species of parasitoids were reared from red clover: *P. digoneutis*, *P. stygicus*, *Peristenus rubricollis* (Thomson) and *Mesochorus* spp. Most often *P. digoneutis* was the dominant parasitoid in red clover, in both the year of cocoon formation and the following year. *Peristenus stygicus* was the second most commonly reared parasitoid. During the year cocoons were formed *Mesochorus* spp. emerged in fairly high proportions. *Peristenus rubricollis* made up a small portion of the parasitoids attacking plant bug nymphs in red clover.

Alfalfa

Six parasitoid species were reared from nymphs collected in alfalfa fields: *P. digoneutis*, *P. stygicus*, *P. rubricollis*, *Peristenus conradi* Marsh, *Peristenus pallipes* (Curtis) and *Mesochorus* spp. In alfalfa, *P. digoneutis* was the dominant parasitoid species in both the year of cocoon formation and the following season. The next most common parasitoid was *P. stygicus*. *Peristenus rubricollis* and *Mesochorus* were never present together as adults in the same season, because of their temporal patterns. *Mesochorus* emerged the year that cocoons were formed and *P. rubricollis* underwent an obligate diapause, emerging the spring following cocoon formation. *Peristenus pallipes* was reared from only two fields, and only one specimen of *P. conradi* was reared.

4.5 Discussion

4.5.1 Abundance of plant bugs in various crops and seasons

Summary statistics (see Appendices 2 & 3) were calculated on a seasonal basis for each field sampled to give a general impression of the sampling effort made in each field, as well as to indicate how abundant the plant bugs and parasitoids were in each field. Neither asparagus nor barley were sampled extensively, and more effort is needed to sample in these crops to determine the pest abundance and parasitoid species attacking hosts in them. The rape fields were sampled fairly frequently; however, there were not many pest nymphs and no parasitoids collected in them. It should be noted that these fields were only sampled in 1999. This was the poorest year in terms of plant bug and parasitoid

abundance, and it is suspected that the low numbers collected in the rape fields could have been due to environmental conditions that year. Varis (1995) reported that development of *Lygus* spp. nymphs in Finland in July was highly dependent on temperature. Also, the fields of rape sampled were of winter rape. Rape is planted twice a year in Europe so there is a winter crop and a summer crop. It would be useful to sample in more rape fields to determine if the numbers of plant bugs and parasitoids collected in this study are actually representative, or if they are merely the result of poor environmental or other conditions affecting the abundance of the hosts and parasitoids. Braun et al. (2001) found that adults and nymphs of *Lygus* spp. were lower in abundance in canola and mustard fields as compared to alfalfa fields in Saskatchewan, Canada. In the other crops sampled, mustard, mixed clover, red clover and alfalfa, hosts and parasitoids were regularly present. In Poland, Bilewicz-Pawińska (1982) found *Lygus* spp. parasitized in alfalfa and in cereal crops such as; barley, oats, rye, and wheat.

4.5.2 Abundance and parasitism in selected fields

The *Lygus* spp. and *A. lineolatus* sampled in Germany appear to have at least two complete generations, and they may have a third generation when conditions are favorable. Bilewicz-Pawińska (1977a) found two generations of *Lygus* spp. and *A. lineolatus* in Poland, and Varis (1995) found that *Lygus* spp. in Finland had one generation. Abundance of *Lygus* spp. nymphs was much greater in the second generation than the first. Bilewicz-Pawińska (1982) found

a similar pattern in *Lygus* spp. in Poland. The abundance of *Lygus* spp. was quite variable from year to year, and Varis (1995) reports similar variability for species in Finland. The number of *A. lineolatus* nymphs did not fluctuate to the same degree as did the *Lygus* spp. nymphs. The number of parasitoid species attacking *Lygus* spp.(5 species) and *A. lineolatus* (7 species) is similar, however, parasitism by larval endoparasitoids was usually lower on *A. lineolatus* (total % parasitism less than 9%, Table 3.7). Bilewicz-Pawińska (1977b) reported similar results in percent parasitism between *Lygus* spp. and *A. lineolatus*. Parasitism by larval endoparasitoids was probably not regulating populations of *A. lineolatus* and some other factor may be responsible, possibly competition with *Lygus* spp. or parasitism of a different life stage.

Total weekly percent parasitism seemed to follow a trend related to the nymphal population levels. The highest rate of parasitism occurred just prior to or at peaks of nymphal abundance. Bilewicz-Pawińska (1977a & b) reported similar trends in seasonal parasitism in Poland. In the first generation of parasitoids present in May, parasitism dropped around 9 June before rising again (Figure 4.2 and 4.9). This marked the end of the first generation of parasitoids and the beginning of the second generation. It is possible that a third generation followed the second one, for *P. stygicus* and for *P. digoneutis*. The growth of the parasitoid populations was closely related to increases in numbers of plant bug nymphs.

Parasitism was calculated for several different time periods, seasonally,

monthly and weekly. Seasonal levels for asparagus and rape were 0%, for barley 23.3% to 56.8% (based on a very small sample size and probably not representative), mustard, 11.5% to 15.1%, mixed clover 27.7% to 42.8%, red clover, 12.7% to 36% and for alfalfa, 3% to 24.8%. Monthly totals fluctuated among the crops (excluding asparagus); May 0% to 20%, June 0% to 27.5% (excluding 53.8% in barley because of small sample size), July 0% to 49.6%, August 2.6% to 48.8% and September 0% to 17.4%. It was normal for the total weekly percent parasitism to range from 0% to 30%, and in the year 2000 it commonly reached up to 40% and sometimes even up to 80%.

The ecology of plant bugs and their parasitoids in cereal crops in Poland was studied by Bilewicz-Pawińska (1976, 1977a & b, 1982). She reported on pest status and population dynamics. Bilewicz-Pawińsk (1982) found that 13% of *L. rugulipennis* were parasitized by *P. digoneutis*, *P. rubricollis* and *P. stygicus* in rye, in June-July, 49% were parasitized in wheat in June-July, 23% were parasitized in oats in July, and 13% were parasitized in barley in June-July-August. The results from this study do not greatly differ from Bilewicz-Pawińska's study, although the crops sampled are quite different. It can be concluded that *Peristenus* spp. are present in many different agroecosystems in Europe.

4.5.3 Temporal evaluation of *Lygus* spp. nymphs

The instar data available for nymphs illustrate that the second generation of *Lygus* spp. is much larger than the first generation. These results are

consistent with the findings of Bilewicz-Pawińska (1977a & b). There is a trend for peaks in percent parasitism to correspond to peaks of the second and third instar *Lygus* spp. nymphs. This is not surprising because these are the stages of nymphs which are attacked. However, mean percent parasitism as determined by dissection of *Lygus* spp. nymphs was almost equal for 3rd, 4th and 5th instars, and mean percent parasitism in *A. lineolatus* nymphs was highest in the 4th instars, then 3rds and 5ths (Tables 3.6, 3.7). In Poland, Bilewicz-Pawińska (1977 a) found peaks in % parasitism of *L. rugulipennis* nymphs in rye corresponded to peaks in 5th instars, and she found parasitoids in 4th and 5th instars and newly emerged adults. In potatoes she found that 2nd, 3rd, 4th, and 5th instars were parasitized, and parasitism was greatest in the 5th instars in late August. In alfalfa in 1973, she found that 2nd, 3rd, 4th and 5th instars were parasitized and that % parasitism peaked in mid August and was highest in the 4th and 5th instars. In alfalfa in 1974 she found that parasitism peaked first in late June and again in late August. The first peak was associated with a peak in 2nd instar nymphs, which also had the highest percent parasitism and that during the peak in August parasitism was highest in the 4th and 5th instars. There is some conflict to which nymphal stage is more heavily parasitized. As noted in Chapter 3, some of the parasitoid stages are missed in dissections, particularly the eggs. The results reported above from Bilewicz-Pawińska (1977a) are based on dissections. The nymphs collected in this study were reared to obtain parasitoid cocoons, therefore the egg stage should not have been missed. During peaks in the population of nymphs, there was a greater proportion of 2nd and 3rd instars

present when parasitism levels were greatest. It is hypothesized that the 2nd and 3rd instars have a greater proportion of parasitoids present in them than can be detected by dissection.

4.5.4 Spatial distribution

The spatial distribution of nymphs and parasitoids in the fields was of interest to determine if there were possible edge effects. Some fields that were sampled were much smaller than others, and this could have an effect on the distribution of either host or parasitoid, or both, in the field. To remove possible effects of size, the fields that were selected for analysis were categorized by size before the analysis was performed. No trends in spatial distribution were found for either *Lygus* spp. and *A. lineolatus*, adults or nymphs, parasitoid cocoons, or percent parasitism. It is possible that fields sampled in this study were too small to exhibit any edge effects. But the results are consistent with the finding of Wise and Lamb (1998b), they found no differences in densities of *Lygus* spp. from the edge to the center of commercial canola fields. From the results presented here it can be concluded that in alfalfa fields of similar sizes, both plant bugs and parasitoids are effective in penetrating to the center of the field.

4.5.5 Parasitoid species composition

Three parasitoid species were reared commonly from nymphs collected in mustard, mixed clover, red clover and alfalfa fields; *P. digoneutis*, *P. stygicus* and the hyperparasitoid *Mesochorus* spp. Bilewicz-Pawińska (1976, 1977a & b, 1982) reported that *P. digoneutis*, *P. stygicus*, *P. rubricollis* and *Mesochorus*

were commonly reared from L. rugulipennis nymphs in Poland. There were more parasitoid species reared from the alfalfa crop than from the other crops studied; *P. conradi*, *P. digoneutis*, *P. pallipes*, *P. rubricollis*, *P. stygicus*, and *Mesochorus*. There were also many more fields of alfalfa sampled compared to other crops. It is possible that more of the rare parasitoid species happened to be collected in this crop because of the volume of collections made in it. Another possible explanation is that the nature of alfalfa is more suitable for a greater number of parasitoid species than are the other crops, in particular because of its stable, perennial nature (Bilewicz-Pawińska 1977a). For example, alfalfa blooms throughout the growing season and may provide nectar for foraging adults throughout the growing season which means that both early season and late season parasitoids could utilize the crop, it is also a suitable overwintering habitat for many insect species. Also because of its stable, perennial nature, it is a habitat for many different insect species, including reservoir populations of potential hosts, such as plant bug nymphs.

The dominant parasitoid reared in this study was; *P. digoneutis*, however *P. stygicus* and *P. rubricollis* were also fairly common. Bilewicz-Pawińska (1976, 1977a) also found *P. digoneutis* to be a dominant parasitoid and the two other species common in agroecosytems in Poland. These species all belong to the same guild; they are larval endoparasitoids of plant bugs, and in particular they attack *Lygus* spp. and *A. lineolatus*. Because of this, there is potential for competition among the species for hosts. It would appear that there is some

niche partitioning which occurs among these species, as they have different numbers of generations (see Chapter 5), which separates them to some degree temporally. For example, *P. rubricollis* is an early season, univoltine species (Bilewicz-Pawińska 1982). There may also be some slight differences in the emergence periods of adults, which will also help to alleviate competition for hosts and food resources. *Peristenus digoneutis*, being the most common species, may have some adaptations which allow it to dominate this guild. Some possibilities are that it may be a better competitor in the larval stage and out-compete other parasitoid larvae inside a host nymph, it may be more efficient at locating hosts or other resources in various habitats or it could have a greater fecundity.

4.6 Conclusions

The number of plant bug nymphs was greatest during their second generation in August. Parasitism tended to increase as host nymph populations increased. A sharp decline in parasitism was noted in the month of May, probably indicating the end of the first generation of larval endoparasitoids attacking plant bug nymphs. Parasitism was highest during the month of July. Parasitism fluctuated due to seasonal conditions. For example, 1999 was a very wet, cold year and the *Lygus* spp. were not abundant and neither were their parasitoids. The dominant parasitoid found was *P. digoneutis*. This species was present in many different crops, including an annual cereal (barley), and annual oilseed brassica (mustard), and perennial forage and seed crops (mixed clover,

red clover and alfalfa).

4.7 Acknowledgments

Several people helped with the collections and rearing of plant bugs and parasitoids for this study; S. Either, H. Goulet, D. Higginson, E. Hunt, S. Lachance, J. Otani and J. Rahn. Dr. Henri Goulet was instrumental in this project by providing identifications of almost all the reared European *Peristenus* adults. Funding for this project was provided by AAFC and ARDI.

Chapter 5 Laboratory emergence patterns of field collected euphorine parasitoids, including several European species of *Peristenus* Foerster (Hymenoptera: Braconidae), a new species of European *Leiophron* Nees and the ichneumonid hyperparasitoid, *Mesochorus* sp.

5.1 Abstract

From 1998 to 2000 cocoons were reared from field collected plant bug nymphs (mostly *Lygus rugulipennis* Poppius and *Adelphocoris lineolatus* (Goeze)) collected in Germany and Switzerland. To obtain parasitoids, the nymphs were reared in the laboratory under natural light supplemented by a 16L:8D artificial light regime. Parasitoid cocoons were monitored for adult emergence during the year that cocoons were formed and any cocoons from which wasps failed to emerge were overwintered and monitored the following year for emergence. Adult emergence was recorded and parasitoid adults were killed in 70% alcohol, prepared by the critical point drying technique, sexed and identified. The following species were collected in this study: *Peristenus adelphocoridis* Loan, *Peristenus conradi* Marsh, *Peristenus digoneutis* Loan, *Peristenus pallipes* (Curtis), *Peristenus rubricollis* (Thomson), *Peristenus stygicus* Loan, two new species of *Peristenus*, one new species of *Leiophron*, and the hyperparasitoid, *Mesochorus* species. The emergence period of each species is given and the proportion of emerging and diapausing individuals is graphed for most of the species reared in this study. Significantly more cocoons of *P. digoneutis* entered diapause than emerged in the year of cocoon formation.

The number of cocoons which produced an adult parasitoid in the year of cocoon formation was not significantly different compared to the number entering diapause for *P. stygicus*. All cocoons of *P. rubricollis* entered diapause and overwintered.

5.2 Introduction

Several species of Palaearctic *Peristenus* (Foerster) have been released as biological control agents in North America against native pest *Lygus* species and the introduced *Adelphocoris lineolatus* (Goeze). The species are, *Peristenus adelphocoridis* Loan, *Peristenus conradi* Marsh, *Peristenus digoneutis* Loan, *Peristenus rubricollis* (Thomson) and *Peristenus stygicus* Loan. There is much variation among these species of parasitoids in terms of the number of generations they can produce in a season, and each species may be best adapted for control programs in different regions of North America. For example, *P. digoneutis* is bivoltine (Bilewicz-Pawińska 1974), *P. rubricollis* is univoltine (Bilewicz-Pawińska 1974) and *P. stygicus* is multivoltine (Drea et al. 1973). Biological control of pest species in North America is still insufficient and further introductions of these and possibly other species are likely. In order to select the most appropriate agent for a biological control program, the biology and phenology of the target pest need to be considered, as well as gaps in existing parasitoid guilds. Based on biological information, the most appropriate species for the target pest can be selected.

The objectives of this study were to document the biology and phenology

of European *Peristenus* species attacking *Lygus* pest species and *A. lineolatus* in Europe. Biological information on the life cycles and developmental periods of these European species will assist biological control workers in assessing these species' potential as biological control agents for use in North America.

5.3 Materials & Methods

Plant bug nymphs were collected in Germany and Switzerland from 1998-2000. Collections were made in agricultural crops such as *Medicago sativa* L. (alfalfa), *Trifolium pratense* L. (red clover), a mixture of *Trifolium* species (clover mixture) and *Sinapis alba* L. (mustard); in non-crop agricultural habitats such as grassy fallow fields, and weeds; and in natural habitats such as riparian vegetation and mountain meadows at high and low elevations.

A standard sized sweep net, 37.5 cm in diameter, was used to collect mirid nymphs, mostly *Lygus* spp. and *A. lineolatus.* The nymphs were collected in the field and transported back to the laboratory in rearing containers with a sprig of alfalfa or other plant material. In the laboratory the nymphs were reared on store-bought green beans and plants taken from the site of collection.

The rearing containers were made from two plastic, tapered, urine sample containers, 6 cm in diameter and 8.5 cm high, and nested one within the other. The bottom of the inner container was cut off and replaced by a piece of mesh (8x8 squares per cm^2). The outer container was filled with fine, moistened vermiculite to a depth of about 1 cm. This served as pupation medium for the emerging parasitoid larvae which, upon leaving the host, would seek a pupation

site and then spin white, spindle shaped cocoons in the moistened vermiculite. The pupation medium provided moisture for both nymphs and the parasitoid pupae in their cocoons. Small pieces of crumpled paper towel were added to the inner container to increase the structural diversity for the plant bug nymphs, and to reduce predation by providing hiding places for molting nymphs. Air circulation in the container was improved by cutting a hole in the lid, 2 cm in diameter, which was screened over with a very finely woven material.

Nymphs were kept on the laboratory bench, under natural light supplemented by a grow light which was set at a 16L:8D light regime. All rearing containers with plant bug nymphs were monitored on average every 3-5 days, when plants and beans were removed and replaced with fresh food and any emerged adult plant bugs were removed. When all the plant bug nymphs had died or completed development the outer container and the vermiculite were inspected for parasitoid cocoons. The number of cocoons was recorded and the mesh lid was replaced with a solid one. The containers which held parasitoid cocoons were monitored almost daily for emerging adult parasitoids. Any cocoons which did not produce adult parasitoids the year the cocoons were formed were kept overwinter and efforts were made during this study to provide natural field conditions for overwintering parasitoids. Cocoons were housed in a subterranean outdoor insectary. The temperature and moisture conditions in this insectary would have fluctuated naturally with seasonal and daily changes. These cocoons were brought inside the following spring, in May, the vermiculite was moistened lightly with water and then the containers were kept on the

laboratory bench and monitored for adult emergence. The date that adult parasitoids emerged was recorded and the adults were killed in 70% alcohol. Most of the samples were shipped to Dr. Henri Goulet, at the Eastern Cereal and Oilseed Research Center, in Ottawa, Canada, where they were prepared by critical point drying, then sexed and identified. Some samples were prepared and identified by the author.

5.4 Results

5.4.1 Parasitoid species collected

Six described species of *Peristenus*; *P. adelphocoridis*, *P. conradi*, *P. digoneutis*, *P. pallipes*, *P. rubricollis* and *P. stygicus* emerged from the cocoons reared from plant bug nymphs. Three undescribed euphorine species were also reared from the plant bugs collected. This included two new species of *Peristenus* and one new species in the closely related genus *Leiophron* (Nees). The numbers of each species reared each year and the emergence period for these species are given in Table 5.1. Data from adult parasitoids which emerged during the 2001 field season, from cocoons collected in 2000 are not presented here. An ichneumonid hyperparasitoid, *Mesochorus* species, was reared from cocoons obtained from rearing plant bug nymphs collected from 1998 to 2000. The number of *Mesochorus* spp. individuals which emerged each year and their emergence periods are given in Table 5.2.

Table 5.1 The number of male and female euphorine parasitoids which emerged from cocoons reared from plant bugs collected in Germany and Switzerland from 1998 - 2000, and the emergence periods for adults of the euphorine species collected. Note that data from cocoons overwintered in 2000 are not presented here.

Species	Year collected	Year emerged	#'s of Males	#'s of Females	Total #	Start of emergence	End of emergence
Peristenus adelphocoridis	1999	2000	2	1	3	9.V.200	26.VI.200
Peristenus conradi	1998	1999	0	1	1	18.V.1999	18.V.1999
Peristenus digoneutis	1998	1998	60	50	110	17.VIII.1998	12.X. 1998
	1998	1999	253	328	581	12.V.1999	1.VIII.1999
	1999	1999	29	15	44	20.V.1999	1.X.1999
	1999	2000	152	125	277	3.V.2000	14.VI.2000
	2000	2000	147	117	264	12.VI.2000	9.X.2000
Peristenus pallipes	1998	1999	5	7	12	27.V.1999	21.VI.1999
	1999	2000	7	8	15	7.V.2000	13.VIII.2000
Peristenus rubricollis	1998	1999	40	38	78	19.V.1999	28.VI.1999
	1999	2000	2	4	6	19.V.2000	2.VI.2000
Peristenus stygicus	1998	1998	6	14	20	3.IX.1998	10.X.1998
	1998	1999	21	17	38	12.V.1999	19.V.1999
	1999	1999	12	20	32	6.VIII.1999	19.IX.1999
	1999	2000	6	11	17	5.V.2000	19.VI.2000
	2000	2000	62	87	149	11.VI.2000	9.X.2000
Peristenus n.sp. near P.digoneutis	1999	2000	0	1	1	5.V.2000	5.V.2000
Peristenus n.sp. near P.pallipes	1999	2000	12	20	32	3.V.2000	19.V.2000
Leiophron n.sp. near Leiophron	1999	2000	1	5	6	2.V.2000	29.VI.2000

Table 5.2 The number of individuals of *Mesochorus* sp., an ichneumonid hyperparasitoid of euphorine braconid wasps, which emerged from cocoons reared from plant bugs collected in Germany and Switzerland from 1998-2000, and the emergence periods for adults of *Mesochorus* sp. Note that data from cocoons overwintered in 2000 are not presented here.

Year collected	Year emerged	# of Males	# of Females	Total #	Start of emergence	End of emergence
1998	1998	-	-	39	21.VIII.1998	30.IX.1998
1998	1999	-	-	1	27.V.1999	27.V.1999
1999	1999	-	-	11	7.VIII.1999	19.IX.1999
1999	2000	-	-	6	11.V.2000	17.V.2000
2000	2000	226	90	316	19.VIII.2000	9.X.2000

5.4.2 Parasitoid emergence

Peristenus adelphocoridis

Few individuals were collected, in total three, and all were collected in 1999. All emerged in 2000 from overwintered cocoons. The emergence period was from 9 May to 26 June, 2000.

Peristenus conradi

Only one specimen of *P. conradi* was collected during this three year study. It was collected in 1998 and emerged from its overwintered cocoon on 18 May, 1999.

Peristenus digoneutis

Peristenus digoneutis was the most commonly reared parasitoid. The emergence patterns of male and female parasitoids collected in 1998 and 1999 are shown in Figure 5.1 and that of 2000 is shown in Figure 5.2. During the season that cocoons were formed, adults of *P. digoneutis* had a peak of emergence in August, which continued into mid September, with emergence sometimes occurring as late as October. In 2000, there was a small emergence period in June and a larger period in August. However, most of the cocoons of *P. digoneutis* entered diapause and failed to emerge in the field season of their collection. Males were protandrous, usually emerging before the females, and in greater numbers. This is difficult to determine from the figures because they present data averaged over 5 day periods and also averaged over more than

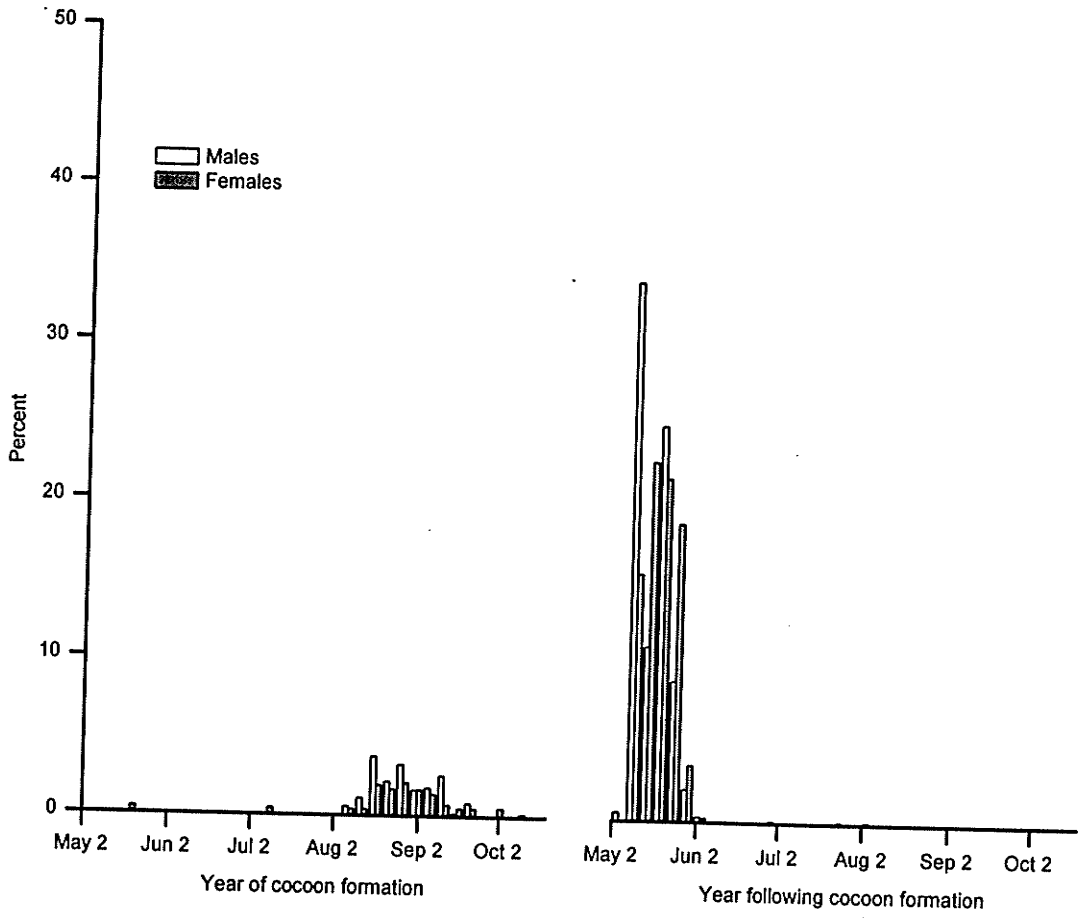

Figure 5.1 Proportion of *Peristenus digoneutis* adults to emerge in five day intervals averaged between two field seasons, from 990 cocoons reared from plant bug nymphs collected in Germany and Switzerland in 1998 and 1999.

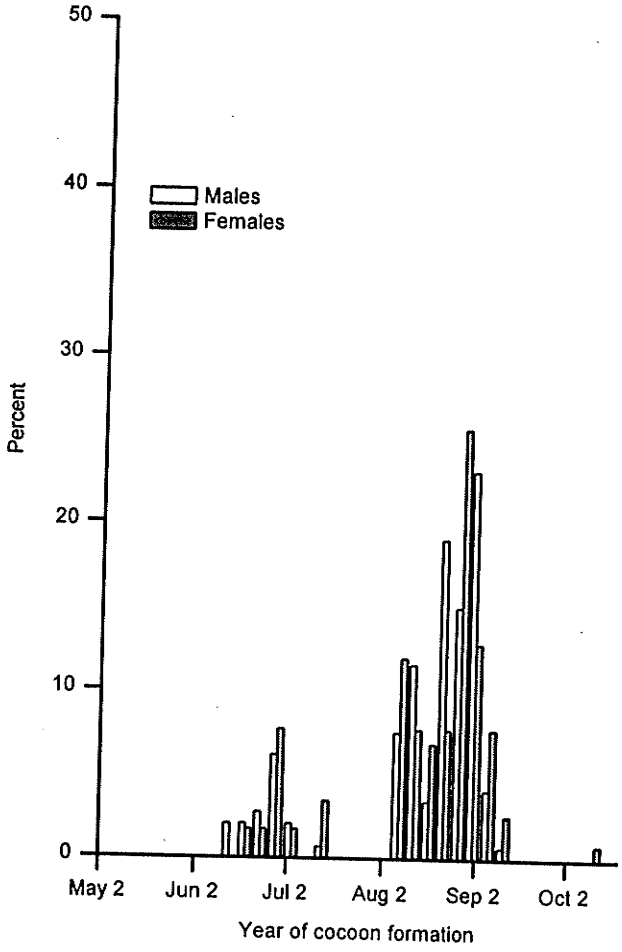

Figure 5.2 Proportion of *Peristenus digoneutis* adults to emerge in five day intervals in 2000, from cocoons reared from plant bug nymphs collected in Germany and Switzerland in 2000. A total of 264 adult *P. digoneutis* emerged from cocoons collected in 2000.

one season. Emergence of overwintered cocoons began in May and continued until August. Most of the overwintered adults emerged in May. The proportion of cocoons entering diapause was 85%, with only 15% emerging in the year of collection (Table 5.3). Chi-square tests were made to determine if the difference in the numbers entering diapause or emerging in the year of collection were different and also to determine if there were differences between the 1998 and 1999 sampling years (as only the 1998 and 1999 data sets are complete). In both years, the number entering diapause is significantly different from and higher than the number emerging in the year of cocoon formation.

Peristenus pallipes

The number of *P. pallipes* reared in 1999 and 2000 was low, 12 and 15 individuals, respectively. None emerged in the year cocoons were formed. Their emergence period was from May to mid July in the year after overwintering (Figure 5.3). There appear to be two periods of emergence, an early one in May and another from mid June to July, although the small sample size, and the combination of two years of data mask this to some degree.

Peristenus rubricollis

Peristenus rubricollis was more common in the 1998 collections, than in the 1999 ones, with 78 individuals in the former and 6 individuals in the latter. In 1999 and 2000, *P. rubricollis* started emerging in late May in the year following cocoon formation and the emergence period lasted for one and a half months (Figure 5.4).

Table 5.3 The proportion of individuals of *Peristenus digoneutis*, *Peristenus stygicus* and *Mesochorus* sp. which were reared from cocoons collected from plant bug nymphs collected in Germany and Switzerland, that either entered diapause or emerged in the year of cocoon collection, the chi-square value for the number entering diapause versus the number emerging, and the chi-square value that indicates if the number entering diapause or emerging is different between 1998 and 1999.

Species	Proportion entering diapause	Proportion emerging in the year of collection	Chi^2 square for # entering diapause versus # emerging	Significance	Chi^2 square # entering diapause or emerging is different between years	Significance
P. digoneutis	85%	15%	539.77	< 0.0001	0.84	Not significant
P. stygicus	52%	48%	0.08	Not significant	10.26	< 0.0025
Mesochorus sp.	12.5%	87.5%	35.42	< 0.0001	10.82	< 0.001

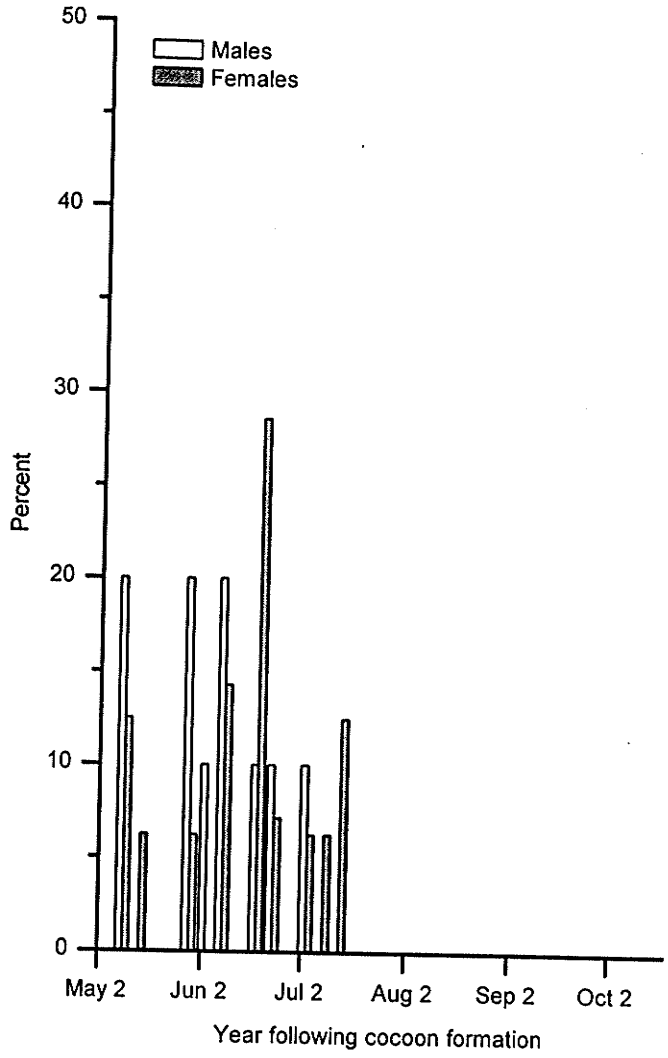

Figure 5.3 Proportion of *Peristenus pallipes* adults to emerge in five day intervals averaged between two field seasons, from 25 cocoons reared from plant bug nymphs collected in Germany and Switzerland in 1998 and 1999.

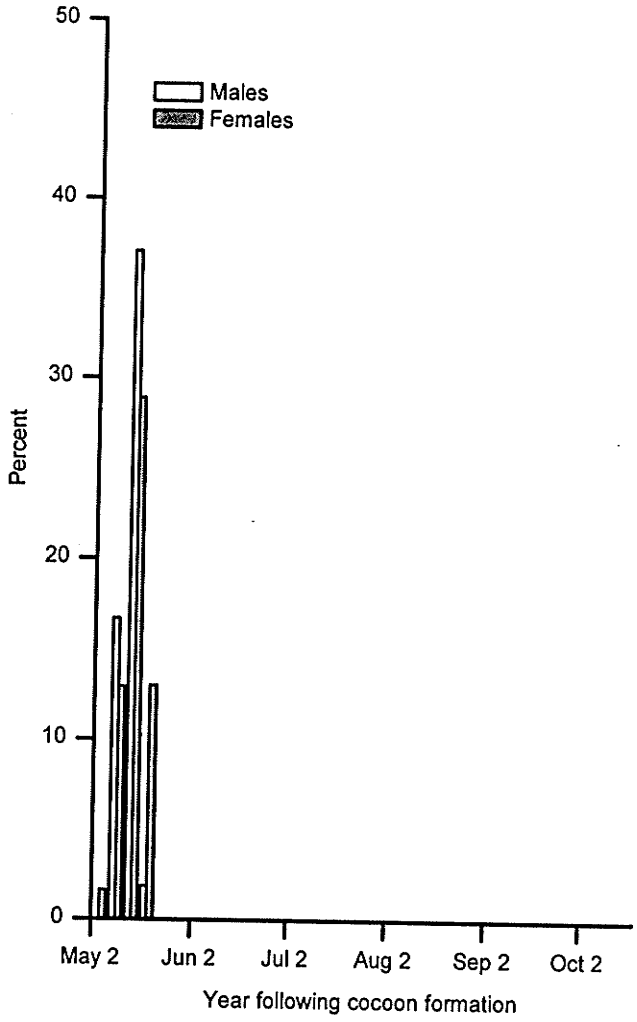

Figure 5.4 Proportion of *Peristenus rubricollis* adults to emerge in five day intervals averaged between two field seasons, from 62 cocoons reared from plant bug nymphs collected in Germany and Switzerland in 1998 and 1999.

Peristenus stygicus

During the season that cocoons were formed, adult emergence peaked in late August early September and sometimes continued until October (Figure 5.5). Of the cocoons collected in 2000, adults began to emerge in June and two peaks in emergence were observed, one in June and another in August (Figure 5.6). Cocoons from 1999 collections which entered diapause and overwintered, emerged within a one week period in early May 2000.

The average proportion of *P. stygicus* to enter diapause was 52%, and 48% emerged the year that cocoons were formed (Table 5.3). However, the actual proportion to enter either category was different between the 1998 and 1999 field seasons: 65% and 30% diapaused respectively.

***Peristenus* n. spp. and *Leiophron* n. sp.**

One specimen of *Peristenus* n. sp. near *P. digoneutis* was reared from a nymph collected in 1999. This individual entered diapause, overwintered and emerged 5 May, 2000, the year following collection. *Peristenus* n. sp. near *P. pallipes*, was reared from cocoons collected in 1999. In total, 32 specimens of this new species emerged in 2000. They emerged from early May until late May in the year following cocoon formation (Figure 5.7). *Leiophron* n.sp. was reared from cocoons collected in 1999. A total of six cocoons formed in 1999 gave rise to parasitoids that entered diapause and emerged the next year, from early May to late June in 2000.

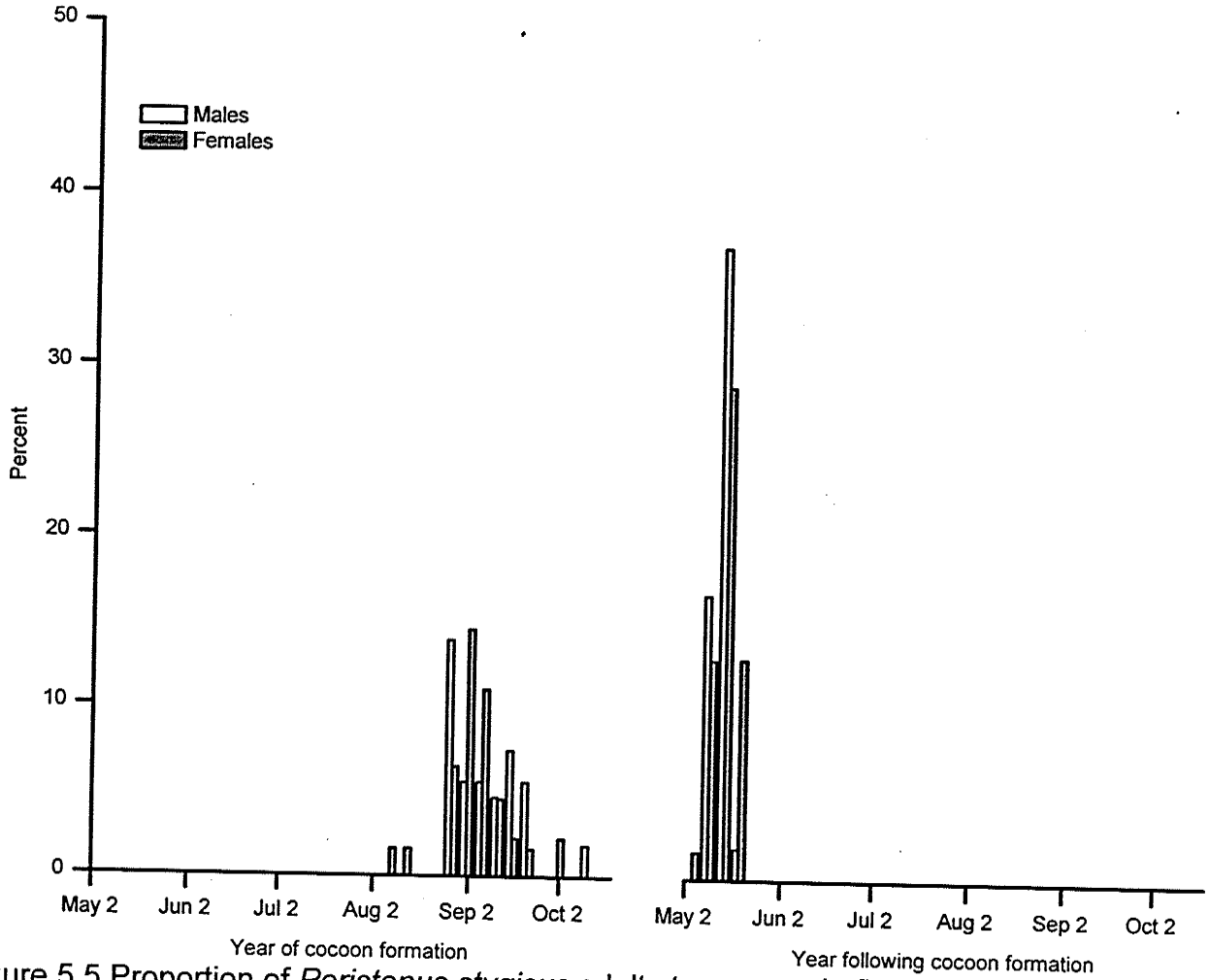

Figure 5.5 Proportion of *Peristenus stygicus* adults to emerge in five day intervals averaged between two field seasons, from 105 cocoons reared from plant bug nymphs collected in Germany and Switzerland in 1998 and 1999.

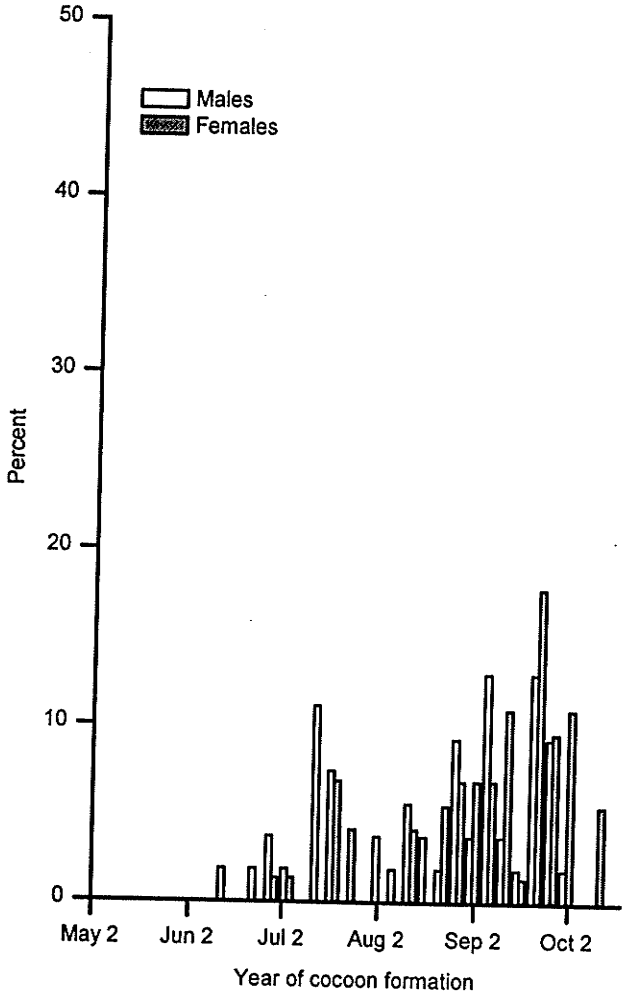

Figure 5.6 Proportion of *Peristenus stygicus* adults to emerge in five day intervals in 2000, from cocoons reared from plant bug nymphs collected in Germany and Switzerland in 2000. A total of 146 *P. stygicus* adults emerged in 2000 from cocoons collected in 2000.

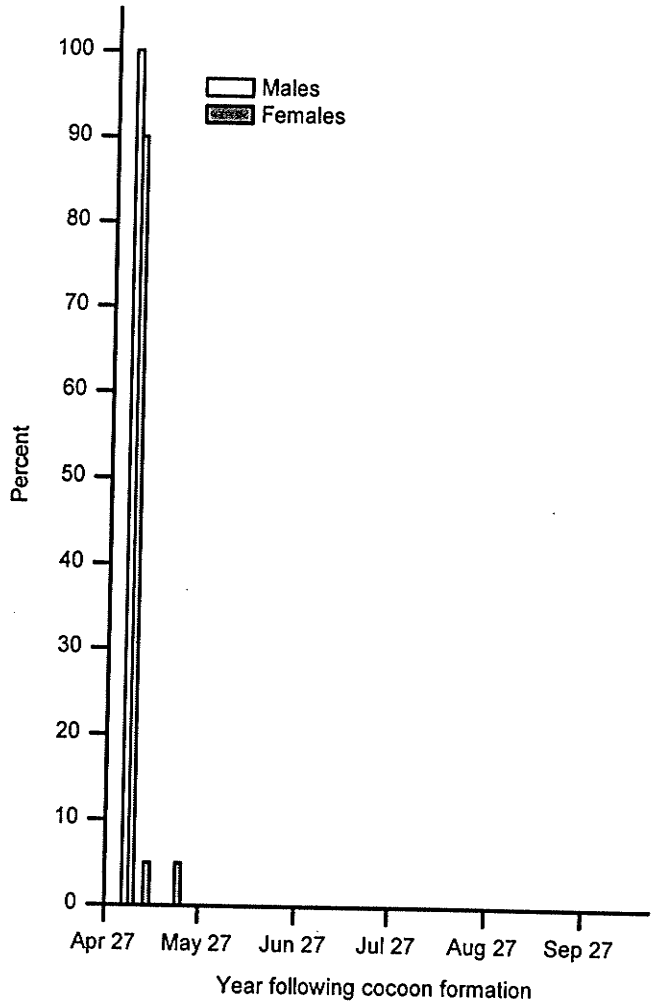

Figure 5.7 Proportion of adults of *Peristenus* n. sp. near *P. pallipes*, to emerge in five day intervals, from 32 cocoons reared from plant bug nymphs collected in Germany and Switzerland, in 1999.

***Mesochorus* sp.**

The number of *Mesochorus* spp. collected each year was variable. The number of adults emerging appears to be greater in the year of cocoon formation, with fewer *Mesochorus* individuals entering diapause. The number of *Mesochorus* collected each year is shown in Table 5.2. In the years cocoons were formed, emergence began in August, peaked in mid September and continued until early October. In the year following cocoon formation, adults emerged from mid to late May (Table 5.2, Figure 5.8). The adults which emerged from cocoons collected in 1998 and 1999 were not sexed so data are for both sexes combined.

The proportion of *Mesochorus* entering diapause the year cocoons were formed was 12.5%, significantly lower than the proportion emerging in the year of cocoon formation which was 87.5% (Table 5.3). The numbers entering diapause or emerging in the year of cocoon formation was also significantly different between 1998 and 1999; however, the analysis is based on a small sample size in 1999.

5.5 Discussion

5.5.1 Laboratory rearing conditions

This study was not conducted in environmental chambers, but on the laboratory bench. Therefore many factors were not controlled. The nymphs and parasitoid cocoons were subjected to the fluctuation in temperatures of the laboratory (20-23°C) which was not air conditioned. Although the nymphs were

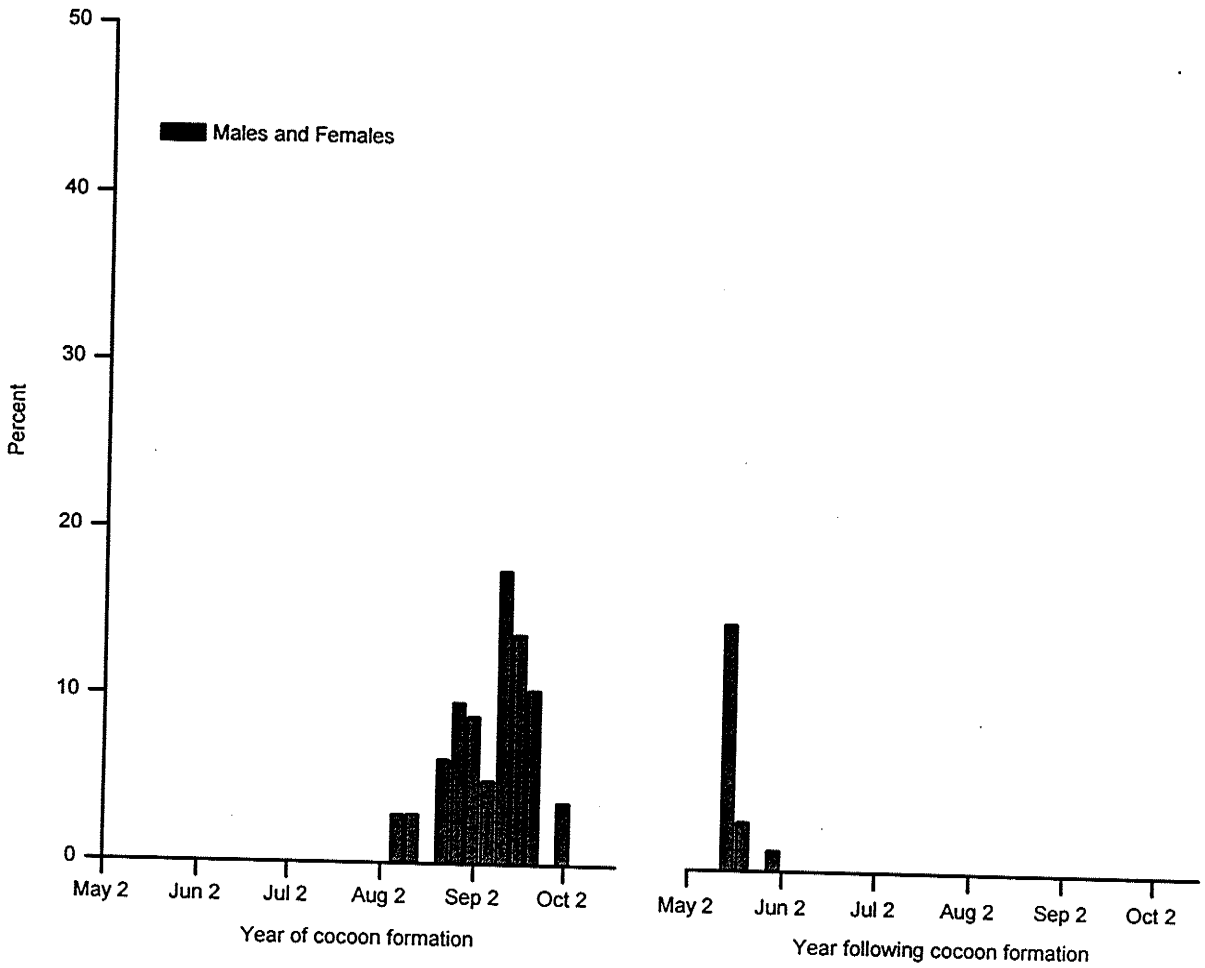

Figure 5.8 Proportion of *Mesochorus* sp. adults to emerge in five day intervals averaged between two field seasons, from 57 cocoons reared from plant bug nymphs collected in Germany and Switzerland in 1998 and 1999.

reared under a 16L:8D photoperiod they were also subject to natural light levels and light regimes because of the windows in the laboratory. Obviously these conditions do not replicate field conditions.

It is thought that the conditions to which nymphs and parasitoid cocoons were subjected may have affected the timing of their emergence periods but not the patterns in which they emerged. Van Steenwyk & Stern (1976) found that the day length to which the host nymphs were exposed would determine if *P. stygicus* went into diapause. In their studies, almost all would enter diapause if they were reared from *Lygus* nymphs exposed to 12-12.75 h light. They found that an experimental photoperiod of 13.5 h resulted in an intermediate incidence of diapause and that the incidence of diapause was very low at 14 h light. Whistlecraft (1997) induced diapause in *P. digoneutis* and *P. stygicus* by rearing parasitized *Lygus* nymphs at 24°C with an 11 hour photophase. Broadbent (1997) was able to induce diapause in *P. stygicus* if the parasitized host nymph was exposed to short day light conditions (11 hours light) before day six after oviposition by the parasitoid. He found that if the host nymph was exposed to short day conditions only after day six after oviposition, diapause was not induced and the parasitoid emerged as if exposed to long day light conditions. It appears that only a small window of time is available in which to alter the diapause condition of *P. digoneutis* and *P. stygicus*.

All of the nymphs collected in this study were exposed to natural light conditions in the field prior to their collection. It is possible that some nymphs

exposed to short day conditions were collected before day six after oviposition. In these cases, the nymphs reared in the laboratory were exposed to long day conditions, so this may have altered their diapause condition, and they may have emerged. This could account for some of the very late season emergence (late September into October) of *P. digoneutis*, and *P. stygicus*. If nymphs collected were exposed to short day conditions in the field during the first six days after oviposition then it is expected that their diapause condition would have been determined prior to collection and laboratory rearing. Therefore they would enter diapause in the laboratory, even after being subjected to long day light conditions in the laboratory. Based on the small window of time in which the diapause condition could be altered by laboratory rearing it is thought that the number of parasitoids entering diapause should underestimate diapause incidence of those individuals collected later in the season.

It is unknown if those parasitoids which emerged in late September and early October could survive, reproduce and complete a third generation. Because of the long field season in Germany and Switzerland, it might be possible for these individuals to complete another generation in plant bugs. However, this is only speculation and until data are collected up to the first frost, the success of these individuals is unknown. The other possibility is that they cannot complete another generation and they are lost from the population. It is also possible that some of these late season emergences were due to exposure in the laboratory, to artificially higher temperatures, which may have affected parasitoid development. It may also be possible that some of the parasitoids

overwinter in adult *Lygus* spp. This has not yet be determined in the field or laboratory.

Lim & Stewart (1976b) found that a diurnally fluctuating temperature regime resulted in greater survival of diapausing cocoons. Broadbent and Whistlecraft (Agriculture and Agri-Food Canada, London, Ontario, pers. comm.) indicated that the emergence of overwintered *Peristenus* cocoons is better in treatments where the temperature in environmental chambers is allowed to fluctuate. Although the insectary conditions are not precisely field conditions, they apparently provided adequate temperature and moisture conditions for the overwintering parasitoids, since emergence success was high, 88% of cocoons overwintered in 2000 (U. Kuhlmann, CabiBioscience, Delèmont, Switzerland, pers. comm.).

The emergence dates of overwintered cocoons may not reflect the true emergence dates because of the rearing conditions. Each spring, overwintered cocoons were removed from the insectary on 1 May and adult emergence started within 2 weeks. Bilewicz-Pawińska (1974) and Hormchan (1977) found that when *Peristenus* cocoons were held at low temperatures for a period sufficient to break diapause and then brought up to higher temperatures the parasitoids would emerge within 2-28 days. A field experiment to monitor emergence under field conditions would provide a much better estimate of the field emergence dates.

5.5.2 Emergence patterns

Biological information currently known for most of the species reported here is presented in Tables 2.1 and 2.2, including the known number of generations. The patterns of emergence are most likely similar to the patterns as they would be observed in the field. Unfortunately there were not enough specimens collected each year, for most of the parasitoid species, to determine their pattern of emergence with confidence. However some general observations can be made.

Univoltine species

Three *P. adelphocoridis* were collected in 1998. They entered diapause and emerged the following May and June. These records are similar to the emergence records for *P. adelphocoridis* reported by Loan (1979). Only one specimen of *P. conradi* was collected during this study. It also entered diapause and emerged the year following collection. Day et al. (1992), indicated that *P. conradi* is univoltine. *Peristenus pallipes* (24 individuals) and *P. rubricollis* (84 individuals) were collected in small numbers but it is felt this is enough data to confirm that they are univoltine. Brindly (1939) and Loan (1965) found that *P. pallipes* did not emerge from cocoons the year they were formed, and that they required a period of diapause before emergence and Bilewicz-Pawińska (1974) stated that *P. rubricollis* is univoltine. For *P. rubricollis* there appears to be two distinct periods of emergence in the spring following diapause, which are separated by about two weeks (Figure 5.4). It is unknown if this biological data is an indication of two cryptic species which are currently identified as *P. rubricollis.*

A detailed taxonomic study of the specimens reared may provide more information to test this hypothesis. All of the individuals of the new species of *Peristenus* and *Leiophron* overwintered in their cocoons before emerging the following spring. More data on the emergence period of the new species are needed to confirm this aspect of their biology, but the data available suggest all are univoltine, entering an obligate diapause and emerging the year following cocoon formation.

Bivoltine species

The information reported here supports the conclusion that *P. digoneutis*, *P. stygicus* and the *Mesochorus* spp. are bivoltine in the Rhine Valley of southern Germany. Adults of these species emerged from cocoons the same year that cocoons were formed, indicating at least two generations exist. Individuals also entered diapause in cocoons, overwintered and emerged the following spring. *Peristenus digoneutis* is reported by most authors to have two generations (Bilewicz-Pawińska 1974, Hormchan 1977, Carignan et al. 1995). *Peristenus stygicus* is bivoltine, and depending on geographic location could be multivoltine (Drea et al. 1979). The data collected in this study confirm that *P. stygicus* in the region of collection is at least bivoltine.

Brindley (1939) reported that *Mesochorus* spp. lay their eggs in the haemoceol of *Euphorus* spp. larvae which are inside the primary mirid host and that the larva is visible in the transparent body of the euphorine host. These hyperparasitoids inhibit the pupation of their host when it has formed its cocoon

and they then pupate inside the cocoon spun by the host (Brindly 1939). The *Mesochorus* spp. collected in this study emerged in greater numbers in the year the cocoons were formed than in the spring following diapause. In 2000, a very large number of *Mesochorus* were collected and emerged the same year.

5.6 Conclusions

The emergence periods of the parasitoid species collected in these areas of Germany and Switzerland will be useful to determine what regions of North America they may be adapted to, and for determining which regions will be suitable for their release in biological control programs. For example, a large proportion of *P. digoneutis* individuals enter diapause each year, establishing that these parasitoids will require that there are hosts available after their emergence in the spring. If the emergence period of the adult could be predicted from existing information then it would be possible to prepare in advance and augment the number of host nymphs available for newly-introduced parasitoids by releasing hosts of appropriate stages into the field.

Information on the number of generations a particular parasitoid species has is also relevant to establishing biological control agents. For example, all species studied here require a stable habitat for more than one season to complete their diapause. Based on this information, stable agricultural habitats should be selected for release sites, where there is control over the physical management of the site for consecutive seasons. The number of generations a parasitoid species has is also of critical importance when considering introducing

the parasitoid into new geographical areas, for the potential biological synchrony with the target host and for seasonal diapause requirements.

In general, the results of this study did not greatly vary from the results of other studies. More detailed studies are needed to confirm the number of generations for the species which were not collected in great numbers. The information obtained in this study will be of use to biological control practioners in Europe and North America. It provides information regarding when parasitoids are entering diapause. If diapause is of concern for laboratory studies using field collected material, field collections can be made at more appropriate times of the season. To improve the success of establishing these biological control agents, biological information must be considered to ensure the best species or strains are selected for release and the actual releases planned accordingly.

5.7 Acknowledgments

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Chapter 6 Ecological host range of selected European *Peristenus* Foerster species in Germany and Switzerland.

6.1 Abstract

The ecological host range of several European species of *Peristenus* Foerster was investigated in Germany and Switzerland from 1998-2000. The study was focused on the species considered for introduction to North America, *Peristenus digoneutis* Loan, *Peristenus rubricollis* (Thomson) and *Peristenus stygicus* Loan. These species are considered for use in biological control programs in Canada against pest *Lygus* (Hahn) species and the introduced *Adelphocoris lineolatus* (Goeze). Adult mirids and nymphs were collected in a variety of habitats including agricultural crops, non-crop agricultural habitats and natural habitats. They were reared in the laboratory to obtain parasitoid cocoons and then these were reared for adult parasitoids. *Peristenus stygicus* had the greatest host range of all the parasitoid species recovered in this study; it attacked six mirid species. *Peristenus rubricollis* had the next greatest host range; it attacked four mirid species. *Peristenus digoneutis* has the smallest host range of those species considered for introduction; it attacked three species. *Peristenus stygicus* should not be released until the potential non-target effects of this species are fully understood. More information on the host range of *P. rubricollis* is needed and any future releases of this species need to be monitored carefully for non target effects. *Peristenus digoneutis* is the safest of these species for release in Canada. It was only found to parasitize *Lygus pratensis* (L.), *Lygus rugulipennis* Poppius and *A. lineolatus* in all habitats in

which it was collected. Future releases of these and other exotic parasitoids need to be carefully planned and executed, and follow up programs need to be designed to maximize the understanding of parasitoid establishment and non-target effects.

6.2 Introduction

Biological control offers the possibility for long term pest management without the adverse effects of pesticide applications. Depending on the pest in questions, a classical biological control approach may be appropriate, or a novel biological control approach may offer a new solution to the pest problem (Pimentel 1963). Biological control today is faced with many challenges. Specifically, the risks of introducing arthropod control agents into new regions need to be weighed, and the most environmentally safe option chosen, within economic limitations. This is a difficult task and requires that all aspects of the biological control program are assessed, including the many biological factors. Among the biological issues which need to be considered when implementing a biological control program are the need to match the appropriate control agent species or strain to the particular pest species in question, the host specificity of that agent (Sands 1998), and possible non-target effects of the agent's introduction (VanDriesche & Hoddle 1997).

Non-target effects may be direct or indirect (Hopper 1998). Considering, specifically, the biological control of arthropods using parasitoids, non-target effects may include direct effects, such as parasitism of non-target species, as well as indirect effects such as competition with other natural enemies of the

target species, and/or effects on predators or hosts of any impacted non-target species. Generally, host-specific parasitoids are favored over generalist parasitoids because there are usually fewer non-target factors to consider.

Assessments of the safety of biocontrol agents, require retrospective studies on previously introduced biocontrol agents (Follett et al. 2000). Analysis of the results of previous studies, should lead to greater insight into what makes a biological control program succeed or fail, as well as a better understanding of the associated risks. Development of such an information base, will lead to better future decisions regarding biological control implementation. Biological control efforts against Nearctic pest *Lygus* species, and the Palaearctic species, *Adelphocoris lineolatus* in North America have been made since the 1970s (Broadbent et al. 2002, Craig & Loan 1984a, b, Soroka & Carl 2002) and offer the possibility for retrospective study. Also, current research opportunities have the potential to explore and test biological and ecological theories relevant to biological control, such as host range expansion, geographic dispersion and host-parasitoid population dynamics.

In North America several plant bugs species are considered serious agricultural pests, including several species of *Lygus* and *A. lineolatus* (Butts & Lamb 1990, 1991a, Craig & Loan 1984a, b,). The species of pest *Lygus* are *Lygus lineolaris* (Palisot de Beavois), *Lygus hesperus* Knight, *Lygus elisus* Van Duzee and *Lygus borealis* (Kelton). In 1917, the alfalfa plant bug, *A. lineolatus,* was accidentally introduced to North America and is now considered a pest of seed alfalfa crops (Craig & Loan 1984a). The *Lygus* and *Adelphocoris* species

feed in a similar way and are found on similar host plants, in particular alfalfa. In the 1970s Loan and Bilewicz-Pawińska (1973) identified several species of Palaearctic *Peristenus* (Hymenoptera: Braconidae) that are larval endoparasitoids of *Lygus* plant bugs and *A. lineolatus* in Europe.

This discovery was of particular interest to crop protection entomologists in North America and it led to efforts to determine if these parasitoids were able to attack and survive in Nearctic pest *Lygus* species which are not part of their natural host range. Studies by Van Steenwyk & Stern (1976,1977), and Hormchan (1977) revealed that these parasitoids could develop in Nearctic *Lygus* species. During the past several decades since these discoveries, efforts to introduce several Palaearctic *Peristenus* species into North America have been made, with the goal of suppressing Nearctic pest *Lygus* species and the parasitoids' natural host, the introduced *A. lineolatus* (Broadbent et al. 2002, Craig & Loan 1984a, b, Hormchan 1977, Soroka & Carl 2002, VanSteenwyk & Stern 1977). The species released were *Peristenus adelphocoridis* Loan, *Peristenus conradi* Marsh, *Peristenus digoneutis* Loan, *Peristenus rubricollis* (Thomson) and *Peristenus stygicus* Loan.

Two of these parasitoid species, *P. conradi* and *P. digoneutis* are now established in North America (Day et al. 1990, 1992). They first established in the northeastern United States (Day et al. 1998) and recently both were discovered in southern Canada (Broadbent et al. 1999). They are found to attack pest Nearctic *Lygus* spp. and *A. lineolatus* in alfalfa (Day 1996). Other exotic *Peristenus* species, *P. rubricollis* and *P. stygicus,* which failed to establish

in North America in the earlier releases, are currently under investigation to determine their potential for use in biocontrol programs across Canada for the control of pest *Lygus* species. They may be useful in alfalfa grown for forage and seed, where biological control agents for other alfalfa pests, including alfalfa leaf blotch miner and alfalfa weevil, are already established. They may also be useful in other crops, such as canola, or in glass house crops.

The Palaearctic species of *Peristenus* have demonstrated the ability to attack and develop in species which are not part of their usual host range, making them polyphagous parasitoids (Condit & Cate 1982, Hormchan 1977, VanSteenwyk & Stern 1976, 1977). Kuhlmann et al. (1998), reviewed the status of biological control of Nearctic *Lygus* and *A. lineolatus* in North America utilizing Palaearctic *Peristenus* species. They highlighted areas of research needed to provide the information necessary to properly assess the risks associated with introducing these parasitoids to North America. Specifically, they advised that biological control agents be accurately identified, a serious problem since the larvae are difficult to identify (Carignan et al. 1995); that confusion in the taxonomy of the species be resolved; that information on the agents origin, distribution, biology, natural enemies and impact in area of origin be studied; and that an analysis of host range expansion of the biological control agents and their possible non-target effects be carried out.

Day (1999) studied the ecological host range of the established *P. digoneutis* in alfalfa crops in northeastern US. Even though *P. digoneutis* and *P. conradi* are established in North America and the host range of *P. digoneutis* in

alfalfa fields in New York state was investigated, more information is needed on these species and other Palaearctic species considered for introduction to North America. Specifically we need to know the range of species they will parasitize in other habitats, including areas in their region of origin. This would be useful to determine habitats which may be suitable for parasitoid establishment and to identify any non-target mirid species which may be at risk in North America and in which habitats they will be at risk.

The aim of the research presented here is to assess the host range of the Palaearctic *Peristenus* species considered for introduction into Canada, *P. digoneutis*, *P. rubricollis* and *P. stygicus*, by conducting field surveys to determine the ecological host range (natural or realized host range) of these parasitoids in a variety of habitats in their native region. The ecological host range is defined as the range of species which actually serve as hosts in the environment.

6.3 Materials & Methods

To assess the host range of *P. digoneutis*, *P. rubricollis* and *P. stygicus*, surveys were conducted from 1998-2000 in a variety of habitats in Germany and Switzerland. Mirids were collected in the field, identified and reared to obtain their parasitoids. Both adults and nymphs were reared to associate the nymphs with the adults of the same species. Parasitoid cocoons were incubated to obtain adult parasitoids to make species identifications. A synoptic collection of all the mirids species collected was prepared and a photographic slide catalogue of the mirids collected was created. Mirid and *Peristenus* voucher specimens

are deposited in the Canadian National Collection (CNC) in Ottawa, at Cabi Bioscience Centre Switzerland in Délemont, Switzerland, and in the J.B. Wallis Museum at the University of Manitoba, Canada.

6.3.1 Mirid surveys

Mirids were collected using sweep nets 37.5 cm in diameter. In 1998, a single sample, consisting of 50 sweeps (180° = 1 sweep) was taken once at three fields of alfalfa and in one red clover field in the Rhine Valley of Germany. The total number of non-target mirids collected in 1998 was low. To obtain more information on the ecological host range of *Peristenus* species, the study was expanded for the 1999 and 2000 field seasons. The main goals were to increase the number of habitats sampled, the diversity of habitats sampled, the number of mirid species collected and the number of nymphs collected. Surveys were made in agricultural crops and non-crop agricultural habitats and in natural habitats in Germany and Switzerland.

6.3.2 Rearing

The mirid adults and nymphs were sorted by morphological characters into morpho species groups. Nymphs and adults were reared separately. The rearing system consisted of two plastic, tapered, urine sample containers, 6 cm in diameter and 8.5 cm high, nested one within the other. The bottom of the inner cup was cut off and replaced by a piece of mesh (8x8 squares per cm^2). The outer cup was filled with fine, moistened vermiculite, to a depth of about 1 cm. This served as pupation medium for the emerging parasitoid larvae, and it provided moisture for nymphs and developing parasitoids. A ventilation hole, 2

cm in diameter and covered with fine netting, was present on the lid of the container.

Approximately 10-15 nymphs or adults were reared together in a container. They were fed plants collected from the survey site and store-bought green beans. Pieces of paper towel were added to the containers to add structural diversity for the nymphs and this was found to reduce predation by providing refuges for molting nymphs. Mirid nymphs and adults were maintained until parasitoids emerged from them, they died, or they were preserved for synoptic collection material. It was critical for some of the nymphs to reach the adult stage to confirm their identity. Most species fed on the green beans provided. For those that did not, the beans nevertheless provided some moisture for them. The success of the mirid rearing in 1999 was not very good but this improved significantly in 2000, the improvement is mostly attributed to the mirids being checked more frequently, every 2-4 days, and by rearing more late instar nymphs.

When all individuals in the sample had finished development, the bottom container and the vermiculite were inspected for parasitoid cocoons. The number of cocoons found was recorded and the lid replaced with a solid one. The cocoons were then monitored almost daily for parasitoid emergence. The date parasitoids emerged was recorded. Adult parasitoids were killed in 70% alcohol and shipped to Dr. Henri Goulet, at the Eastern Cereal and Oilseed Research Center (ECORC) in Ottawa, where they were prepared using the critical point drying technique, sexed and identified. Any cocoons which did not

produce adults during the collection year were placed in an outdoor insectary in October of that year. They were removed from the insectary the following 1 May. Each container was misted with water, stored in the laboratory and monitored daily for emerging parasitoids. Any emerged adults were treated as above.

6.3.3 Habitats surveyed

The habitat types are classified into three subcategories, agricultural crop habitats, non-crop agricultural habitats or natural habitats. The agricultural crops were: *Medicago sativa* L. (alfalfa), *Trifolium pratense* L. (red clover), a mixture of *Trifolium* species (clover mixture), *Lotus corniculatus* L. (bird's-foot trefoil) and *Sinapis alba* (L.) (mustard). The other agricultural habitats were: grassy fallow field, *Erigeron* species (Fleabane sp.), *Matricaria* sp. (scentless camomile) and *Lythrum salicaria* L. (purple loosestrife). The natural habitats were: mountain meadow - low elevation, mountain meadow - high elevation, and riparian vegetation.

6.4 Results

6.4.1 Mirid surveys

In 1998, three fields of alfalfa and one of red clover were surveyed. In 1999, 23 surveys were made in Germany and 20 in Switzerland, for a total of 43. In 2000, 14 surveys were made in Germany and 17 in Switzerland, for a total of 31. A complete list of the collection sites and a description of each are given in Appendices 4, 5 and 6. A total of 78 surveys were made for mirids and their parasitoids from 1998-2000, at 45 different sites, and 12 different habitat types were sampled (Table 6.1).

Table 6.1 Summary statistics for surveys of mirid host range for *Peristenus* parasitoids made during the 1998, 1999 and 2000 field seasons.

Year	1998	1999	2000	1998-2000 combined
Number of surveys in Germany	4	23	14	41
Number of surveys in Switzerland	0	20	17	37
Total # of surveys	4	43	31	78
Total # of survey sites	4	29	16	45*
Total # of habitat types surveyed	2	8	8	12*

* Numbers reflect the total # of survey sites and habitats surveyed through the duration of the study. There was some degree of overlap of sites and habitats sampled from year to year, therefore they are not simply additive from 1998-2000.

In this three year survey, 45 mirid species were collected. These comprised five different subfamilies and included phytophagous and predaceous species. A list of all the mirid species collected, along with information on their global distribution, habitat records, host plants and habit, as given by Schuh (1995), are found in Tables 6.2a, 6.2b, 6.3, 6.4 and 6.5.

6.4.2 Mirid/habitat associations

The mirid species collected in agricultural crop habitats are listed in taxonomic order in Table 6.6a, and the habitats in which they were collected in are indicated. Table 6.6b contains the same information for mirids collected in non-crop agricultural habitats and natural habitats. The habitats can be ranked based on the number of mirid species collected in them, from the greatest number of mirid species to the lowest number of mirid species as: mountain meadow - high elevation, grassy fallow field, alfalfa, mountain meadow - low elevation, red clover, scentless camomile, clover mixture, fleabane, mustard, bird's-foot trefoil, purple loosestrife and riparian vegetation.

6.4.3 Parasitoid/habitat associations

The habitats in which each species of mirid was found to be parasitized in are indicated in Tables 6.6a & 6.6b. The habitats can be ranked from highest to lowest based on the number of mirid species parasitized in the habitat as: mountain meadow - high elevation and alfalfa, grassy fallow field, clover mixture,

Table 6.2a Species of the subfamily Mirinae, tribe Stenodemini, which were collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland in 1998, 1999 and 2000, their distribution, habitat, host plants and feeding habits. Information taken from R.T. Schuh, 1995. Plant bugs of the world (Insecta: Heteroptera: Miridae).

Species	Distribution	Habitat and/or feeding habit
Leptopterna dolobrata (L.)	Holarctic	grasses, meadows, *Vaccinium* sp. [Ericaceae], vetch [Fabaceae], *Calamagrostis* sp. [Poaceae], *Omoni spinosa* [Poaceae], *Phleum* sp. [Poaceae], [Poaceae]
Megaloceroea recticornis (Geoffroy)	Holarctic	[Poaceae]
Notostira elongata (Geoffroy)		*Ononis spinosa* [Fabaceae], [Poaceae]
Pithanus maerkelii (Herrich-Schaeffer)	Holarctic	grasses and sedges in damp areas, *Carex* sp. [Cyperaceae], *Juncus* sp. [Juncaceae], [Poaceae]
Stenodema calcerata (Fallén)	Palaearctic	grasses, meadows, *Ononis spinosa* [Fabaceae], *Quercus* sp. [Fagaceae], [Poaceae], xerophilous sp. [Poaceae]
Stenodema holsata (Fabr.)	Palaearctic	grasses, meadows, *Sorgham halopense* [Poaceae], [Poaceae]
Stenodema laevigata (L.)	Palaearctic	grasses, meadows, *Ononis spinosa* [Fabaceae], *Quercus* sp. [Fagaceae], [Poaceae]
Stenodema virens (L.)	Holarctic	*Ononis spinosa* [Fabaceae], *Elymus cinereus* [Poaceae], [Poaceae]
Trigonotylus caelestialium (Kirkaldy)	Holarctic	grasses, sedges, damp areas, [Brassicaceae], *Zea* sp. [Poaceae]

Table 6.2b Species of the subfamily Mirinae, tribe Mirini, which were collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland in 1998, 1999 and 2000, their distribution, habitat, host plants and feeding habits. Information taken from R.T. Schuh, 1995. Plant bugs of the world (Insecta: Heteroptera: Miridae).

Species	Distribution	Habitat and/or feeding habit
Adelphocoris lineolatus (Goeze)	Holarctic	*Medicago* and herbaceous plants, *Artemesia dracunculus* [Asteraceae], *Medicago sativa* [Fabaceae], *Melilotus* sp. [Fabaceae], *Ononis spinosa* [Fabaceae], Oxytropis sp. [Fabaceae], *Trifolium* sp. [Fabaceae], *Hyssopus seravschanicus* [Lamiaceae], *Nepeta pannonica* [Lamiaceae], *Origanum tyttanthum* [Lamiaceae], *Perovskia* sp. [Lamiaceae], *Eremus* sp. [Liliaceae]
Adelphocoris seticornis (Fabr.)	Palaearctic	*Medicago*, meadows, *Ononis spinosa* [Fabaceae], *Vicia* sp. [Fabaceae], [Fabaceaea]
Calocoris norvegicus (Gmelin)	Holarctic	sub alpine meadows, *Ferula* sp. [Apiaceae], *Solanum melongena* [Solanaceae]
Calocoris roseomaculatus (De Geer)	Palaearctic	sub alpine meadows
Calocoris striatellus (Fabr.)	Palaearctic	*Quercus* sp. [Fagaceae]
Capsus ater (L.)	Holarctic	meadows, *Ononis spinosa* [Fabaceae], *Agropyron repens* [Poaceae], *Phleum pratense* [Poaceae], *Poa compressa* [Poaceae], [Poaceae]
Lygocoris pabulinus (L.)	Holarctic	herbaceous plants, *Alnus* sp. [Betulaceae], *Ononis spinosa* [Fabaceae], *Quercus pedonculata* [Fagaceae], *Salix* sp.[Salicaceae]
Lygus gemellatus (H.-S.)	Palaearctic	forests, semi steppe, *Artemesia* sp. [Asteraceae], *Ononis spinosa* [Fabaceae]
Lygus pratensis (L.)	Palaearctic	meadows, steppe, montane steppe, agricultural crops, *Artemesia* sp. [Asteraceae], *Ononis spinosa* [Fabaceae], [Euphorbiaceae], *Quercus* sp. [Fagaceae], *Nepeta* sp. [Lamiaceae], *Origanum* sp. [Lamiaceae], *Atraphaxis* sp. [Polygonaceae], *Nicotiana* sp. [Solonaceae], *Solanum melongena* [Solonacaea]
Lygus punctatus (Zetterstedt)	Palaearctic	
Lygus rugulipennis Poppius	Holarctic	meadows, alpine, sub alpine, agricultural crops, rape [Brassicaceae], sugar beet [Chenopodiaceae], *Ononis spinosa* [Fabaceae], *Quercus* sp. [Fagaceae]
Lygus wagneri Remane	Palaearctic	boreal, montane, marshes, meadows
Orthops sp.		
Phytocoris longipennis Flor	Palaearctic	*Acer* sp. [Aceraceae], *Quercus pedonculata* [Fagaceae], *Ulmus* sp. [Ulmaceae]
Polymerus micropthalamus (Wagner)	Palaearctic	
Stenotus binotatus (Fabr.)	Holarctic	grass spp. [Poaceae], vetch [Fabaceaea], *Dactylis glomerata* [Poaceae], *Phleum pratense* [Poaceae]

Table 6.3 Species of the subfamily Orthotylinae, which were collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland in 1998, 1999 and 2000, their distribution, habitat, host plants and feeding habits. Information taken from R.T. Schuh, 1995. Plant bugs of the world (Insecta: Heteroptera: Miridae).

Subfamily	Tribe	Species	Distribution	Habitat and/or feeding habit
Orthotylinae	Halticini	*Halticus apterus* (L.)	Palaearctic	*Vicia striata* [Fabaceae]
		Strongylocoris steganoides (Sahl.)	Palaearctic	
		Orthocephalus brevis (Panzer)	Palaearctic	*Campanula rapunculoides* [Campanulaceae] *Campanula* sp. [Campanulaceae]
		Orthocephalus coriaceus (Fabr.)	Holarctic	[Asteraceae], *Quercus pedonculata* [Fagaceae], *Quercus* sp. [Fagaceae]
		Orthocephalus saltator (Hahn)	Palaearctic	[Asteraceae], [Poaceae]
	Orthotylini	*Dryophilocoris flavoquadrimaculatus* (De Geer	Palaearctic	*Quercus pedonculata* [Fagaceae]
		Globiceps fulvicollis (Jakovlev)	Palaearctic	*Genista* sp. [Fabaceae], *Thymus* sp. [Lamiaceae], [Poaceae]
		Heterotoma plannicornis (Pallas)	Palaearctic	
		Mecomma ambulans (Fallén)	Palaearctic	
		Orthotylus flavosparsus (Sahl.)	Holarctic	*Chenopodium album* [Chenopodiaceae], *Atriplex* sp. [Chenopodiaceae]

Table 6.4 Species of the subfamily Phylinae, which were collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland in 1998, 1999 and 2000, their distribution, habitat, host plants and feeding habits. Information taken from R.T. Schuh, 1995. Plant bugs of the world (Insecta: Heteroptera: Miridae).

Subfamily	Tribe	Species	Distribution	Habitat and/or feeding habit
Phylinae	Hallodapini	*Systellonotus triguttatus* (L.)	Palaearctic	*Thymus vulagaris* [Lamiaceae]
	Phylini	*Amblytylus nasutus* (Kirsch.)	Holarctic	*Agrostis* sp. [Poaceae], *Phleum pratense* [Poaceae], [Poaceae]
		Lepidargyrus ancorifer Fieber	Holarctic	*Centaurea* sp. [Asteraceae], *Chrysanthemum* sp. [Asteraceae]
		Megalecoleus molliculus (Fallén)	Holarctic	*Achillea milleifolium* [Asteraceae], *Achillea* sp. [Asteraceae], *Anthemis* sp. [Asteraceae], *Tanecetum* sp. [Asteraceae], [Poaceae]
		Megalecoleus pilosus (Schrank)	Palaearctic	*Achillea* sp. [Asteraceae], *Tanecetum vulgare* [Asteraceae]
		Plagiognathus arbustorum (Fabr.)	Holarctic	*Corylus avellana* [Betulaceae], *Quercus pedonculata* [Fagaceae], *Urtica dioica* [Urticaceae]
		Plagiognathus chrysanthemi (Wolff)	Holarctic	[Asteraceae], *Medicago sativa* [Fabaceae], *Trifolium* sp. [Fabaceae], *Lotus corniculatus* [Fabaceae]

Table 6.5 Species of the subfamily Deraeocorinae, and subfamily Bryocorinae, which were collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland in 1998, 1999 and 2000, their distribution, habitat, host plants and feeding habits. Information taken from R.T. Schuh, 1995. Plant bugs of the world (Insecta: Heteroptera: Miridae).

Subfamily	Tribe	Species	Distribution	Habitat and/or feeding habit
Deraeocorinae	Deraeocornini	*Deraeocoris ruber* (L.)	Holarctic	Probably predaceous on aphids, *Echium* sp. [Boraginaceae], *Symphytum* sp. [Boraginaceae], *Prunus* sp. [Rosaceae], *Rubus* sp. [Roseaceae], *Urtica* sp. [Urticaeae]
		Deraeocoris scutellaris (Fabr.)	Palaearctic	Probably predaceous on aphids, *Calluna* sp. [Ericaceae], *Erica* sp. [Ericaceae]
Bryocorinae	Dicyphini	*Dicyphus hyalinipennis* (Burmeister)	Palaearctic	Probably predaceous on other insects, *Senecio viscosa* [Asteraceae], *Ononis natrix* [Fabaceae], *Epilobium* sp. [Onograceae], *Atropa belladona* [Solonaceae]

Table 6.6a Mirid species collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland from 1998-2000, the crop habitats they were collected (X) and parasitized in (X*).

Species	Crop habitats				
	Alfalfa	Red clover	Clover mixture	Bird's- foot trefoil	Mustard
Leptopterna dolobrata	X	X			
Megaloceroea recticornis	X				
Notostira elongata	X*	X	X*		
Pithanus maerkeli					
Stenodema calcerata	X*	X	X		
Stenodema holsata					
Stenodema laevigata					
Stenodema virens	X*	X			X
Trigonotylus caelestialium	X*	X	X*		
Adelphocoris lineolatus	X*	X*	X	X	X
Adelphocoris seticornis	X	X		X	
Calocoris norvegicus	X				
Calocoris roseomaculatus			X		
Calocoris striatellus					
Capsus ater	X	X			
Lygocoris pabulinus					
Lygus gamellatus	X				
Lygus pratensis	X*	X*	X*	X	X*
Lygus punctatus					
Lygus rugulipennis	X*	X*	X*	X	X*
Lygus wagneri					
Orthops sp.					
Phytocoris longipennis					
Polymerus micropthalamus		X			
Stenotus binotatus	X	X			
Halticus apterus	X				
Orthocephalus brevis	X	X			
Orthocephalus coriaceus					
Orthocephalus saltator					
Strongylocoris steganoides					
Dryophilocoris flavoquadrimaculatus					

Table 6.6a continued

Globiceps fulvicollis					
Heterotoma plannicornis					
Mecomma ambulans					X
Orthotylus flavosparsus					
Systellonotus triguttatus					
Amblytylus nasutus	X				
Lepidogyrus ancifor	X	X			
Megalecoleus molliculus					
Megalecoleus pillosus					
Plagiognathus arbustorum	X				
Plagiognathus chrysanthemi	X	X			
Deraeocoris ruber	X	X			
Deraeocoris scutellaris					X
Dicyphus hyalinipennis					
# of mirid sp.	21	16	7	4	6
# of mirid sp. parasitized	7	3	4	0	2

Table 6.6b Mirid species collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland from 1998-2000, the non-crop agricultural habitats and natural habitats they were collected (X) and parasitized in (X*).

Species	Other agricultural habitats				Natural habitats		
	Grassy fallow field	Fleabane sp.	Scentless camomile	Purple loosestrife	Mountain meadow, low elev.	Mountain meadow, high elev.	Riparian vegetation
Leptopterna dolobrata	X*	X	X		X*	X*	
Megaloceroea recticornis	X		X			X	
Notostira elongata	X			X	X	X	
Pithanus maerkeli	X				X	X	
Stenodema calcerata	X				X	X	
Stenodema holsata					X	X*	
Stenodema laevigata	X					X	
Stenodema virens	X*			X	X*	X*	
Trigonotylus caelestialium	X				X	X	
Adelphocoris lineolatus	X*	X	X		X	X	
Adelphocoris seticornis	X				X*	X	
Calocoris norvegicus	X		X		X	X*	
Calocoris roseomaculatus	X					X*	
Calocoris striatellus						X	
Capsus ater	X		X		X	X	
Lygocoris pabulinus						X	
Lygus gamellatus	X		X		X		
Lygus pratensis	X*	X	X*	X*	X	X*	
Lygus punctatus						X	
Lygus rugulipennis	X*	X	X*	X*	X	X*	
Lygus wagneri						X	
Orthops sp.						X	
Phytocoris longipennis							X
Polymerus micropthalamus	X					X	
Stenotus binotatus	X		X		X	X	
Halticus apterus	X	X	X		X	X	
Orthocephalus brevis	X	X				X	
Orthocephalus coriaceus						X	

Table 6.6b continued

Orthocephalus saltator						X	
Strongylocoris steganoides	X					X	
Dryophilocoris flavoquadrimaculatus						X	
Globiceps fulvicollis	X						
Heterotoma plannicornis							
Mecomma ambulans							
Orthotylus flavosparsus						X	
Systellonotus triguttatus	X						
Amblytylus nasutus	X		X		X		
Lepidogyrus ancifor	X	X	X			X	
Megalecoleus molliculus			X			X	
Megalecoleus pillosus						X	
Plagiognathus arbustorum	X					X	
Plagiognathus chrysanthemi			X			X	
Deraeocoris ruber	X		X		X		
Deraeocoris scutellaris						X	
Dicyphus hyalinipennis	X					X	
# of mirid sp.	28	7	15	4	17	37	2
# of mirid sp. parasitized	5	0	2	2	3	7	0

red clover, mountain meadow - low elevation, mustard, scentless camomile and purple loosestrife. No mirids were found to be parasitized in the bird's-foot trefoil, fleabane, or riparian habitats. The mirids which were parasitized are listed in Tables 6.7a, 6.7b, and 6.7c, based on the habitat subcategories and includes the identity of the parasitoid species attacking each mirid species. The greatest number of parasitoid species was found in the alfalfa habitat. The habitats can be ranked as having the greatest number of parasitoid species present to the lowest number of parasitoid species present as, alfalfa, mountain meadow low-elevation, red clover, scentless camomile, mountain meadow - high elevation, clover mixture, grassy fallow field, mustard and purple loosestrife. The number of collection sites surveyed for each habitat are listed in Table 6.8, along with the number of collections made in each habitat and the numbers of specimens and species collected, for mirids and parasitoids found in each habitat.

6.4.4 Parasitoid/mirid associations

The number of individuals of each mirid species collected, as well as the number of parasitoids obtained from rearing these individuals over three field seasons from 1998-2000 are given in Table 6.9. Parasitoid identifications from cocoons reared in 2000 are pending for the following mirid species; *A. lineolatus, Adelphocoris seticornis* (Fabr.) *Calocoris roseomaculatus* (De Geer), *Leptopterna dolobrata* (L.), *L. pratensis, L. rugulipennis, Notostira elongata*

Table 6.7a Mirid species and their parasitoids in crop habitats collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland from 1998-2000. X indicates the presence of the mirid in the absence of any parasitoids.

Species	Alfalfa	Red clover	Clover mixture	Bird's-foot trefoil	Mustard
Leptopterna dolobrata	*P. rubricollis*	X			
Stenodema calcerata	X*	X	X		
Stenodema virens	X*	X			X
Trigonotylus caelestialium	X*	X	*P. stygicus*		
Adelphocoris lineolatus	*P. pallipes* (spring), *P. digoneutis* *P. rubricollis, P. stygicus* n.sp.nr. *L.defeciens*	*P. digoneutis, P. rubricollis* *P. stygicus*	X	X	*P. digoneutis*
Lygus pratensis	*P. pallipes* (spring), *P. digoneutis,* *P. rubricollis, P. stygicus* n.sp.nr. *L.defeciens*	*P. digoneutis, P. rubricollis* *P. stygicus*	*P. stygicus,* *P. digoneutis*	X	*P. digoneutis,* *P. stygicus*
Lygus rugulipennis	*P. pallipes* (spring), *P. digoneutis* *P. rubricollis, P. stygicus* n.sp.nr. *L.defeciens*	*P. digoneutis, P. rubricollis* *P. stygicus*	*P. stygicus,* *P. digoneutis*	X	*P. digoneutis,* *P. stygicus*
Notostira elongata	X*	X	*P. stygicus*		
# parasitoid species	At least 5	3	2	0	2

* Parasitoid species presently unidentified from this host, pending 2001 emergence data.

Table 6.7b Mirid species and their parasitoids in non-crop agricultural habitats collected in mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland from 1998-2000. X indicates the presence of the mirid in the absence of any parasitoids.

Species	Grassy fallow field	Fleabane sp.	Scentless camomile	Purple loosestrife
Leptopterna dolobrata	X*	X	X	
Stenodema virens	X*			X
Adelphocoris lineolatus	*P. pallipes* (spring)	X	X	
Lygus pratensis	*P. pallipes* (spring), *P. digoneutis*	X	*P. digoneutis, P. pallipes* (spring) *P. stygicus*	X*
Lygus rugulipennis	*P. pallipes* (spring), *P. digoneutis*	X	*P. digoneutis, P. pallipes* (spring) *P. stygicus*	X*
# parasitoid species	At least 2	0	3	At least 1

* Parasitoid species presently unidentified for this host, pending 2001 emergence data.

Table 6.7c Mirid species and their parasitoids in natural habitats collected in mirid host range surveys for *Peristenus* parasitoids in Germany and Switzerland form 1998-2000. X indicates the presence of the mirid in the absence of any parasitoids.

Species	Mountain meadow, low elevation	Mountain meadow, high elevation	Riparian vegetation
Leptopterna dolobrata	*P. stygicus*, n.sp.nr. *P. pallipes* n.sp.nr. *P.digoneutis*	X*	
Stenodema holsata	X	X*	
Stenodema virens	*P. pallipes* (summer)	X*	
Adelphocoris seticornis	X*	X	
Adelphocoris lineolatus	X	*P. adelphocoridis*	
Calocoris norvegicus		*P. adelphocoridis*, n.sp.nr. *P.pallipes* X*	
Calocoris roseomaculatus			
Lygus pratensis	X	*P. pallipes* (spring)	
Lygus rugulipennis	X	*P. pallipes* (spring)	
# parasitoid species in natural habitats	At least 4	At least 3	0

* Parasitoid species presently unidentified for this host, pending 2001 emergence data.

Table 6.8 Mirid host range surveys for *Peristenus* parasitoids: Number of collection sites and collections made in each habitat type, the number of mirids and parasitoids collected and the number of mirid and parasitoid species collected from each habitat type over three field seasons in Germany and Switzerland, from 1998-2000.

Habitat	# of collection sites	# of collections	# of mirid nymphs	# of parasitoid cocoons	# of mirid species	# of parasitoid species
Alfalfa	9	15	930	130	21	5
Red clover	8	11	345	65	16	3
Clover mixture	1	1	73	6	7	2
Birds foot trefoil	1	1	10	0	4	0
Mustard	1	1	11	4	6	1
Grassy fallow field	10	21	747	21	28	2
Fleabane sp.	1	1	4	0	7	0
Scentless camomile	2	3	80	3	15	3
Purple loosestrife	1	1	502	47	4	1
Mountain meadow low elevation	2	5	589	68	17	4
Mountain meadow high elevation	9	17	566	5	37	3
Riparian vegetation	1	1	10	0	2	0

Table 6.9 The number of adults and nymphs collected of each mirid species and the number of parasitoids reared from each mirid species, from host range surveys made in Germany and Switzerland during 1998-2000.

Species	# adults collected	# nymphs collected	# parasitoids reared
Leptoptema dolobrata (L.)	362	864	70
Megaloceroea recticornis (Geoffroy)	62	2	0
Pithanus maerkeli (H.-S.)	5	0	0
Notostira elongata (Geoffroy)	207	151	4
Stenodema calcerata (Fallén)	113	11	0
Stenodema holsata (Fabr.)	4	4	1
Stenodema laevigata (L.)	2	0	0
Stenodema virens (L.)	133	300	7
Stenodema calcarata or *S. virens*	15	393	8
Trigonotylus caelestealium (Kirkaldy)	67	100	2
Adelphocoris lineolatus (Goeze)	203	529	98
Adelphocoris seticornis (Fabr.)	48	36	1
Calocoris norvegicus (Gmelin)	10	4	3
Calocoris roseomaculatus (De Geer)	75	57	3
Calocoris striatellus (Fabr.)	1	0	0
Capsus ater (L.)	36	21	0
Lygocoris pabulinus (L.)	1	0	0
Lygus gemellatus (Herich-Schäffer)	5	0	0
Lygus pratensis (L.)	72	33	0
Lygus punctatus (Zetterstedt)	10	0	0
Lygus rugulipennis Poppius	262	147	31
Lygus rugulipennis or *L. pratensis*	275	940	121
Lygus wagneri Remane	52	124	0
Orthops sp.	1	0	0
Phytocoris longipennis Flor	1	2	0
Polymerus micropthalamus (Wagner)	13	0	0
Stenotus binotatus (Fabr.)	52	16	0
Halticus apterus (L.)	41	1	0
Orthocephalus brevis (Panzer)	41	1	0
Orthocephalus coriaceus (Fabr.)	1	0	0
Orthocephalus saltator (Hahn)	1	0	0
Strongylocoris steganoides (Sahl.)	16	0	0
Dryophilocoris flavoquadrimaculatus (De Geer)	1	0	0
Globiceps fulvicollis (Jakovlev)	1	0	0
Heterotoma planicornis (Pallas)	2	0	0
Orthotylus flavosparsus (Sahlberg)	1	0	0
Mecomma ambulans (Fallén)	1	0	0
Systellonotus triguttatus (Linne)	1	0	0
Amblytylus nasutus Kirschbaum	461	47	0
Lepidargyrus ancorifer Fieber	28	0	0
Megalecoleus molliculus (Fallén)	2	0	0
Megalecoleus pilosus (Schrank(	1	0	0
Plagiognathus arbustorum (Fabr.)	12	0	0
Plagiognathus chrysanthemi (Wolff)	105	42	0
Deraeocoris ruber (L.)	10	0	0
Deraeocoris scutellaris (Fabr.)	7	1	0
Dicyphus hyalinipennis (Burmeister)	4	13	0
Unknown	93	8	0

(Geoffroy), *Stenodema calcarata* (Fallén), *Stenodema holsata* (Fabr.), *Stenodema virens* (L.) and *Trigonotylus caelestialium* (Kirkaldy). Parasitoids were reared from 12 mirid species during this three year study. A list of the parasitoid species and their hosts associated during this study is given in Table 6.10. In this study, the parasitoid species can be ranked according to the number of hosts they parasitize from highest to lowest as, *P. stygicus*, *Peristenus pallipes* (Curtis) (both spring and summer generations), *P. rubricollis*, *P. digoneutis,* a new species of *Leiophron* (Nees) similar to *L. defeciens, Peristenus adelphocoridis* Loan, a new species similar to *P. pallipes*, and a new species similar to *P. digoneutis.*

The host mirid species can be ranked according to the number of species of parasitoids attacking them, from highest to lowest as; *A. lineolatus* (6 species) *L. rugulipennis* and *L. pratensis* (5 species), *Leptopterna dolobrata* (3 species), *Calocoris norvegicus* (2 species), and *Stenodema calcarata/virens* (1 species), *Trigonotylus caelestialium* (1 species) and *Notostira elongata* (1 species). A diagram of parasitoid/mirid host associations known for those parasitoids collected during this study is presented in Figure 6.1.

6.5 Discussion

6.5.1 Mirid surveys

Surveys made in 1998 were not very intensive and served as a starting point from which improvements to the study could be made. The crop habitats in

Table 6.10 Parasitoid species and the mirid species they were reared from, based on mirid host range surveys for *Peristenus* parasitoids made in Germany and Switzerland during 1998-2000.

Parasitoid species	Mirid host species	# of mirid host species
Peristenus adelphocoridis Loan	*Adelphocoris lineolatus, Calocoris norvegicus*	2
Peristenus digoneutis Loan	*Lygus rugulipennis/ Lygus pratensis, A. lineolatus*	3
Peristenus pallipes (Curtis) (spring)	*L. rugulipennis/ L. pratensis, A. lineolatus*	3
Peristenus pallipes (Curtis) (summer)	*Stenodema calcarata/ Stenodema virens*	2
Peristenus pallipes (Curtis)	total	5
Peristenus rubricollis (Thomson)	*L. rugulipennis/ L. pratensis, A. lineolatus, Leptopterna dolobrata*	4
Peristenus stygicus Loan	*L. rugulipennis/ L. pratensis, A. lineolatus, L. dolobrata, Trigonotylus caelestialium, Notostira elongata*	6
new species similar to *P. digoneutis*	*L. dolobrata*	1
new species similar to *P. pallipes*	*C. norvegicus. L. dolobrata*	2
new species similar to *Leiophron defeciens*	*L. rugulipennis/L. pratensis, A. lineolatus*	3

* Parasitoid from the following species have yet to be identified from cocoons reared in 2000; *A. lineolatus, Adelphocoris seticornis, Calocoris roseomaculatus, L. dolobrata, L. pratensis, L. rugulipennis, N. elongata, S. calcarata, Stenodeam holsata, S. virens, T. caelestialium.*

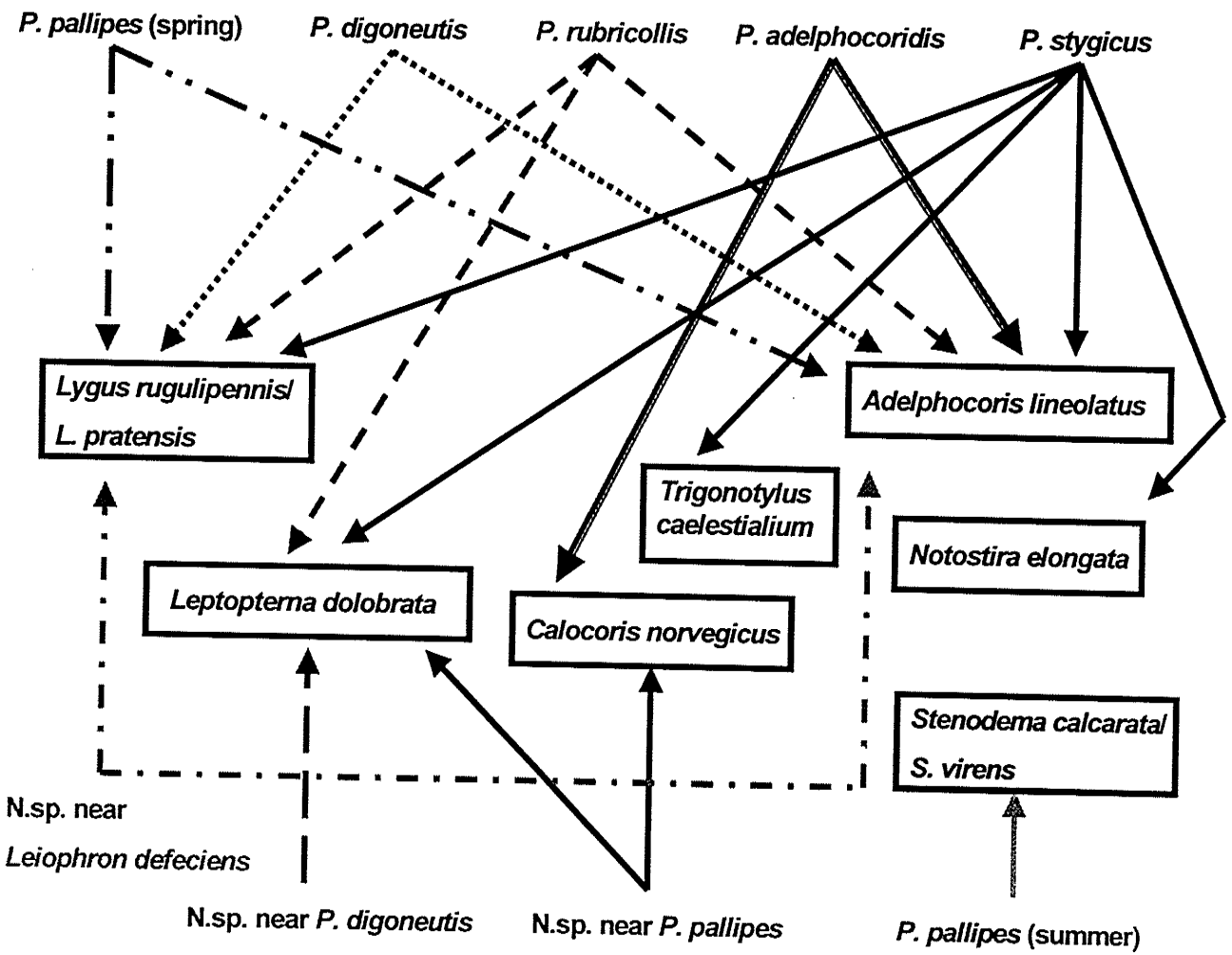

Figure 6.1 A diagram of parasitoid and host relationships based on host associations determined from mirid host range surveys for *Peristenus* parasitoids made in Europe from 1998-2000. Mirid host species are surrounded by text boxes.

the Rhine Valley were selected because they were similar to biological control target crops in North America. One of these crops, alfalfa, has historically served as the main release crop in North America (Day et al. 1990), because of its perennial nature, the typically high levels of target hosts resident in it, and the abundant nectar sources for foraging adult parasitoids. Non-crop agricultural habitats, such as fallow fields and weed species, were selected because they may harbor reservoir populations of the target plant bug species and possibly other hosts of the parasitoids. The natural habitats, mountain meadows and riparian vegetation, were selected because they were expected to have a higher mirid species diversity than the agricultural crops, and it was thought that a greater number of non-target species might be collected in them as compared with agricultural habitats.

The wide range of habitats selected also allowed determination of the range of habitats that the parasitoids naturally occupy, and the range of hosts they utilize in these habitats. This information can be used to assess the risk to non-target mirids by Palaearctic *Peristenus* species. This study also provides information as to which type of habitats should be surveyed in North America to determine if parasitoids are established and/or parasitizing non-target mirids.

In the mirid surveys, a diverse group of mirid species was collected, and included both adults and nymphs. It is important to note that while it was not intentional, many more specimens of the subfamily Mirini were collected and

reared as compared with the other subfamilies. This is, in part, due to the large number of species of Mirinae as compared with the other subfamilies, they are simply more speciose (Schuh 1995). Bilewicz-Pawińska (1982) studied Heteroptera in agroecosystems in Poland and collected 34 species of Heteroptera from cereal crops, 15 of these were mirid species. In all the crops studied *Lygus* spp. were found to be the dominant mirid and heteropteran present.

6.5.2 Mirid/habitat associations

Most of the mirids collected in this study are associated with grasses and flowering host plants found in meadow habitats (Tables 6.2 - 6.5). The greatest number of mirid species were found in the mountain meadow - high elevation sites. The next highest habitat was the grassy fallow fields. These habitats were both very diverse in plant species, and had many flowering species present throughout the season, creating a structurally diverse habitat. The perennial alfalfa crop was third highest in mirid species diversity and was also a structurally complex habitat. Although the alfalfa was low in plant species diversity, flower blooms were present for most of the season which provided nectar and a nutrient rich resource for many insect species, including adult *Peristenus* spp. The high diversity in the top three habitats could be the result of sampling many sites for each of these habitats (Table 6.8), resulting in greater mirid diversity as the number of individuals collected increased. The mountain meadow - low

elevation, red clover and scentless camomile had similar numbers of mirid species collected. As more field collections of mirids are made in these habitats, the number of mirid species collected from them might be expected to rise, resulting in a clearer picture of the mirid and parasitoid diversity present in these habitats. The mirid diversity appeared to be greatest in structurally diverse habitats which had a diverse plant community, and were dominated by flowering plants or grasses.

6.5.3 Parasitoid/habitat associations

On average, 2.25 species of *Peristenus* were found in each habitat. Even though more mirid species were collected in mountain meadow habitats the greatest number of parasitoid species were collected in the alfalfa habitat. It was in this habitat where the greatest number of *L. rugulipennis*, *L. pratensis* and *A. lineolatus* were collected. The alfalfa habitat is a very stable agricultural habitat. The plant is perennial and the stand is usually grown for successive seasons in 3-5 year rotations. It is cut periodically throughout the season, and continually blooms throughout the growing season. These qualities make alfalfa an ideal habitat for many herbivorous insect species and their predators in agricultural regions. It is probably because the crop harbors a fairly permanent population of the common host species that the number of parasitoids collected in it was greater in this monoculture crop.

Each of the parasitoid species considered for introduction attacked hosts in the alfalfa and red clover agricultural habitats. *Peristenus digoneutis* only attacked *A. lineolatus*, *L. pratensis* and *L. rugulipennis* and only in the following agricultural habitats; alfalfa, red clover, clover mixture, mustard, grassy fallow field, and scentless camomile. Bilewicz-Pawińska (1982) reported that *P. digoneutis* attacked *L. rugulipennis* in rye, wheat and barley. *Peristenus rubricollis* only parasitized its hosts in the alfalfa and red clover habitats and attacked the same three host species as *P. digoneutis*, plus *L. dolobrata.* Bilewicz-Pawińska (1982) found *P. rubricollis* attacked *L. rugulipennis* in rye, wheat, barely and oats and *A. lineolatus* in alfalfa. *Peristenus stygicus* attacked hosts in alfalfa, red clover, clover mixture, scentless camomile and in the mountain meadow low elevation habitats. This species attacked the same three hosts as *P. digoneutis*, plus *L. dolobrata*, *N. elongata* and *T. caelestialium.* Bilewicz-Pawińska (1982) found *P. stygicus* attacked *L. rugulipennis* in rye, wheat, barley and oats, and *T. caelestialium* in wild grasses.

6.5.4 Mirid/parasitoid associations

Most *Peristenus* species under study here are polyphagous, that is they attack more than one host species. However, it appears that *Peristenus* species are specific to species of the subfamily Mirinae. This may be a true phylogenetic host-parasitoid relationship, or it could be an artifact of rearing many more Mirinae nymphs compared to the other subfamilies (Table 6.9).

Peristenus stygicus has the widest host range of all parasitoids collected in this study and it attacks its hosts in all the habitat subcategories. These results were similar to those reported by Bilewicz-Pawińska (1982). In another host range study (Condit & Cate 1982), this species was found to have a fairly wide host range. *Peristenus pallipes* has the second highest host range. It has been speculated that this species is a complex of species which are similar in morphology (Dr. H.Goulet pers. comm.). The possible temporal disjunction seen in *P. pallipes* in this study (Figure 5.3, Chapter 5) and the host data generated by this study (Table 6.10) may lend some biological data to support this idea. *Peristenus rubricollis* has the third largest host range detected so far in this study. It appears that the abundant *Lygus* species and *A. lineolatus* were preferred hosts for *P. digoneutis.* Out of the three species considered for introduction, the already established *P. digoneutis*, utilized hosts only in the agriculture habitats.

Several predaceous mirids, *Deraeocoris* species and *Dicyphus hyalinipennis* (Burmeister), were collected, and no parasitoids were reared from these species. Parasitoids were rarely reared from adult mirids, only two parasitoids were reared from adults of *S. virens* and one parasitoid was reared from an adult *T. caelestialium*. Parasitoids were reared from several non-target mirid species for the first time in this study in the summer of 2000. The

identification of the parasitoids collected during the 2000 field season which overwintered is forthcoming.

6.6 Conclusions

Of the European *Peristenus* considered for introduction into Canada, *Peristenus stygicus* has the greatest host range, next is *Peristenus rubricollis* and then *Peristenus digoneutis*. *Peristenus digoneutis* should be of little threat to non-*Lygus*, non-target mirids in a variety of habitats. It is felt that more detailed studies are needed to determine the host range of *P. rubricollis*, and that *P. stygicus* should not be released because of its wide host range and its potential as a biological control agent should be reevaluated.

The ecological consequences of establishing European *Peristenus* species in North America are not known today. It would be appropriate to test the physiological host range by laboratory and field methods of any exotic parasitoids prior to their introduction, and to conduct more detailed studies on the ecological host range of these parasitoid species in their geographic regions of origin. Physiological host range is defined as those species which a parasitoid may attack and in which they successfully develop, in a laboratory setting (Condit & Cate 1982). It is known that the physiological host range may be greater than the ecological host range, and if so, the dangers of host switching in a new environment becomes a concern. Therefore both ecological and physiological studies are needed to understand the potential impact of a parasitoid species.

Studies on the mirid host range of these parasitoids should be carried out in more agricultural habitats, crop and non-crop habitats, including hedgerows and windbreaks, as well as in undisturbed habitats.

Efforts should be focused on collecting and rearing mirid nymphs from more of the other mirid subfamilies to determine if the host range is truly related to the phylogeny of the host group. It is also very important to determine the extent to which the Palaearctic species of *Peristenus* may compete with Nearctic *Peristenus*; and laboratory and field competition studies need to be conducted to assess this.

It is critical that future field releases of these parasitoids are planned and executed carefully. One Palaearctic *Peristenus* species, *P. conradi*, has already been accidentally introduced and is established in North America. Efforts to reduce the number of accidental establishments of exotic organisms need to be made. It may be appropriate to screen all parasitoids prior to field releases. There is an opportunity in Canada to conduct pre-release surveys prior to more releases, to determine levels of parasitism by Nearctic parasitoids and to confirm the identity of Nearctic parasitoids parasitizing mirids in agricultural and non agricultural habitats. Post release surveys are also needed to determine parasitoid establishment, geographic range expansion and non-target parasitism evaluation, as well as non-target parasitoid competition. New Nearctic *Peristenus* species are being identified, such as *Peristenus howardi* Shaw (Day

et al 1999). Some of these species may be suitable as native biological control agents for North America. We need to make an effort to understand their ecological roles prior to more exotic *Peristenus* releases, because we may already have adequate biological control agents present in North America, if we take the time to look.

6.7 Acknowledgments

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Chapter 7 General discussion

In this chapter the results of the research presented in the thesis will be used to evaluate the prospects for successful biological control for pest *Lygus* species and *A. lineolatus* in North America.

7.1 Estimating parasitism levels

There are a few techniques which researchers can employ to assess percent parasitism in the host population being studied. The two most common methods are rearing and dissection. Both methods were compared and their effectiveness at detecting parasitoids and their strengths and weaknesses were evaluated. Each method was found to provide different types of data and either method may be found to be superior to the other depending on the research objectives. In this study, eggs were not detected using the dissection method, however, individual nymphs that were reared which contained a parasitoid egg at the time of collection would probably produce a parasitoid. Therefore the rearing method was determined to be superior at detecting parasitoids. Day (1994) compared the rearing and dissection methods for detecting parasitoids in *Lygus* spp. He concluded that dissection was better for estimating parasitism because of the very high mortality in the rearing, 52% of parasitoids died either with their mirid host or upon emergence from the host. This type of rearing mortality is unacceptable for any study, and possibly the rearing methods used in his study were flawed. Day (1994) also had problems detecting parasitoid eggs using the

dissection method and he found that estimates of parasitism using this method were 25% lower than the percent parasitism determined by a laboratory experiment, due to the lower detection rate by dissection. In this study there was no correlation in percent parasitism calculated using either method and % parasitism was found to range from 0% - 40% using either method. The main conclusion from the study was that the method for parasitoid detection should be determined by the research objectives and it is recommended that some form of preliminary study is made to determine the limitations of each method under the constraints of individual studies to aid in this decision.

The rearing method was selected for use in the studies presented here because the rearing method provided the most appropriate data to meet the objectives of the research. In particular, it was necessary to have adult specimens of the parasitoids for species identification. This allowed the identity of each parasitoid reared to be determined and therefore the parasitoid community structure could be evaluated and the ecological host range of the parasitoids could be investigated.

7.2 Parasitoid effectiveness

Several of the parasitoid species collected in this study were reared from plant bug nymphs found in many different crops, including forages and other crops representing both annual and perennial crops. The diversity of crops in which these parasitoids were found to attack their hosts is important when

considering their potential as biological control agents for pest species which are pests in a wide variety of crops, as *Lygus* spp. are (Broadbent et al. 2002). Some of these parasitoids can effectively find hosts in a number of different crops (Bilewicz-Pawińska 1976, 1977a, 1982). The crop itself may be very important in influencing the diversity of insects found in it, as well as levels of parasitism.

Parasitoids were consistently found to attack pest mirids in crops like alfalfa and red clover. These crops are similar to naturally occurring patches of wild flowers, because of great abundance of flowers found throughout the season. These flowers provide a reliable source of nectar and pollen for foraging adult parasitoids. Adult female parasitoids often require these resources to mature their eggs (van Emden 1990). These crops are also attractive to hosts of the parasitoids, and may serve as host reservoirs. Alfalfa and red clover crops may be reliable habitats where parasitoids can mature their eggs and find hosts, and they may disburse from these crops to other more attractive crops in order to find hosts. In an effort to try to increase parasitism in other crops, flower borders could be planted around the target crop. It is also possible that weed patches serve as reservoirs for parasitoids (van Emden 1990), and selectively spraying the weeds within a crop, while leaving those at the edges unsprayed may also increase parasitism. Considering that the edges

of crops are often low yielding this practice may not have a negative economic impact to the grower and may be beneficial for parasitoids (van Emden 1990).

The spatial distribution of the host and parasitoids was evaluated and both were found to penetrate the crops to the center of the field. No trends in edge effects were detected for either hosts or parasitoids. It should be noted that fields which were sampled for this study were very small by North American standards and the results of this study may not reflect spatial dynamics as they would occur in larger fields. However, Wise & Lamb (1998b) did not find *Lygus* spp. to exhibit edge effects in large commercial canola fields in Canada.

Parasitism levels were found to be variable throughout the season in all crops studied. Bilewicz-Pawińska (1977a) found similar results. Despite this variability parasitism was detected in fairly high levels, throughout the field seasons, 0% - 49% on a per month basis, and 0% - 80% when calculated each week. Parasitism levels were also found to be highly variable among years. This is thought to be related to the abundance of host nymphs (Hawkins 1994), which may be affected by climatic factors such as temperature and precipitation (Varis 1995). The combination of *Peristenus* spp. attacking *Lygus* spp. and *A. lineolatus* in agricultural crops produced parasitism levels up to 80%. This is higher than the mean maximum parasitism levels calculated by Hawkins (1994) for externally feeding hosts eating herbs (40%) and parasitism in cultivated crops (40%).

Many parasitoid species are found to have alternative hosts which are necessary to allow the parasitoid population to survive during seasonal and yearly fluctuations in the population size of their main host species (van Emden 1990). This study found that *A. lineolatus* was not as heavily parasitized by *Peristenus* spp. compared to *Lygus* spp. nymphs, yet it was present in the same crops, and other habitats, at the same time as the *Lygus* spp. It is possible that *A. lineolatus* is an alternative host of these parasitoids and it may be necessary for parasitoid survival when populations of *Lygus* spp. are low. Lawton (1986) reported that polyphagous parasitoids may have a greater impact on the targeted host when their alternative species is present. In order to establish European *Peristenus* spp. it may be necessary to find release habitats containing established populations of *A. lineolatus*.

7.3 Parasitoid communities

Parasitoid communities were detected in the agricultural ecosystems and natural habitats studied. In crops the dominant parasitoid species was *P. digoneutis*, usually making up 30-60% of the parasitoids in a collection. Bilewicz-Pawińska (1976) also found *P. digoneutis* to be the dominant parasitoid in Poland. Next was *P. stygicus* and then *P. rubricollis*. Other parasitoid species, such as *P. conradi*, *P. pallipes* and *Leiophron* sp., were rare in the agroecosystems studied. There appears to be some temporal separation among the species making up the agricultural parasitoid community, with *P. rubricollis*

emerging very early in the spring, followed by *P. digoneutis* and *P. stygicus*. However, there may be some overlap in the emergence times of these species. *Peristenus rubricollis* only attacks the first generation of plant bug nymphs as it is univoltine, whereas both *P. digoneutis* and *P. stygicus* are at least bivoltine in southern Germany and attack the second, larger, generation of plant bugs. Most likely this reduces competition between *P. rubricollis* and the other species for hosts, however *P. digoneutis* and *P. stygicus* may be in direct competition throughout the season for hosts.

A very similar number of parasitoid species were reared from hosts collected in agricultural crops and natural habitats. The parasitoid community in agricultural crops included: *P. conradi*, *P. digoneutis*, *P. pallipes* (spring), *P. rubricollis*, *P. stygicus*, and a new species near *L. defeciens*. In natural habitats the community consisted of: *P. adelphocoridis*, *P. digoneutis*, *P. pallipes* (spring), *P. pallipes* (summer), *P. stygicus*, new species near *P. digoneutis*, and a new species near *P. pallipes*. The similar levels in diversity could be explained by similarities between the crops sampled, such as alfalfa and red clover, and natural habitats, both having abundant sources of host populations, nectar and pollen resources and they are a stable habitat for overwintering parasitoids. The differences in parasitoid species found could be related to differences in host species preferences and to each species ability to recognize the different habitats as potential host habitats (van Alphen & Vet 1986). Some parasitoid

species select highly disturbed, agricultural type habitats, while others select more stable, natural type habitats (Hawkins 1994). There may also be some competitive displacement among the parasitoid species.

The parasitoid species composing the guild found in agroecosystems in Europe will be influenced by interspecific competition and also by the habitat (Milller & Ehler 1990). These ecosystems can be considered to be regularly disturbed habitats, as the forages are cut for hay throughout the growing season, and the annual crops undergo removal of crop biomass and soil disturbance and compaction. The forage seed crops would be the most stable out of all the habitats studied. The parasitoid guild observed in the agricultural crops in Germany is then adapted to these disturbances which are also typical of North American agriculture. Therefore the species studied should be suitable for attacking *Lygus* spp. in similar crops in North America.

7.4 Emergence patterns of parasitoid species

Several different emergence patterns of adult parasitoids were observed. More individuals of *P. digoneutis* overwintered than emerged during the year cocoons were formed. *Peristenus digoneutis* was found to have two generations a year. The number of individuals of *P. stygicus* entering diapause was variable each year and this species was also found to have two generations a year. *Peristenus rubricollis* was univoltine and entered diapause the year in which cocoons were formed. Bilewicz-Pawińska (1974) found that both *P. digoneutis*

and *P. rubricollis* were protandrous, with males emerging slightly earlier than females and in larger numbers and that near the end of the emergence period the number of females emerging was greater. The emergence patterns reported here also conform to this pattern for *P. digoneutis*, *P. rubricollis* and also for *P. stygicus*. These life history and emergence patterns are very important when considering which species are suitable for various regions of North America. The geographical region in which the parasitoids are intended for release needs to be considered and biological information evaluated to determine the most appropriate species for the region. For instance in Canada, at Vegreville and Fairview Alberta, one generation of *Lygus* is produced, whereas in Saskatoon, Saskatchewan two generations are produced. This is thought to be related to differences in heat unit accumulation among the three locations (Butts & Lamb 1991b). Parasitoid overwintering requirements also need to be considered when selecting release habitats. Seed and forage alfalfa fields appear to be very good choices of release habitats because they are stable, perennial systems, where these parasitoids could safely overwinter undisturbed.

7.5 European species potential as biocontrol agents

Many researchers report that there is a growing concern in the general public regarding the negative impacts by biological control agents, including problems arising from their host range (Harris 1990, Philogène 1998, Solter & Maddox 1998). Harris (1990) indicated that the potential for biological control

agents to establish permanently, and to disperse independently may be ideal from a biological control perspective, but very undesirable when there is concern regarding the safety of the biological control agent. He also noted that the level of concern increases with the number of species and genera attacked by the agent. Usually parasitoids will attack a group of host species that have some relation to each other, ecological or phylogenetic (Shaw 1994). As a general rule idiobiont parasitoids (those which complete their development on only one stage of the host) typically have a broader host range as compared to koinobiont parasitoids (those which complete their development on the host as the host continues to molt and grow) probably because they do not require the same physiological compatibility with their host (Askew & Shaw 1986). The *Peristenus* species studied are koinobiont parasitoids.

The ecological host range of these parasitoids was studied to provide information to assess their potential risks to non-target mirids. Other host range studies were made by Bilewicz-Pawińska (1982), Condit & Cate (1982) and Day (1999). In this study, *P. stygicus* attacked the greatest number of mirid species and attacked them in a wide variety of habitats. Condit and Cate (1982) reported *P. stygicus* to have a wider host range determined by laboratory tests. *Peristenus rubricollis* had the next highest recorded host range and then *P. digoneutis*. From this study, it appears that *P. digoneutis* attacks a small number

of hosts and does so in a limited number of habitats. This is consistent with the findings of Bilewicz-Pawińska (1982).

Hawkins (1994) reports that on average koinobiont parasitoids attack 6 species and 5 genera, whereas idiobiont parasitoids attack on average 11 host species and 10 host genera. The findings of the host range study are similar to the averages of host ranges reported by Hawkins (1994) for koinobiont parasitoids. Price (1994) indicated that mean parasitoid species richness for herbivorous host species is significantly lower for hemipterans (2.64 species) compared to homopterans (4.02 species), hymenopterans (6.95 species) and lepidopterans (4.59 species). It appears that both *Lygus* spp. and *A. lineolatus* have more than the average number of parasitoid species attacking them, five and six species respectively.

It is also important to assess the contact frequency between host and parasitoid when discerning host range relationships (Shaw 1994). It is difficult to present any quantitative information based on the host range work reported here because the sampling effort varied greatly between habitats sampled. It was also difficult to rear the different mirid species collected since plant hosts were either unknown or not available for feeding the mirids during rearing. The rearing methods need to be refined to improve rearing survival for many of the mirid species collected. Any improvements to the rearing methods would help in

making comparisons with regards to parasitism levels in the mirid species sampled.

Nevertheless, information gained is useful when determining the risk to non-targets and gives an indication of where to look for non-target effects in the release environment. At this point, it is advised that the host range of *P. stygicus* and *P. rubricollis* be thoroughly investigated before planning any more releases of these species. Consideration needs to be given to their potential impacts on non-targets as the ecological role of many mirids is unknown and the consequences of affecting their populations is also unknown.

Peristenus digoneutis is currently established in North America. This species appears to be reducing the size of *Lygus* populations in alfalfa in the eastern US (Day 1996) and has recently been found in southern Canada (Broabent et al. 1999). The risk to non-target mirids by this species appears to be low (Day 1999) but it should be noted that its potential for competition with native parasitoids is currently unknown.

It should be noted that the parasitism levels reported in this study reflect the level of parasitism by several species. A consideration for biological control programs is, whether one parasitoid species, or more than one parasitoid species, is required to achieve the desired results. Based on the larval-endoparasitoid guild studied in agricultural habitats in Germany, it is recommended that only one species of *Peristenus* be established as a biological

control agent for pest *Lygus* spp. in North America. Establishing a co-evolved guild, such as the European *Peristenus* spp. studied, may saturate the available parasitoid niches and result in more interspecific competition for the host resources without achieving the desired results (Ehler 1990). For example, winter moth *(Operophtera brumata* L.) in its native range in Europe, has over 20 species of parasites and it still reaches pest status. While in eastern Canada, it is controlled by two introduced natural enemy species (Ehler 1990), most likely because of reduced interspecific competition. In order to determine the possible competitive interactions between the European *Peristenus* species studied, the impact of each species on the host population needs to be determined individually. Day (1996) has determined that *P. digoneutis* is responsible for 35-50% parasitism of *Lygus* spp. nymphs in alfalfa in New York during 1988-1993. These levels suggest that *P. digoneutis* is established and is effective by itself. Considering *P. digoneutis* and *P. stygicus* are the most likely species to be undergoing competition, and that *P. digoneutis* is already established, it is recommended that *P. stygicus* is not released as a biological control agent.

7.6 Contributions to knowledge

These parasitoids will attack plant bug nymphs in many different crops, forage, cereals and oilseed crops, as well as annual and perennial crops (Bilewicz-Pawińska 1977a & b, 1982). This research provides observational data on parasitism levels in various crops in Europe, which gives an indication of what levels of parasitism could be expected from these parasitoids when introduced into North America. Studies on the ecological host range of these parasitoid species provide general ecological knowledge of parasitoid host use in the field which can be used to estimate impact to potential hosts in ecosystems where these parasitoids may be introduced.

7.7 Future research

Suggestions for future research include:

1. Studies on native parasitoids' effectiveness in limiting target pest populations in field crops and glasshouse crops in North America.
2. Laboratory and field studies on parasitoid host range in North America and Europe.
3. Field studies in North America to determine introduced parasitoid species' impact on target pests and non-target organisms (mirids and native parasitoids) in crop and non-crop habits.
4. Studies to improve establishment techniques for introduced parasitoids.
5. Taxonomic studies on both North American and European parasitoid species.

6. Molecular identification of all parasitoid species.

7. Keys to mirid nymphs.

8. Parasitoid competition studies (within host).

9. Parasitoid/host synchrony studies.

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Appendix 1. Descriptions of fields sampled weekly for the mirid bugs, *Lygus* spp. and *Adelphocoris lineolatus*, and their parasitoids, *Peristenus* spp., in Germany and Switzerland during the 1998, 1999, and 2000 field seasons.

Field code	Location	Crop	Length meters	Width meters	Years sampled	Other	GPS
St1	Steinenstadt	Alfalfa	167.05	16.9	1998, 1999, 2000		N 47° 45. 515' E 007° 33. 527', Elevation 355.4 m
Mu1	Hügelheim	Mustard	86.45	81.25	1999		N 47° 50. 387' E 007° 37. 191', Elevation 239.6 m
Mu2	Hügelheim	Mustard	260	54.6	1999		N 47° 50. 387' E 007° 37. 191', Elevation 239.6 m
Mu3	Nuenburg	Mustard	204.75	50.05	2000		N 47° 47. 644' E 007° 33. 545', Elevation 243.8 m
Mu4	Nuenburg	Mustard	150.8	56.55	2000		N 47° 48. 092' E 007° 33. 785', Elevation 235.3 m
Nu1	Nuenburg	Alfalfa	150.8	58.50	1999, 2000		N 47° 48. 092' E 007° 33. 785', Elevation 235.3 m
Nu2	Nuenburg	Red clover & alfalfa	131.3	9.1	1999, 2000		N 47° 48. 092' E 007° 33. 785', Elevation 235.3 m
Hu1	Hügelheim	Alfalfa	172.25	68.90	1998, 1999		N 47° 50. 185' E 007° 37. 184', Elevation 240.2 m
Hu2	Hügelheim	Alfalfa	176.8	42.25	1998		N 47° 50. 404' E 007° 37. 325', Elevation 240.2 m
Hu3	Hügelheim	Alfalfa	176.8	22.75	1998. 1999		N 47° 50. 506' E 007° 37. 370', Elevation 238.4 m
RC2	Hügelheim	Red clover	234	59.15	2000		N 47° 49. 395' E 007° 36. 699', Elevation 257.3 m
Air1	Hügelheim	Alfalfa	144.95	79.95	2000		N 47° 49. 493' E 007° 35. 616', Elevation 242.9 m
Air2	Hügelheim	Alfalfa	174.2	32.5	2000		N 47° 49. 806' E 007° 34. 955', Elevation 242.9 m
PT1	Hügelheim	Mixed alfalfa, red clover & pink Trifolium sp.	128.7	26.65	2000		N 47° 49. 680' E 007° 36. 941', Elevation 238.0 m
PT2	Hügelheim	Mixed alfalfa, red clover & pink Trifolium sp.	100.1	93.6	2000		N 47° 49. 623' E 007° 37. 070', Elevation 243.8 m
Asp1	Steinenstadt	Asparagus	171.6	13.65	1999	8 rows	N 47° 45. 515' E 007° 33. 527', Elevation 355.4m
Asp2	Hügelheim	Asparagus	207.35	84.45	1999	46 rows	N 47° 49. 680' E 007° 36. 941', Elevation 238.0 m

Appendix 1 continued

Ca1	Hügelheim	Rape	153.4	81.25	1999		N 47° 50. 828' E 007° 35. 553', Elevation 246.0 m
Ca2	Hügelheim	Rape	200.2	148.85	1999	Near shed	N 47° 50. 986' E 007° 36. 157', Elevation 236.8 m
Ba1	Steinenstadt	Barley	167.05	78.65	1999		N 47° 45. 515' E 007° 33. 527', Elevation 355.4 m
Ba2	Hügelheim	Barley	153.4	122.2	1999		N 47° 50. 828' E 007° 35. 553', Elevation 246.0 m
Ma1	Mappach	Red clover	121.55	26.65	1998		N 47° 41.118' E 007° 36. 073', Elevation 320.3 m
Ma5	Mappach	Alfalfa	176.15	76.05	1998		N 47° 40. 640' E 007° 35. 315', Elevation 325.5 m

Appendix 2. The total number of plant bugs collected and parasitoids reared each year from fields of asparagus, barley, rape seed, mustard, mixed clover and red clover fields, from 1998 - 2000.

Crop	Field Code	Year	# of sampling weeks	# of adult *Lygus* spp.	# of adult *Adelphocoris lineolatus*	# of *Lygus* spp. nymphs	# of *Adelphocoris lineolatus* nymphs	Total # of nymphs	Total # of nymphs reared	Total # of parasitoid cocoons	Total % parasitism
Asparagus	Asp1	1999	2	15	0	29	0	29	29	0	0.0
	Asp2	1999	2	2	0	2	0	2	2	0	0.0
Barley	Ba1	1999	2	34	3	8	6	14	14	8	57.1
	Ba2	1999	1	52	0	30	0	30	30	7	23.3
Rape seed	Ca1	1999	5	9	0	3	0	3	3	0	0.0
	Ca2	1999	6	20	1	11	0	11	11	0	0.0
Mustard	Mu1	1999	7	247	35	418	27	445	445	67	15.1
	Mu2	1999	6	145	4	481	10	491	491	67	13.6
	Mu3	2000	5	207	44	270	0	270	270	31	11.5
	Mu4	2000	6	243	170	191	9	200	200	29	14.5
Mixed clover spp.	Pt1	2000	6	330	52	1117	191	1308	1056	452	42.8
	Pt2	2000	6	295	131	588	222	810	810	224	27.7
Red clover	Ma1	1998	12	529	150	1285	74	1359	1359	259	19.0
	Nu2	1999	7	777	126	1768	136	1904	1904	241	12.7
	Nu2	2000	13	713	1368	1110	1146	2256	1669	293	17.6
	RC2	2000	8	755	118	876	95	971	971	350	36.0

Appendix 3. The total number of plant bugs collected and parasitoids reared each year from fields of alfalfa, from 1998 - 2000.

Crop	Field Code	Year	# of sampling weeks	# of adult *Lygus* spp.	# of adult *Adelphocoris lineolatus*	# of *Lygus* spp. nymphs	# of *Adelphocoris lineolatus* nymphs	Total # of nymphs	Total # of nymphs reared	Total # of parasitoid cocoons	Total % parasitism
Alfalfa	Hu1	1998	9	422	217	426	581	1007	1007	97	9.6
	Hu1	1999	8	423	636	523	800	1323	1323	68	5.1
	Hu2	1998	3	157	41	265	35	300	300	40	13.3
	Hu3	1998	8	403	366	1176	521	1697	1697	262	15.4
	Hu3	1999	7	48	12	22	4	26	26	4	15.4
	Ma5	1998	9	370	255	1261	320	1581	1581	340	21.5
	St1	1998	9	403	138	546	188	734	734	104	14.2
	St1	1999	14	295	84	146	50	196	196	11	5.6
	St1	2000	13	694	645	1042	485	1527	1438	304	21.1
	Nu1	1999	5	229	150	588	249	837	837	25	3.0
	Nu1	2000	10	1116	548	391	1093	1484	1014	166	16.4
	Air1	2000	14	747	1045	1037	1767	2804	2212	291	13.2
	Air2	2000	11	515	309	864	383	1247	1087	270	24.8

Appendix 4. Locality information for sites where surveys of mirid host range for *Peristenus* parasitoids were made during the 1998 field season.

Country	Region	Locality	Habitat type	No. of surveys
Germany	Rhine Valley	near Hügelheim	Alfalfa (Hu1)	1
Germany	Rhine Valley	near Hügelheim	Alfalfa (Hu3)	1
Germany	Rhine Valley	near Mappach	Red clover (Ma1)	1
Germany	Rhine Valley	near Mappach	Alfalfa (Ma5)	1
			Total # of surveys in 1998	4

Appendix 5. Locality information for sites where surveys of mirid host range for *Peristenus* parasitoids were made during the 1999 field season.

Country	Region	Locality	Habitat type	No. of surveys
Germany	Rhine Valley	near Hügelheim	*Matricaria*, fallow field	1
		near Hügelheim	Alfalfa field (Hu1)	1
		near Hügelheim	Alfalfa field (old mass collection site)	1
		near Neuenburg	*Matricaria* in fallow field	2
		near Neuenburg	Grassy fallow field beside Neuenburg *Matricaria* field	1
		near Neuenburg	Grassy field margin of mustard field	1
		near Neuenburg	Trefoil field	1
		near Neuenburg	*Erigeron* sp. flowers	1
		near Neuenburg	Red clover field	1
		Steinenstadt	Grassy fallow field (beside St1)	10
		Steinenstadt	Grassy field near park	1
		Steinenstadt, beside swimming pool	Grassy field beside swimming pool	2
			Germany total # of surveys	23
Switzerland	Bern	Schelten Pass, near Stirenburg restaurent	Grassy mountain meadow, high elevation	2
	Jura	Develier	Alfalfa field	1
		Develier-Desuss	Red clover patch	2
		near Develier	Red clover field	1
		near Develier	Red clover field	1
		near Develier	Red clover and grass field	1
		near Develier	Gorge creek	1
	Neuchâtel	Las Varrieres Pass, Val des Traverese	Mountain meadow - high elevation	1
		Las Varrieres Pass, Val des Traverese	Grassy mountain meadow - high elevation	1
		Las Varrieres Pass, Val des Traverese	Wild flowers and red clover field - low elevation	1
		Vue des Alpes	Mountain meadow, high elevation	1
	Solothurn	Öber Grenchenburg	Mountain meadow, high elevation	2
		Weissenstein	Mountain meadow near highway, low elevation	1
	Valaise	Les Haudéres	Mountain meadow, high elevation	1
		near Sion	Alfalfa field, high elevation	1
		near Sion	Mountain meadow, high elevation	1
		near Sion	Mountain meadow, high elevation	1
			Switzerland total # of surveys	20
			Total # of surveys 1999	43

Appendix 6. Locality information for sites where surveys of mirid host range for *Peristenus* parasitoids were made during the 2000 field season.

Country	Region	Locality	Habitat type	No. of surveys
Germany	Rhine Valley	Hügelheim	Alfalfa field (Air1)	3
		Steinenstadt	Alfalfa field (St1)	4
		Hügelheim	*Trifolium* spp. mixture (PC1)	1
		near Neuenburg	Alfalfa (Nu1)	1
		Neuenburg	Red clover (Nu2)	2
		Hügelheim	Red clover (RC2)	1
		Gupf, Tannenkirch	Mustard	1
		Gupf, Tannenkirch	Purple loosestrife (*Lythrum salicaria*)	1
			Germany total # surveys	14
Switzerland	Bern	Schelton Pass	Mountain meadow, high elev.	1
	Jura	Délemont, CABI	Grassy field	3
		Develier	Grassy field, with *Ranunculus* sp.	1
		Develier-Dessus	Grassy field, with *Ranunculus* sp.	1
		Chantillion	Grassy field	1
	Solothurn	Weissenstein	Mountain meadow, low elev.	4
		Weissenstein	Mountain meadow, high elev.	2
		Öber Grenchenburg	Mountain meadow, high elev.	4
			Switzerland total # of surveys	17
			Total # of surveys 2000	31