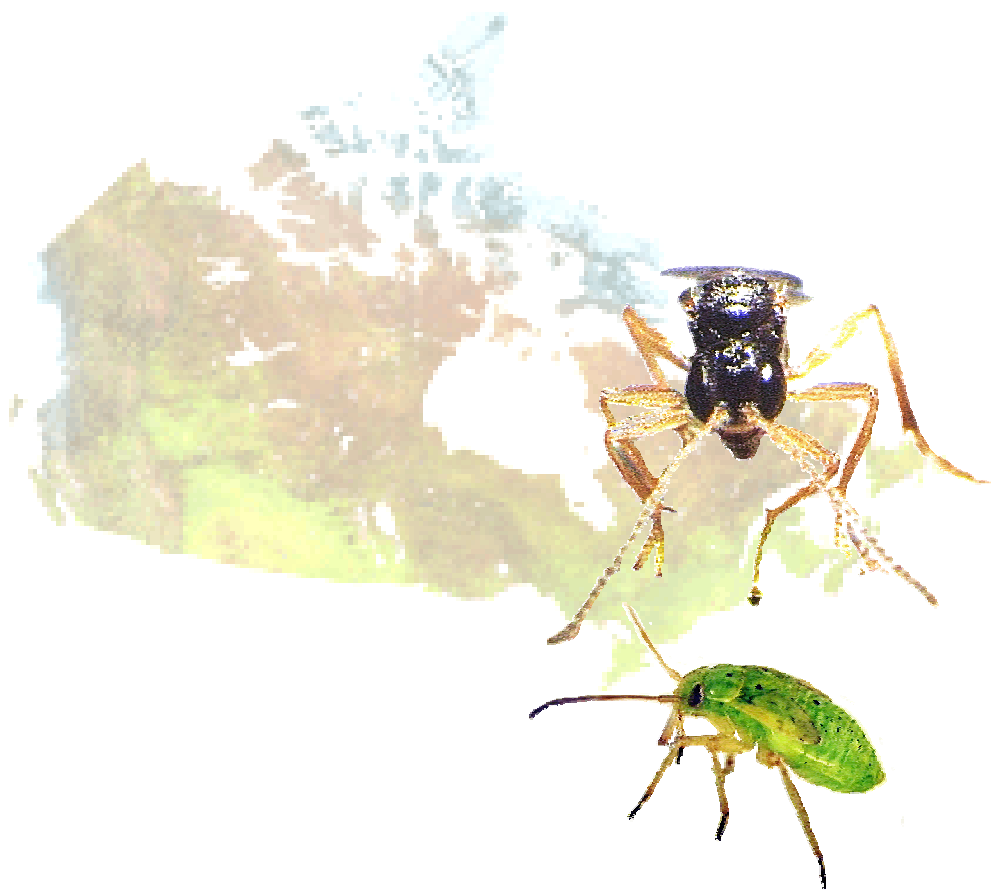


Aus dem
Zoologischen Institut
-Zoophysiologie-

**Studies on the ecology of European *Peristenus* spp.
(Hymenoptera: Braconidae) and their potential for the biological
control of *Lygus* spp. (Hemiptera: Miridae) in Canada**



Dissertation
zur Erlangung des Doktorgrades
der Mathematisch-Naturwissenschaftlichen Fakultät
der Christian-Albrechts-Universität
zu Kiel

vorgelegt von
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Kiel, Mai 2004

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1 General Introduction

1.1 Biology and pest status of *Lygus* plant bugs in Canada

In Canada, the *Lygus* Hahn plant bug complex (Heteroptera: Miridae) contains 27 native species, of which 14 are recorded as agricultural field pests (Maw et al., 2000). In North America, the most abundant and important species as pests are the tarnished plant bug, *L. lineolaris* (Palisot de Beauvois) (Fig. 1.1), the western tarnished plant bug, *L. hesperus* Knight, the lucerne (pale legume) bug, *L. elisus* van Duzee, *L. borealis* (Kelton), and occasionally *L. keltoni* Schwartz (Schwartz & Foottit, 1992; Broadbent et al., 2002).

Adult *Lygus* bugs overwinter under plant litter at the soil surface in field margins, brush piles or shelterbelts (Craig & Loan, 1987; Cermak & Walker, 1992). When temperatures increase in the spring, adults leave their hibernation sites and move to flowering or budding weeds and crop plants, e.g. canola and alfalfa, where females immediately start to oviposit (Jones, 2000; Broadbent et al., 2002). The first nymphs appear at the end of May, and the first adults of the new generation are present as early as the end of June (Jones, 2000). These adults usually spread out to colonize new flowering plants, including many agricultural crops (Broadbent et al., 2002). Although difficult to document, the number of generations of *Lygus* per year has been assumed to vary between one and five depending on the regional climate (Schwartz & Foottit, 1998).

Figure 1.1

Adult *Lygus lineolaris* (Palisot de Beauvois) (photo: Scott Bauer; USDA ARS; www.forestryimages.org)



Lygus adults and nymphs feed on and cause damage to 130 economically important host plants in North America (Young, 1986). The piercing-sucking mouthparts cause damage to plants by puncturing tissue, secreting digestive enzymes, and sucking out plant juices (Tingey & Pillemer, 1977). Feeding on vegetative and reproductive parts of the plant causes a wide variety of damage, e.g. stunting, bud-blast, premature flower drop, distortion of seeds and death of meristematic tissue (Craig & Loan, 1987; Butts & Lamb, 1990a; Cermak & Walker, 1992; Gillespie & Footitt, 1997; Soroka, 1997; Chaput & Uyenaka, 1998). In all cases, damage leads to poor crop quality resulting in economic losses.

Historical records of *Lygus* damaging agricultural crops in Canada go back to the 1940's (Carlson, 1940; McMahon & Arnason, 1947); since then, *Lygus* plant bugs have been reported as pests of several crops throughout the country. The tarnished plant bug *L. lineolaris* is the most widespread *Lygus* species in Canada (Schwartz & Footitt, 1998). In eastern Canada it is the only species causing economic damage on various vegetable and fruit crops (Cermak & Walker, 1992; Chaput & Uyenaka, 1998). In the province of Ontario, it is estimated that 5% of vegetable and fruit crops are damaged annually by *Lygus*, resulting in more than Can\$ 12 million annual losses (Broadbent et al., 2002). In the prairie provinces (western Canada) *Lygus* plant bugs, principally *L. lineolaris*, *L. borealis*, *L. elisus*, and *L. hesperus*, have been recognized throughout the alfalfa seed growing areas as one of the most important reasons of reduced alfalfa seed production (McMahon & Arnason, 1947; Soroka, 1997). Soroka (1997) suggested that the entire alfalfa seed growing industry, valued at Can\$ 50 million, may be lost unless treated with insecticides. Since the late 1980s, *Lygus* spp. have also been identified as serious pests of oilseed rape, *Brassica napus* L. and *B. campestris* L. in Alberta (Butts & Lamb, 1990b, 1991), Saskatchewan (Braun et al., 2001) and Manitoba (Timlick et al., 1993). In 1997, approximately 20% of the canola crop in southern Alberta was lost due to heavy *Lygus* outbreaks, and estimated economic losses were more than Can\$ 70 million to the canola industry (Mason & Soroka, 1998; Broadbent et al., 2002). Relatively recently increasing problems with the occurrence of *Lygus* spp. in greenhouses and conifer nurseries have been noticed in British Columbia (Shrimpton, 1985; Gillespie & Footitt, 1997); however, economic losses have not yet been estimated.

1.2 Classical biological control of insects

Every year an increasing number of invasive species spread to new habitats by human activities and cause serious harm to ecosystems and agriculture (van Lenteren 1995; Bright, 1999). For example, the annual cost associated with invasive species is estimated at US\$ 137 billion in the United states alone (Knight, 2001). The concept of biological control has been regarded as one appropriate method to reduce the impact of invasive species below economic thresholds.

Biological control is defined as the use of parasitoids, predators, pathogens, antagonists, or competitors to suppress a pest population, making it less abundant and thus, less damaging than it would otherwise be (van Driesche & Bellows, 1996). The concept of using natural enemies to reduce the densities of pest populations is not new and dates back several thousand years when in China and Yemen ant colonies were used to control pests in tree crops (Coulson et al., 1982). While in the beginning of biological control mainly predators were used, the role of parasitoids was discovered in the 19th century (van Driesche & Bellows, 1996). The American entomologist Fitch was the first to suggest in 1855 the importation of European parasitoids to control an introduced insect pest in North America. However, this idea was first used in 1893, when Riley introduced the European braconid *Cotesia glomerata* Linneaus into the United States against the accidentally imported cabbage pest *Pieris brassicae* (Linneaus) (Coppel & Mertins, 1977). This introduction of an antagonist from the area of pest origin was the first example in history for “classical biological control”. Two different strategies in biological control have to be distinguished: (1) inoculative control, in which natural enemies are introduced with the expectation that they will establish in the new environment and provide a long-term pest suppression by self-reproduction; and (2) inundative control, in which mass-produced biological control agents are released to cause a short-term control without achieving establishment (van Driesche & Bellows, 1996; van Lenteren et al., 2003). The advantage of inoculative biological control over other methods for controlling insect pests is the permanency of the control system, which makes classical biological control programs highly economic (van Lenteren, 2001).

Searching for possibilities to improve the success of biological control, Hokkanen & Pimentel (1984) stated that the success of introducing natural enemies for biological control was about 75% higher employing new parasitoid-host (predator-prey) associations versus those based on

long-evolved associations between parasitoids and hosts. This concept for biological control, suggesting the use of parasitoids that have never been associated with the targeted host, has been defined as “new association”, whereas the introduction of an antagonist from the area of pest origin is defined as “old association”.

Up until 1992, over 2000 species of parasitoids and predators have been introduced against 543 insect pest in almost 200 different countries or islands. Approximately 12% of all parasitoid introductions have led to successful control, but the majority of introductions have failed to control the targeted pest (Greathead & Greathead, 1992). In comparison, for biological control of weeds 30% of the species introductions have been judged as successful (Pimentel et al., 2000). Although the success of biological control programs has been limited in the past, biological control of invasive species is still growing in popularity (Knight, 2001) because concerns over problems associated with chemical control of insect pests, particularly contamination of the environment, increasing development of resistance of insects to pesticides, destruction of natural enemies, and the damage to human health are increasing (van Driesche & Bellows , 1996).

1.3 Risk assessment in biological control

Within the last ten years concerns regarding the safety of biological control have increased, and the ongoing debate recently received public attention (Knight, 2001; van Lenteren et al., 2003). Environmental conservationists argue that classical biological control could have a major environmental cost if introduced natural enemies colonize and disrupt native systems (Hawkins & Marino, 1997). The main areas of concern include the irreversibility of alien introductions, the dispersal of biological control agents to new habitats, and the potential expansion of the agent’s host range and harm to non-target hosts, including native or beneficial insects (“host switching”) (e.g. Howarth 1983, 1991, 2000; Simberloff & Stilling, 1996; Folett et al., 2000; Lockwood, 2000; Michaud, 2002; Kuris, 2003). Furthermore, it has been argued that if chemical and biological control methods were equally specific, then chemical control could pose an even smaller risk to the environment than a living organism that is subject to selection over evolutionary time (Secord & Karieva, 1996). However, negative environmental effects from releases of exotic natural enemies have rarely been reported (Onstad & McManus, 1996; van Lenteren et al., 2003).

In many publications focusing on non-target effects of biological control agents, target specificity has been pointed out as the key to safety in biological control (Nechols et al., 1992; Onstad & McManus, 1996; Secord & Kareiva, 1996; Strand & Obrycki, 1996; Sands, 1997; Keller, 1999; Knight, 2001; van Lenteren et al., 2003). Host specificity can be defined as the degree to which a species restricts its diet (Nechols et al., 1992). To understand host specificity in a natural enemy, it is necessary to know the species' host range which is broadly defined as the set of species that can support development of a parasitoid or serve as prey for a predator (Strand & Obrycki, 1996). Thus, species with one or only a few hosts are defined as specialists, whereas species with many hosts are generalists. The release of generalists has been considered to be more risky for biological control because they are expected to be polyphagous not only in the area of origin but also in the new environment in which they are released. In particular, the use of "new associations" for biological control has been assumed to increase the potential harm to non-targets because this strategy already requires control agents that have more than one host (Onstad & McManus, 1996). Consequently, host specificity assessments are a requirement for all pre-release evaluation programs of arthropod natural enemies (Onstad & McManus, 1996; Kuhlmann et al., 1998, 2000; van Lenteren et al., 2003). To oppose the critics and to ease the fears about insect biocontrol getting out of control, safety of natural enemy releases has been improved recently by the design of testing schemes for host specificity, as were already implemented for weed biological control (Sands, 1988; Barratt et al., 1999; van Lenteren et al., 2003).

1.4 Current control methods for *Lygus* spp. in Canada

Recent outbreaks of *Lygus* spp. in Canada have mainly been controlled by the use of chemical pesticides. After serious problems with *Lygus* had emerged in southern Alberta in 1997, 200,000 ha of canola were sprayed with insecticides; in 1998 the treated area increased to 400,000 ha (Mason & Soroka, 1998; Braun et al., 2001). Few insecticides are registered for *Lygus* spp. in Canada, and their use has raised concerns because insecticides are often sprayed during the peak pollination periods and thus, may present a danger to pollinating insects (Broadbent et al., 2002). Furthermore, Gillespie et al. (2000) stated that the use of insecticides to control *Lygus* would kill beneficial insects that are used for controlling other greenhouse pests. Another risk of using chemical control is the development of resistance of insect pests to insecticides, as it has been noticed recently for *Lygus* populations from cotton in the U.S. (Godfrey et al., 2001).

The idea of using biological control to suppress *Lygus* populations in North America dates back to the 1960s. North American efforts for biological control have been summarized for the United States (Coulson, 1987) and Canada (Craig & Loan, 1987; Carl & Mason, 1996; Broadbent et al., 2002). Among pathogens, the entomophagous fungus *Beauveria bassiana* (Balsamo) Vuillemin has shown some success when used against *Lygus* in cotton fields in the southern US (Ruberson & Williams, 2000). North American predators and parasitoids attacking *Lygus* spp. have been listed in Mason & Soroka (1998). One of these natural enemies, the egg parasitoid *Anaphes iole* Girault, is already commercially available in the USA (Broadbent et al., 2002). Extensive investigations were also focused on the potential of native nymphal parasitoids belonging to the genus *Peristenus* Foerster and *Leiophron* Nees. These parasitoids attack all nymphal instars of *Lygus* spp. and kill their hosts in the L5 stage. In Canada, four native species were found and considered relatively ineffective (Lachance, 2000; Broadbent et al., 2002). Thus, surveys for exotic parasitoids were extended to the Palaearctic where three promising European *Peristenus* species were discovered (Bilewicz-Pawinska, 1982; Coutinot & Hoelmer, 1999); the idea of reducing plant bugs in North America by introducing European parasitoids was born. However, releases of several *Peristenus* species in the late 1970s in the Canadian provinces Alberta and Saskatchewan were not successful (Craig & Loan, 1987). In contrast, the European nymphal parasitoid *Peristenus digoneutis* Loan (Hymenoptera: Braconidae), released against *Lygus lineolaris* in the 1980s in the north-eastern USA (Day et al., 1990; 1992; 1998), caused high levels of parasitization and a significant decrease of *Lygus* densities on alfalfa (Day, 1996). Releases in the US have already resulted in the spread of *P. digoneutis*, and in fact its range of distribution has expanded northwards into southern Quebec (eastern Canada) (Broadbent et al., 1999).

The success of *P. digoneutis* in the USA, increasing problems with *Lygus* spp. in western Canada, and the concern regarding chemical control have renewed interest in research into the potential for the establishment of additional European species of *Peristenus* for biological control of *Lygus* pests in Canada (Kuhlmann, 2000; Broadbent et al., 2002).

Besides *P. digoneutis* Loan, the most promising candidates for biological control of *Lygus* nymphs are regarded to be *P. stygicus* Loan and *P. rubricollis* (Thomson) (Hymenoptera: Braconidae), all known to attack the European tarnished plant bug *Lygus rugulipennis* (Bilewicz-Pawinska, 1982; Coutinot & Hoelmer, 1999). However, the use of these species would be an example of a “new association” and as mentioned above, this concept is assumed

to increase the risk of non-target effects. Thus, host range studies in the area of origin and host specificity testing in Europe and Canada have been recommended prior to future introduction of these species into North America (Kuhlmann et al., 1998; Coutinot & Hoelmer, 1999; Broadbent et al., 2002). Furthermore, Coutinot & Hoelmer (1999) suggested the investigation of plant-host-parasitoid associations and the exploration of promising locations like Scandinavia to locate appropriate climatic strains.

1.5 Thesis outline

With this thesis I want to provide data on the host specificity of *Lygus* parasitoids belonging to the genus *Peristenus*, in an effort to assess potential risks for introducing these European parasitoids as biological control agents of native *Lygus* species in Canada. I also want to elucidate additional aspects of the parasitoid's biology relevant for biological control, to guide the choice of parasitoid species for possible introduction, and to improve the chances of successful releases.

Although there has been much research conducted on *Lygus* species in Schleswig-Holstein (northern Germany), the *Lygus* parasitoid complexes have never been defined in this region. Therefore, chapter 2 presents data on the distribution, seasonal abundance and synchronization of different *Lygus* hosts and their associated nymphal parasitoids collected in various habitats. These results are discussed in context with data from other European regions.

In the following chapters (3 and 4) two different procedures are demonstrated to examine the host specificity of the potential *Lygus* control agents that were found in the area of investigation. Chapter 3 addresses results from laboratory tests on selected non-target species with the aim of defining the physiological host range of the control agents. In contrast, extensive data from field studies of parasitoid-host complexes are presented in chapter 4 in order to define the ecological host range of the parasitoids. These field data provide the basis for interpreting host range estimates made via the laboratory tests described in the previous chapter.

Following a reductionist approach to agent selection, chapters 5 and 6 are focused on two characteristics of parasitoids which are considered to embody their potential as biological control agents. In chapter 5, aspects of the reproductive biology of *Lygus* parasitoids are

compared; this has been largely neglected in previous studies, and is important for successful establishment of a biological control agent. The question whether European *Lygus* parasitoids have the ability to survive extended periods of temperatures below 0°C is essential when considering releases in western Canada; this aspect is examined in chapter 6 which presents data on parasitoid cold-hardiness.

In chapter 7 all information accumulated on host specificity, fecundity, and cold-hardiness of nymphal *Lygus* parasitoids is discussed in a broader context.

2 Seasonal occurrence of *Lygus* spp. (Hemiptera: Miridae) and parasitization of their nymphs by *Peristenus* spp. (Hymenoptera: Braconidae) in Schleswig-Holstein (Germany)

2.1 Introduction

In many European countries, the most common *Lygus* species is the European tarnished plant bug, *Lygus rugulipennis* Poppius (Bilewicz-Pawinska & Varis, 1985; Boness, 1963; Afscharpour, 1960; Varis, 1995, 1997). It occurs throughout the Palaearctic region from Spain to Far East Russia and Japan (Schwartz & Footitt, 1998) and has been recorded from 437 host plants in 57 families (Holopainen & Varis, 1991). Recently it has been described from the nearctic region (western cordilleran system from Alaska to southern Colorado), where it is widely distributed at high elevation and latitude (Schwartz & Scudder, 1998). In Europe, *Lygus rugulipennis* holds nearly the same ecological niche as the economically important pest *Lygus lineolaris* in North America (Day, 1987) and thus, parasitoids of the European species have been suggested for biological control of North American *Lygus* populations (Drea et al., 1973; Day, 1987; Coutinot & Hoelmer, 1999).

In Schleswig-Holstein (northern Germany) *L. rugulipennis* (Fig. 2.1) and occasionally *L. pratensis* are very common, but are rarely reported as serious pests, suggesting that they are kept below the economic threshold by natural enemies. However, there is only limited information on parasitoids and predators attacking *Lygus*, despite intense investigations on the ecology of *L. rugulipennis* and *L. pratensis* on cultivated plants in Schleswig-Holstein in the 1960s (Afscharpour, 1960; Boness, 1963).

Figure 2.1

Adult *Lygus rugulipennis*
Poppius

(photo: T. Haye)



This chapter presents data on the seasonal occurrence and synchronization of *Lygus rugulipennis* and associated nymphal parasitoids collected in various habitats in Schleswig-Holstein. Furthermore, the nymphal parasitoid complexes of *Lygus pratensis* and *L. maritimus* are defined.

2.2 Material and methods

2.2.1 Study area and field sites

Sampling of *L. rugulipennis* and *L. pratensis* was conducted in clover and camomile sites in the eastern parts of Schleswig-Holstein (“östliches Hügelland”), Northern Germany, during the 2001-2003 growing seasons. For *L. maritimus*, surveys and collections were made along the west coast of Schleswig-Holstein (“Marsch”).

During the 2001/2 field seasons a continuous sampling program was carried out from May to September to determine the seasonal abundance of *L. rugulipennis* and its parasitoids. For that reason, a single red clover field (*Trifolium pratense* L.), two fields dominated by clover and vetches (*Vicia* sp.), and two non-crop sites with stands of *Matricaria recutita* (L.) (= “Real camomile”) and *Tripleurospermum perforatum* (Mér.) (= “Scentless camomile”) Wagner were selected. Stands with *Matricaria recutita* were chosen because this weed is one of the preferred host plants of *Lygus* in Sweden, Finland and northern Germany (Kullenberg, 1944; Boness, 1963; Varis, 1972). Clover/vetches fields were chosen because leguminous crops are also known to be preferred habitats for *Lygus* plant bugs (Boness, 1963). Clover fields sampled and adjacent areas were not treated with insecticides at any time. Collections at exactly the same sites during successive years were not possible, because field crops were rotated after each growing season. The following briefly summarizes the sampling sites:

1. Lütjenbrode-Eichtal, (N 54°21'52 E 11°01'49), (2001): At the beginning of the collections (May 27th –June 14th) the field was planted with a mixture of canola, red clover, Persian clover and vetches (“Landsberg”- mixture). After June 14th, the field has been cut and sampling was continued one month later (July 15th) after the plants had grown again. At this time the field vegetation was dominated by red clover and a little Persian clover (Fig. 2.2). Adjacent fields were planted with mustard and wheat. In late season, when the quality of the clover plants had deteriorated and almost no *Lygus* nymphs were found, additional samples were taken from an adjacent, blooming mustard field.



Figure 2.2 Red clover sampling site at Lütjenbrode - Eichtal (August 11th, 2001).

2. Lütjenbrode II, (N 54°21'52 E 11°01'49), (2002): This field was located approximately 100 m away from the first field, where samples were taken in 2001. It was also planted with “Landsberg” mixture at the beginning of the season, and was cut in early June. However, sampling was continued on a small field strip the farmer had left uncut. The site was adjacent to a wheat field and uncultivated grass land.

3. Lindhöft, (N 54°27'32 E 09°58'37), (2002): In contrast to the previously described fields, this location was a red clover monoculture. Parts of the field had been cut at the beginning of June, but sampling was still possible in the remaining areas. Adjacent fields were planted with canola and oats.

4. Rastorf, (N 54°21'52 E 11°01'49), (2002): The location at Rastorf (Fig. 2.3) was a natural stand of *Matricaria recutita* with only single plants of *Tripleurospermum perforatum*. It was located in a small gravel pit surrounded by forest, an unmanaged weedy area, and a canola field. At the beginning of June nearly all *Matricaria* plants were senescent or dried out and only *Tripleurospermum* plants were still in good condition. Additional collections in late season were carried out in a small area where the soil had been tilled in June, allowing new *Matricaria* plants to grow.

5. Sophienhof, (N 54°12'30 E 10°20'19), (2002): This site was adjacent to a wheat field in a small, damp hollow. The vegetation was dominated by *M. recutita* in the first half (June) and - *T. perforatum* in the second half of the summer (July, August). Collections were stopped in late August when the field had been ploughed.



Figure 2.3 Camomile sampling site at Rastorf (June 10th, 2002).

2.2.2 Sampling methods

Two types of sampling methods were used:

Continuous collections were conducted at the field sites previously described (see 2.2.1) throughout the field season at intervals of 5 to 10 days, depending on the weather conditions. The data yielded information on the annual changes in density of *Lygus* nymphs and adults, distribution of different age classes, parasitization of nymphs and parasitoid species composition. In each of the five test fields, sweep net samples (20 sweeps) were taken in two transects of five samples each. A standard sweep net (38 cm diameter) was used for all collections. The sweep net samples were first transferred into white plastic trays, and *Lygus* adults were counted and released. Nymphs were then separated from other insects and spiders using a mouth aspirator. Collected nymphs were maintained on foliage in ventilated, clear

plastic containers and later transported in a cooler to the laboratory, where nymphs were separated by instar to analyze the frequency distribution of the five nymphal instars.

To compare parasitoid species composition in camomile and clover on a larger scale, *mass collections* were made at numerous clover and camomile sites in eastern Schleswig-Holstein when high infestations with late instar *Lygus* nymphs were observed. However, these sites were usually sampled only once during a season. *Lygus* nymphs (L3-L5) collected with sweep nets were transferred into plastic transport buckets containing large strips of paper and some fresh food (beans or host plants) to reduce cannibalism among nymphs. Each bucket contained a maximum of 1000 nymphs; L1-L2 nymphs were not collected. The top rims of the buckets were coated with Fluon® GP1 (polytetrafluorethylen, a Teflon based polymer produced by Whitford Worldwide) to prevent escape during transport.

2.2.3 Determination of parasitization level and parasitoid species composition

From each collection, subsamples of 30 to 70 late instar nymphs (L3-L5) were taken randomly and dissected for parasitoid eggs or larvae in order to establish the parasitization level in nymphs. The nymphs were dissected either on the same day or after storage at -20°C . Since the immature stages of the parasitoid species found in dissections are morphologically indistinguishable (Bilewicz-Pawinska & Pankanin, 1974), the remaining nymphs from each site were reared until adult parasitoids emerged in the same or the following year. Morphological characteristics of the adults were then used to identify the parasitoid species responsible for the observed parasitization level. When nymphal densities were low during the continuous collections, additional sweeps were taken to enlarge sample size for rearing. As an exception, the parasitization levels at Lütjenbrode-Eichtal (2001) were only estimated by rearing instead of both rearing and dissections. On some occasions when small instar nymphs were dominant in the field, samples of L1 and L2 nymphs were taken additionally to compare the parasitization levels among early and late instar nymphs. In this case, small nymphs were reared at least to the third instar before being dissected in order to facilitate detection of the developing parasitoid.

2.2.4 Rearing

Depending on the sample size, three different rearing systems were used. The rearing system for small sample sizes (1 –10 nymphs) consisted of a single container made by stacking two

120 ml clear polyethylene cups that close with a screw top lid (Fig. 2.4 a). The bottom of the inside cup was removed and replaced with a piece of gauze. The bottom of the outer container was filled with moist vermiculite (opticult*, optima-Werke, Switzerland) to offer the parasitoid larvae a pupation site. The container lid had a hole covered with fine gauze for air circulation.

The rearing system for samples ranging from 11 to 50 nymphs consisted of 1.2 L plastic containers fitted with removable Petri dishes on the bottom (Fig. 2.4 b). The Petri dishes were filled with moist vermiculite and separated from the rest of the container by a round piece of gauze which allowed larval parasitoids gain access to the Petri dish to pupate. In this way, *Lygus* nymphs were kept away from the freshly emerged parasitoid larvae, which was necessary because *Lygus* can also be entomophagous (Boness, 1963).

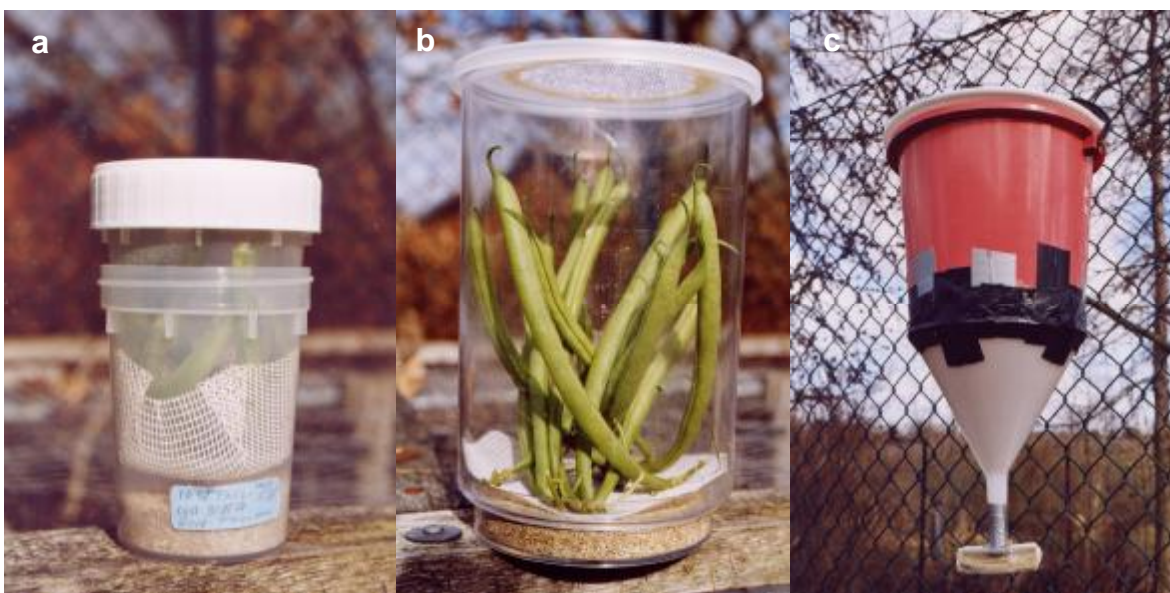


Figure 2.4 *Lygus* rearing systems for (a) small (1 - 10 nymphs), (b) medium (11 - 50 nymphs), and (c) large sample sizes (max. 500 nymphs).

Mass collected samples were kept in plastic buckets with the bottoms removed and replaced with fine white gauze instead (Fig. 2.4 c). Plastic funnels terminating in vermiculite-filled Petri dishes were attached to the bottom of the buckets in order to collect emerging parasitoid larvae that fall through the gauze. Each bucket was filled with no more than 500 nymphs to reduce cannibalism among nymphs. Petri dishes were checked for parasitoids cocoons every day and were exchanged when more than 100 cocoons were counted.

In order to provide food for the nymphs, fresh beans were added to all rearing systems every three or four days, depending on the food quality. After all nymphs had reached the adult stages or parasitoid larvae had emerged, the Petri dishes and cups containing the vermiculite with the parasitoid cocoons were removed and stored in an outdoor wooden shelter.

2.2.5 Overwintering

In September, after the last nymphs had disappeared in the field and parasitoid emergence had ended, the remaining parasitoid cocoons from all samples were counted and the vermiculite was replaced with fresh, clean, slightly moistened vermiculite to reduce the risk of mortality caused by fungus during the overwintering period. All samples were stored in a subterranean insectary, where temperatures never dropped below + 2 °C, or in an outdoor wooden shelter until the following spring. In late December, samples were checked for moisture and if necessary a few drops of water were added to the vermiculite. The storage temperature was monitored with Testostor 175 mini loggers.

2.2.6 Monitoring of parasitoid emergence

In April 2002, after cold storage for six months all overwintering cocoons from 2001 were transferred into a climatic chamber (temperature: 21°C, light period: 16 h, RH: 60%) to induce parasitoid emergence. Over a period of six weeks the emergence of parasitoids was checked daily. Emerged adults were removed from containers and individually placed in small, labelled glass vials containing 70% alcohol for later identification. In 2003 the same procedure was followed, but samples from 2002 were overwintered outside in an open wooden shelter to monitor the emergence under natural temperature conditions in order to examine if the parasitoid emergence is synchronized with the appearance of their *Lygus* hosts in the field. The parasitoid cocoons obtained from the 1st *Lygus* generation in June had to be monitored daily throughout the summer for emergence of plurivoltine parasitoids. In 2001, all samples were stored outside, whereas in 2002, samples were transferred into a climatic chamber (21°C) in July, after the first parasitoid had emerged outside.

2.2.7 Data analysis

The following biocenotic indices (Mühlenberg, 1989) were used to compare the structure of nymphal parasitoid communities at different camomile and clover sites:

1. Dominance index (d) for measuring the relative frequency of each species in comparison with the total number of individuals at each site
2. JACCARD's coefficient (JC) for comparing species identity among species communities
3. RENKONEN's coefficient (RC) for comparing the correspondence of dominance structures among species communities
4. Similarity index after WAINSTEIN (K_w) for comparing not only the numbers of species common to each site, but also their relative frequencies

Differences in parasitoid species composition and parasitization levels were analysed with the statistical software SPSS 8.0 for Windows.

2.3 Results

2.3.1 Seasonal occurrence of *Lygus rugulipennis* in clover and camomile

Hibernated *Lygus* adults first appeared in the second half of April in clover and camomile stands, where they immediately started ovipositing in newly grown plants. The number of adults per 50 sweeps was usually not higher than one or two. At all clover sites the first nymphs (L1) were observed in the last week of May or first week of June (Fig. 2.5). This early period was not monitored in camomile stands, but the occurrence of nymphs was likely similar. Depending on the collection site, the peak of the 1st nymphal generation was reached in the third week of June (Lütjenbrode II, Fig. 2.6 a) or in the first week of July (Lindhöft, Fig. 2.6 b).

In clover the density of 1st generation nymphs never exceeded 20 nymphs / 20 sweeps at all three sites. In comparison, nymphal densities in *Matricaria* stands (Figs. 2.7 a,b) were considerably higher (40-60 nymphs / 20 sweeps). At all sites the peak of new adults occurred in the first week of July and densities observed were between 20 and 50 adults / 20 sweeps.

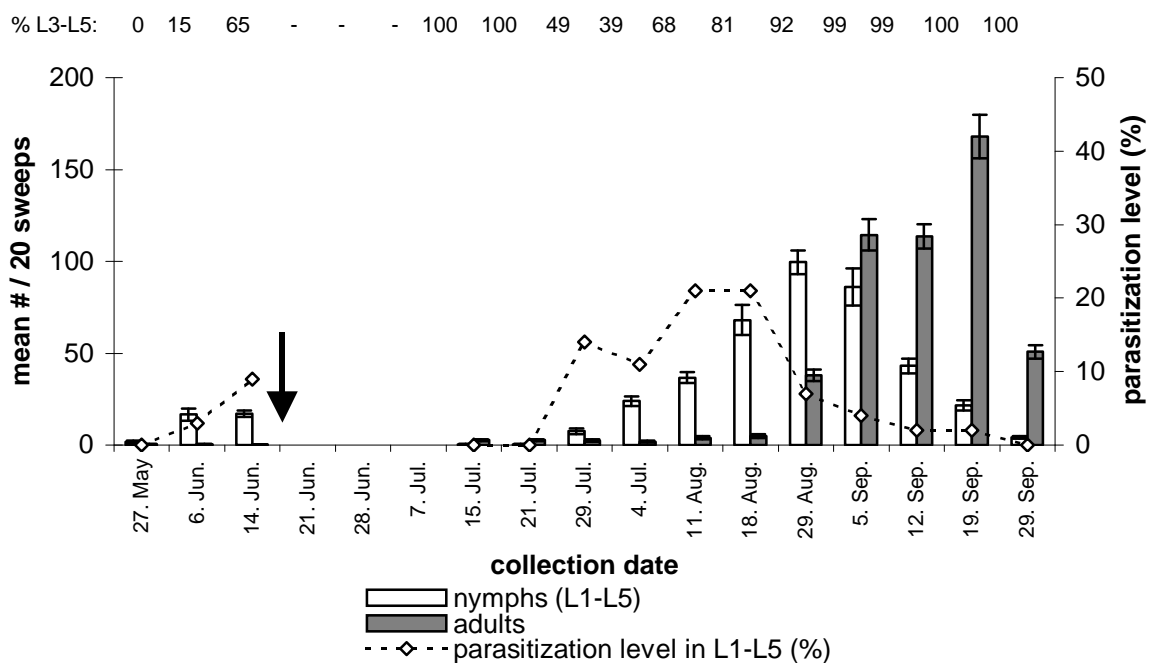


Figure 2.5 Nymphs and adults of *L. rugulipennis* per 20 sweeps (mean \pm SE) and parasitization level in nymphs by *Peristenus* spp. (estimated by rearing) from samples collected in clover at Lütjenbrode-Eichtal in 2001 (total number of nymphs collected: 3303 ; % L3-L5 = proportion of L3-L5 nymphs relative to the entire number of nymphs; 100% = all larval instars (L1-L5) at each date of sampling; the arrow indicates field cut).

After the adult peak nymphal densities were always low and did not increase before the end of July, when the 2nd generation of nymphs emerged (indicated by a high proportion of L1-L2 nymphs in the collections). Depending on the collection site, nymphal density (2nd generation) peaked between the second week of August (Sophienhof) and the first week of September (Lindhöft). In Lütjenbrode II nymphal densities of the 2nd generation were up to five times higher (approximately 100 nymphs / 20 sweeps) than in the 1st generation (fig. 2.6 a), whereas in Lindhöft they were only two times higher (Fig. 2.6 b). In Sophienhof, where old *Matricaria* plants were almost replaced by newly grown *Tripleurospermum* plants, only minor differences between nymphal densities of both generations were observed. In Rastorf, however, where at the same time the majority of the *Matricaria* plants were already dried out and only a few new plants were grown, the nymphal density was much lower than in the first generation and not more than 20 nymphs / 20 sweeps were counted (Fig. 2.7 a). The second peak of new adults occurred between late August and mid September. In 2001, on average 160 adults / 20 sweeps were found in Lütjenbrode-Eichtal (Fig. 2.5); this was the highest adult density ever observed during these studies. At two other sites high numbers of nymphs were also found, even in September. In early September 2001, when nymphal densities in red clover at Lütjenbrode-Eichtal (Fig. 2.5) were already low and plants almost deteriorated,

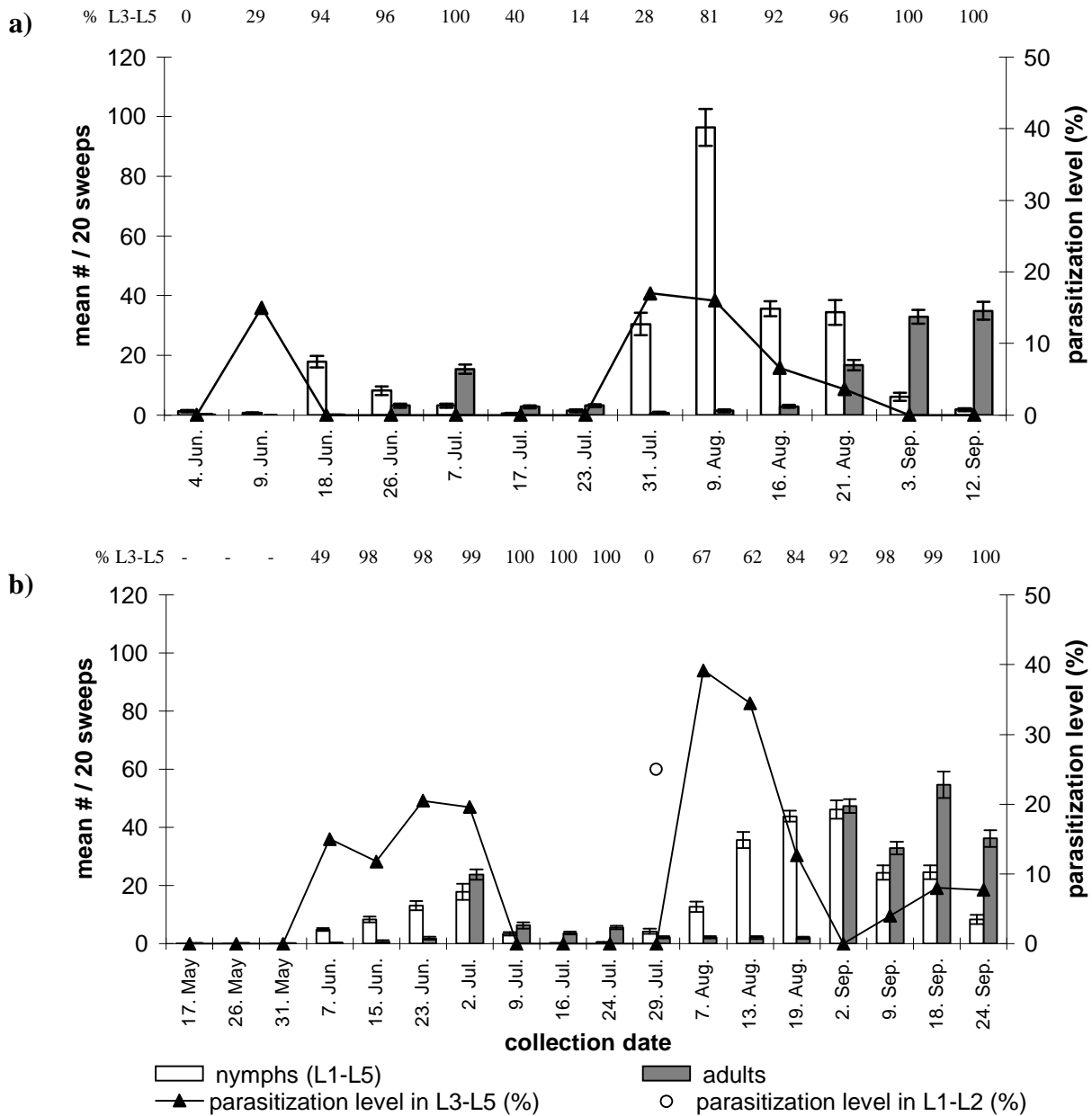


Figure 2.6 Nymphs and adults of *L. rugulipennis* per 20 sweeps (Mean \pm SE) and parasitization level in nymphs by *Peristenus* spp. from samples collected in 2002 in clover fields at a) Lütjenbrode II (total number of nymphs collected: 2629) and b) Lindhöft (total number of nymphs collected: 2512); (% L3-L5 = proportion of L3-L5 nymphs relative to the entire number of nymphs; 100% = all larval instars (L1-L5) at each date of sampling).

higher densities of small nymphs were recorded in an adjacent field with flowering mustard plants (Fig. 2.8 b). In Rastorf a similar situation was observed in 2002 (Fig. 2.8 a). In an area that was only 20 m away from the first collection site, soil had been removed in June, allowing new *Matricaria* plants to grow. In this area the nymphal density reached a remarkable 100 nymphs / 20 sweeps. Adults of the late summer generation were observed

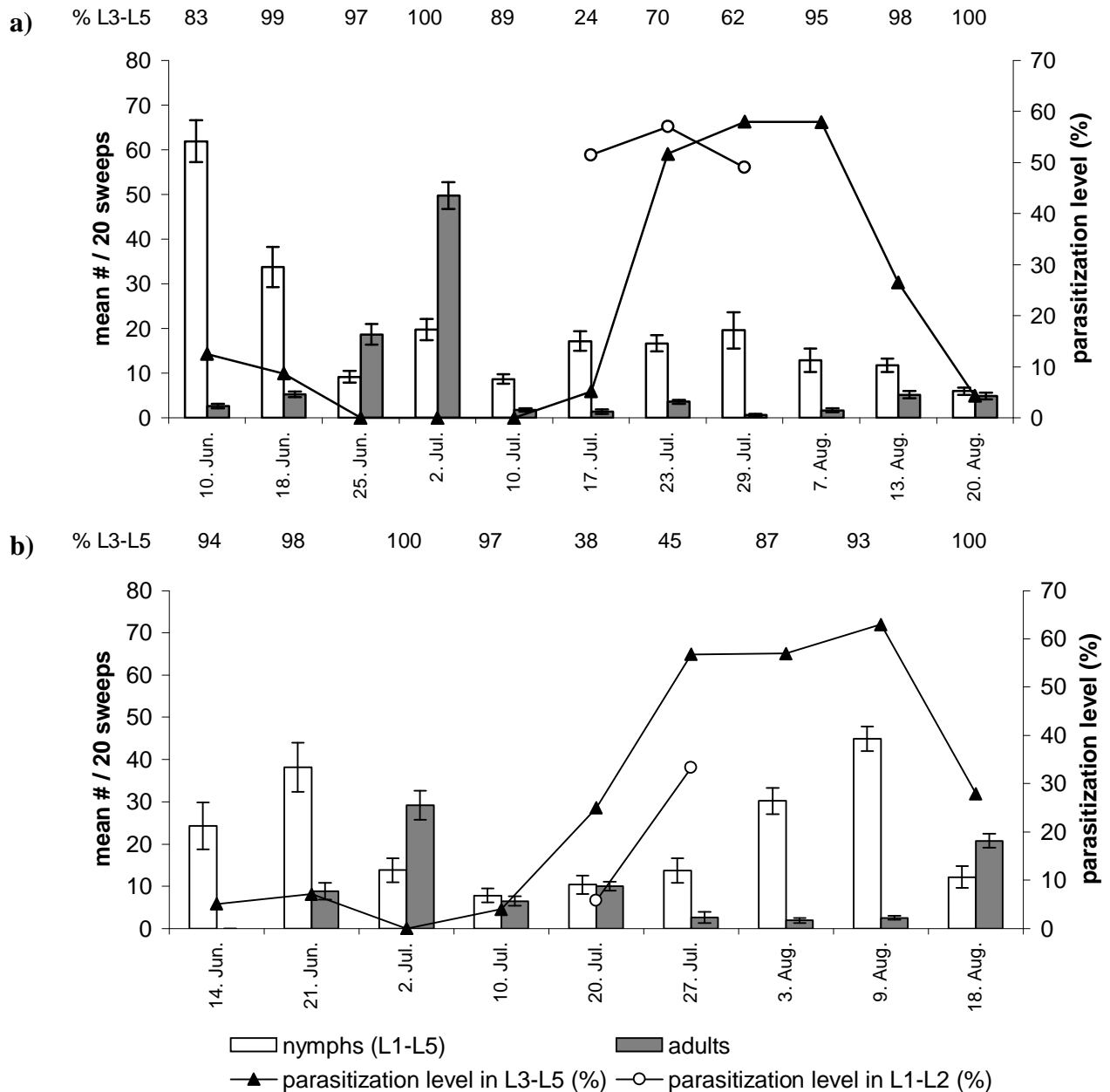


Figure 2.7 Nymphs and adults of *L. rugulipennis* per sweeps (Mean \pm SE), and parasitization level in nymphs by *Peristenus* spp. from samples collected in camomile stands in 2002 at a) Rastorf (total number of nymphs collected: 4133) and b) Sophienhof (total number of nymphs collected: 4482); (% L3-L5 = proportion of L3-L5 nymphs relative to the entire number of nymphs; 100% = all larval instars (L1-L5) at each date of sampling).

feeding on their host plants until late autumn, and they often accumulated at small patches where fresh plant material was still available.

2.3.2 Parasitization level of *L. rugulipennis* nymphs in clover and camomile

In Lütjenbrode-Eichtal (Fig. 2.5) the first parasitized nymphs occurred in early June (observed by rearing all instars in 2001). The highest density of parasitized nymphs of the 1st generation

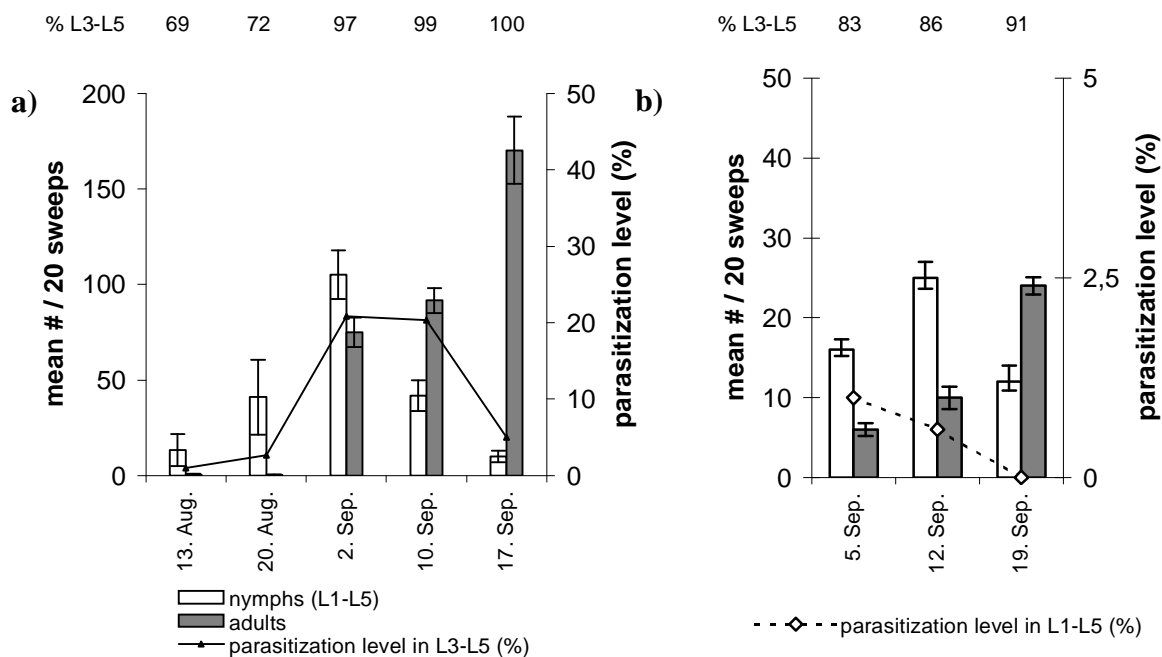


Figure 2.8 Nymphs and adults of *L. rugulipennis* per 20 sweeps (Mean \pm SE) and parasitization level in nymphs by *Peristenus* spp. from samples collected in a) newly grown camomile at Rastorf in 2002 (total number of nymphs collected: 2089) and b) blooming mustard at Lütjenbrode-Eichtal in 2001 ((total number of nymphs collected: 523); (% L3-L5 = proportion of L3-L5 nymphs relative to the entire number of nymphs; 100% = all larval instars (L1-L5) at each date of sampling).

(L3-L5) was usually found in the second and third week of June. The maximum parasitization level (in L3-L5) measured at clover and camomile sites ranged from 5 to 22% and 8 to 12%, respectively. The parasitization level found in single mass collections in camomile was similar and varied between 4 and 27 % (n = 8 sites, Appendix, Table 1). In the 2nd nymphal generation, the highest density of parasitized nymphs (L3-L5) was found between the end of July and mid-August. In clover the highest parasitization levels observed during regular collections ranged from 17% to 39% (Figs. 2.5, 2.6). Remarkably, at all clover sites the parasitization level (in L3-L5) always peaked before the highest density of nymphs was observed in the field. At both continuously sampled camomile sites the parasitization level in the 2nd nymphal generation increased to 63% and was markedly higher than in the 1st generation. At two additional camomile sites (Heikendorf, Gettorf; Appendix, Table 1 and 3), where mass samples were also taken from both *Lygus* generations, a similar increase of the parasitization level was noticed.

At Rastorf (Fig. 2.7 a) small instar nymphs (L1-L2) were greatly dominating (17 July), and the parasitization level in late instar nymphs (L3-L5) was low (5%), whereas in small nymphs it was high (56%). At Sophienhof (Fig. 2.7 b) the opposite trend was observed. Although the

proportion of small nymphs was high (62%, 20 July), the parasitization level in small nymphs was lower (6%), than in late instar nymphs (25%).

In general, a significant decrease of the parasitization level was noticed when adult densities were increasing. The last parasitized nymphs were found in the last week of September (Fig. 2.6 b). In late season the parasitization level was maximum 1% in mustard (Lütjenbrode-Eichtal), whereas in newly grown *Matricaria* (Rastorf) it was 21% (Fig. 2.8).

No significant difference in parasitization levels was found in mass samples from the second nymphal generation (Appendix, Table 2 and 3) collected in clover and chamomile stands (Mann-Whitney test: $Z = -0.773$; $P = 0.439$; n.s., $n = 14$ clover and 12 chamomile sites). In clover and chamomile sites surveyed over time, parasitization levels ranged from 6 to 62% (mean = 32.6%) and from 14 to 63 % (mean = 36.1%), respectively (Fig. 2.9). In Lütjenbrode-Eichtal where several mass collections were conducted, a drastic decrease of the parasitization level from 24% (August 11th) to 9% (August 29th) was observed (Appendix, Table 4).

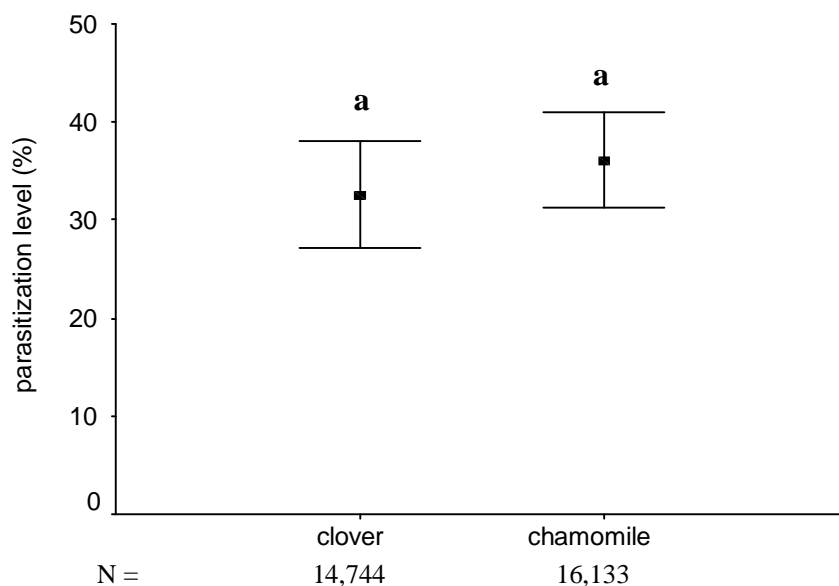


Figure 2.9 Parasitization level (%) in samples from *Lygus* mass collections (2nd nymphal generation) in 14 clover and 12 chamomile stands (N = total number of nymphs collected; Mann-Whitney test: $P = 0.44$, n.s.; vertical bars show standard errors and means with the same letter are not different).

2.3.3 Parasitoid species composition

All parasitoids reared from the first and second generation of *Lygus* nymphs were the primary

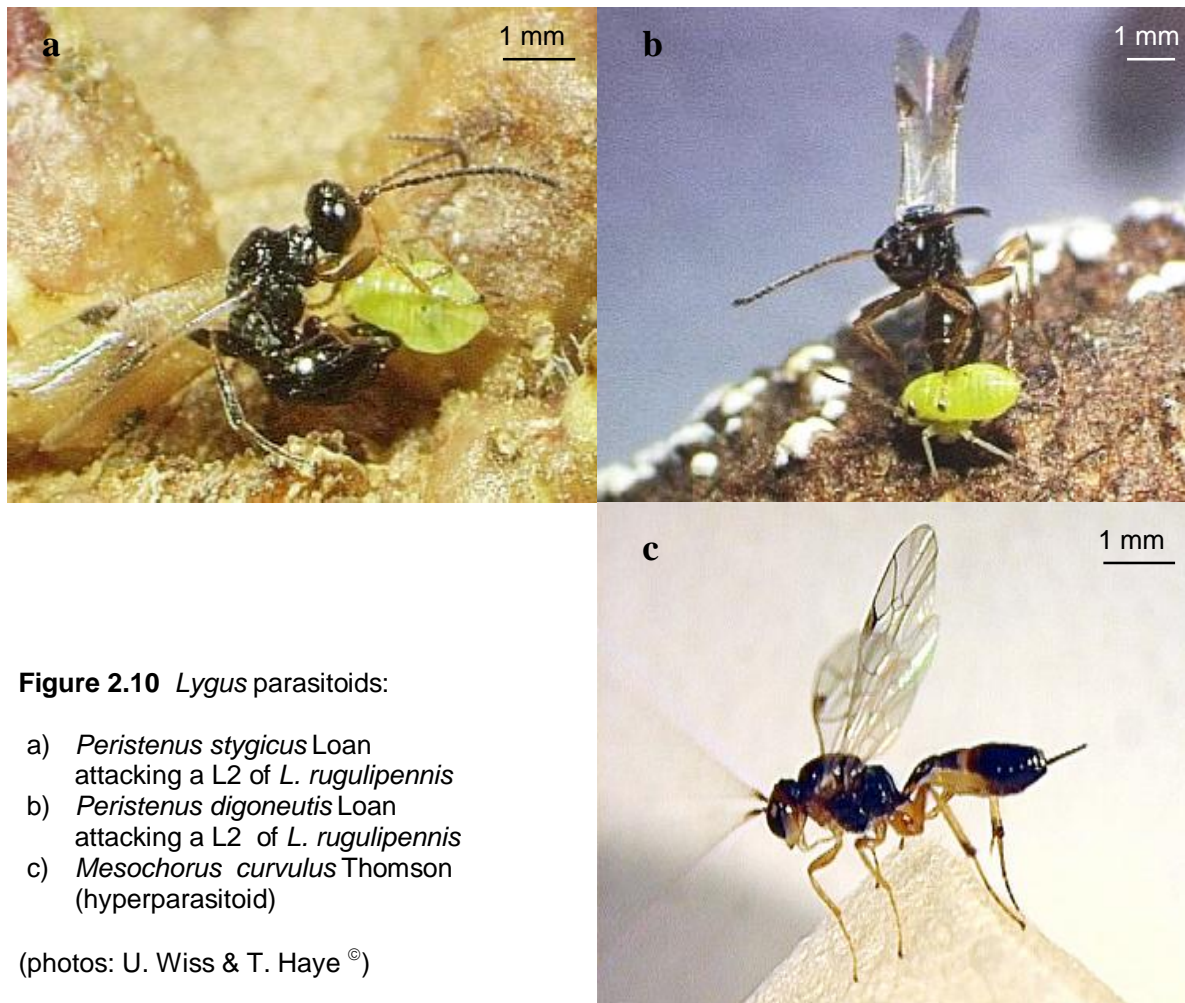


Figure 2.10 *Lygus* parasitoids:

- a) *Peristenus stygicus* Loan attacking a L2 of *L. rugulipennis*
- b) *Peristenus digoneutis* Loan attacking a L2 of *L. rugulipennis*
- c) *Mesochorus curvulus* Thomson (hyperparasitoid)

(photos: U. Wiss & T. Haye ©)

parasitoids *Peristenus stygicus* Loan and *Peristenus digoneutis* Loan (Hymenoptera: Braconidae) or the hyperparasitoid *Mesochorus curvulus* Thomson (Hymenoptera: Ichneumonidae) (Fig. 2.10). The numbers of parasitoids reared and the proportions of each parasitoid species varied greatly with each collection date; thus, in the following the proportion of each parasitoid is related to the entire number of parasitoids that were reared from each *Lygus* generation. Proportions of every single collection are presented in Figure 2.11 and 2.12.

In red clover at Lindhöft (Fig. 2.11 a; n = 42 parasitoids), *P. digoneutis* was the most frequently occurring species in the first nymphal generation of *Lygus* (54%), followed by *P. stygicus* (42%) and *Mesochorus curvulus* (4%). From collections at Lütjenbrode-Eichtal (first nymphal generation) only 14 parasitoids emerged in 2001, 11 belonging to *P. digoneutis* and 3 to *P. stygicus* (Fig 2.11 c); in 2002, two adults emerged (1 *P. stygicus* and 1 *P. digoneutis*; Fig. 2.11 b). In Lindhöft and Lütjenbrode-Eichtal *P. digoneutis* was the first species found to cause parasitization of nymphs in the field. At the continuously sampled camomile sites

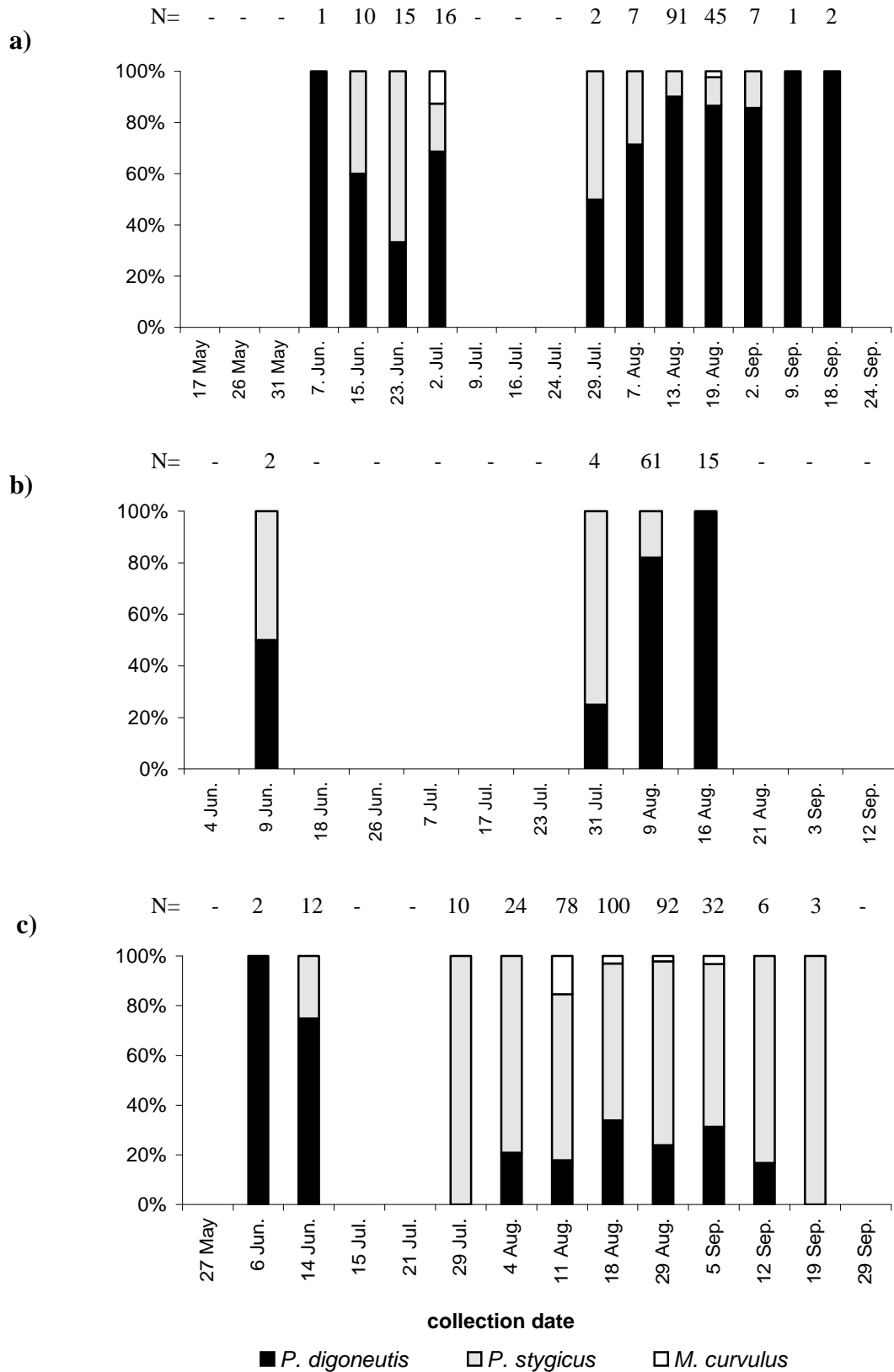


Figure 2.11 Temporal pattern of nymphal parasitoid occurrence at three clover sites: a) Lindhöft, 2002; b) Lütjenbrode II, 2002 and c) Lütjenbrode-Eichtal, 2001 (N = no. of parasitoids reared). The large gaps between the groups of bars indicate the period between the 1st and 2nd parasitoid generation.

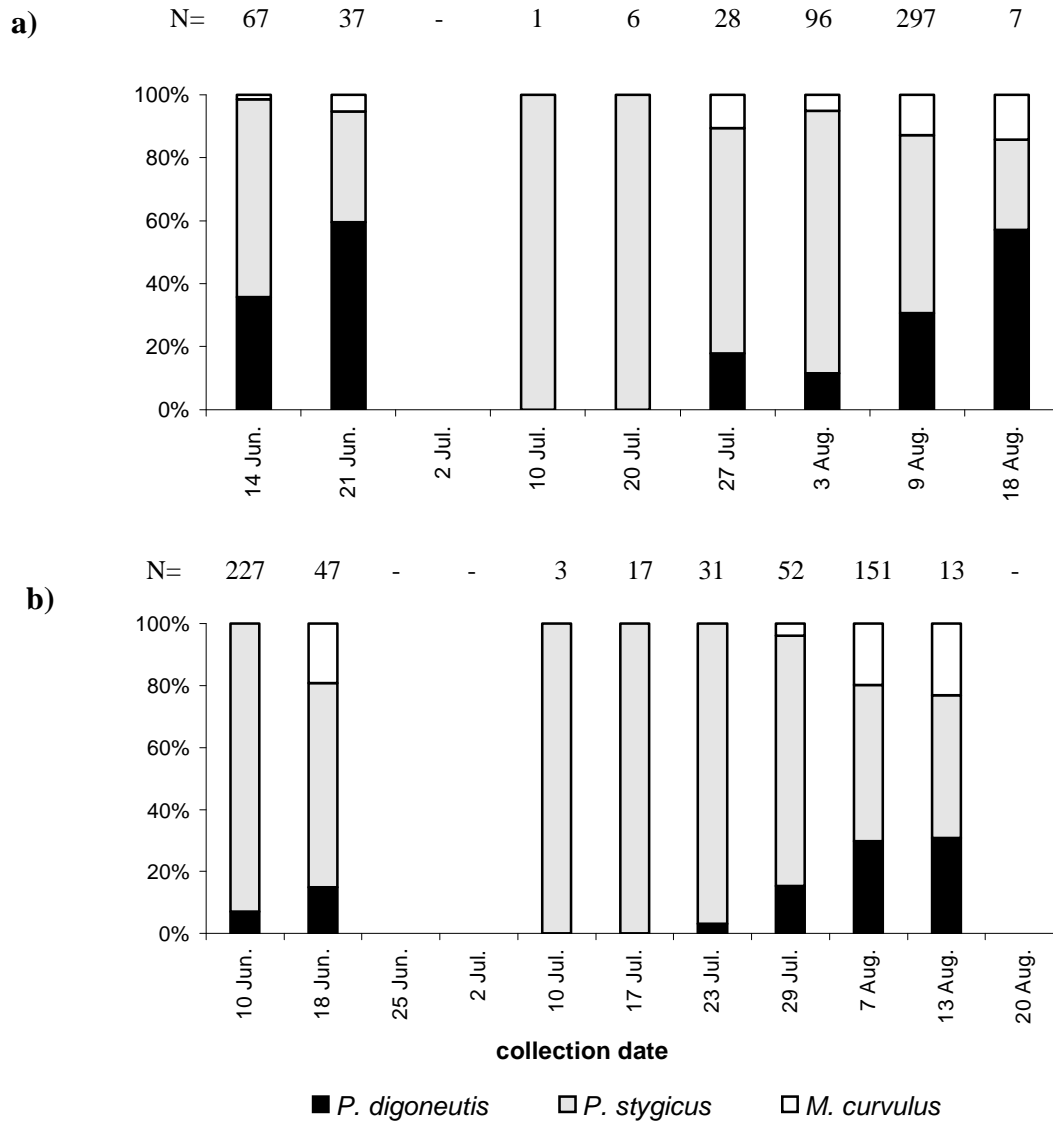
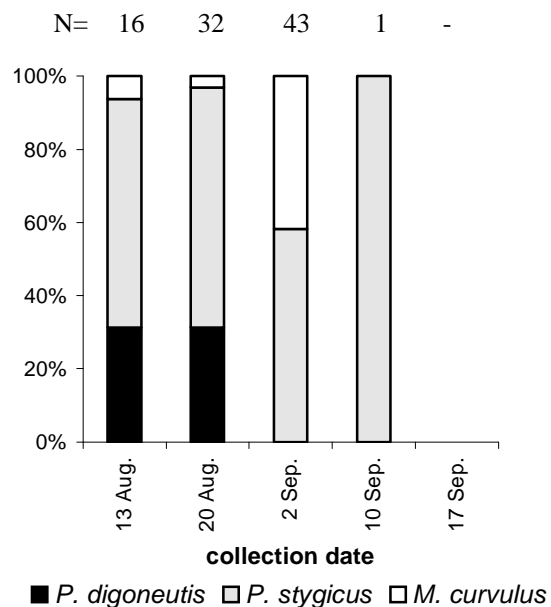


Figure 2.12

Temporal pattern of nymphal parasitoid occurrence at two camomile sites in 2002 (N = no. of parasitoids reared):

- a) Sophienhof
- b) Rastorf
- c) Rastorf (late season)

The large gaps between the groups of bars indicate the period between the 1st and 2nd parasitoid generation.



(Figs. 2.12 a,b) *P. stygicus* was not only dominant in Rastorf (88%), but also in Sophienhof (53%); the portions of *P. digoneutis* were 9 and 44%, respectively. *Mesochorus curvulus* represented 3% of all specimens from each site. In the second nymphal generation, *P. digoneutis* was clearly the dominant species at the clover sites in Lindhöft (88%) and Lütjenbrode II (82%) (Figs. 2.11 a,b). However, at Lütjenbrode-Eichtal *P. stygicus* was more frequent (69%) and the proportion of *P. digoneutis* was only 25% (Fig. 2.11 c). The total amount of hyperparasitoids was 5% or less at all three clover sites. From samples collected in mustard (Lütjenbrode-Eichtal) no parasitoids emerged.

Patterns of parasitoid occurrence and species composition at both camomile sites sampled in 2002 (Figs. 2.12 a, b, c) were very similar to those observed in red clover at Lütjenbrode-Eichtal in 2001 (Fig. 2.11 c). *Peristenus stygicus* was not only the first parasitoid that caused parasitization of nymphs in the field (2nd week of July), but with a proportion of 64% (Sophienhof) and 65% (Rastorf), it was also the most common species. Parasitization caused by *P. digoneutis* was first observed in the third week of July. However, in Sophienhof and Rastorf the proportion of *P. digoneutis* increased with almost every collection and represented in total 26% and 22%, respectively. The total proportion of hyperparasitoids was 13% and 10%, respectively.

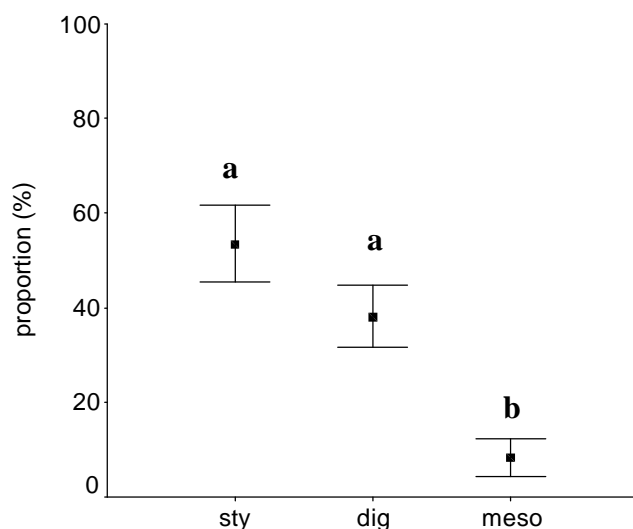


Figure 2.13

Proportion of parasitoids (%) emerged from *Lygus* mass samples (first nymphal generation) collected in camomile stands ($n = 8$; sty = *P. stygicus*; dig = *P. digoneutis*; meso = *M. curvulus*; Wilcoxon signed ranks tests: sty-dig: $P = 0.33$; dig-meso: $P < 0.05$; sty-meso: $P < 0.05$; vertical bars show standard errors and means with the same letter are not different).

The mean proportions of *P. stygicus*, *P. digoneutis*, and *M. curvulus* in mass collections in camomile ($n = 8$; 1st nymphal generation of *Lygus*) were 54% (range: 26 to 88%), 38% (range: 8 to 66%), and 8% (range: 0 to 35%), respectively (Fig. 2.13; Appendix, Table 1). The

mean proportion of a species is equivalent to the dominance index d (e.g. the mean proportion of species A is 54%, then the mean dominance index for species A is 0.54). The mean proportions of the primary parasitoids did not differ significantly (Wilcoxon signed ranks test: $P = 0.33$). The RENKONEN's coefficient (RC) is used to compare the correspondence of dominance structures among species communities. The RC can range from 0 to 1; a RC of 1 indicates that the dominance structure among the species communities is identical. In the present study, a relatively low mean RC (0.69) shows the variation in the dominance structure among the parasitoid species found at each camomile site (see Appendix, Table 5 a). In more than half of the sites *P. stygicus* dominated, whereas *P. digoneutis* dominated at three sites. The JACCARD's coefficient (JC), which can range from 1 to 100, is used to compare the species identity among species communities. If a species community consists of exactly the same species, the JC is 100. In almost all camomile sites sampled, all three parasitoids occurred and thus, the JC was found to be high (mean JC = 92; Appendix, Table 5 a). In order to determine the similarity index after WAINSTEIN (K_W), not only the numbers of species common to each site, but also their relative frequencies are considered ($K_W = JC \times RC$). The K_W can vary between 1 and 100. K_W -values close to 100 suggest that species communities are very similar. The mean K_W in camomile stands was 64 (Appendix, Table 5 b) and thus, the similarity of parasitoid communities was relatively low.

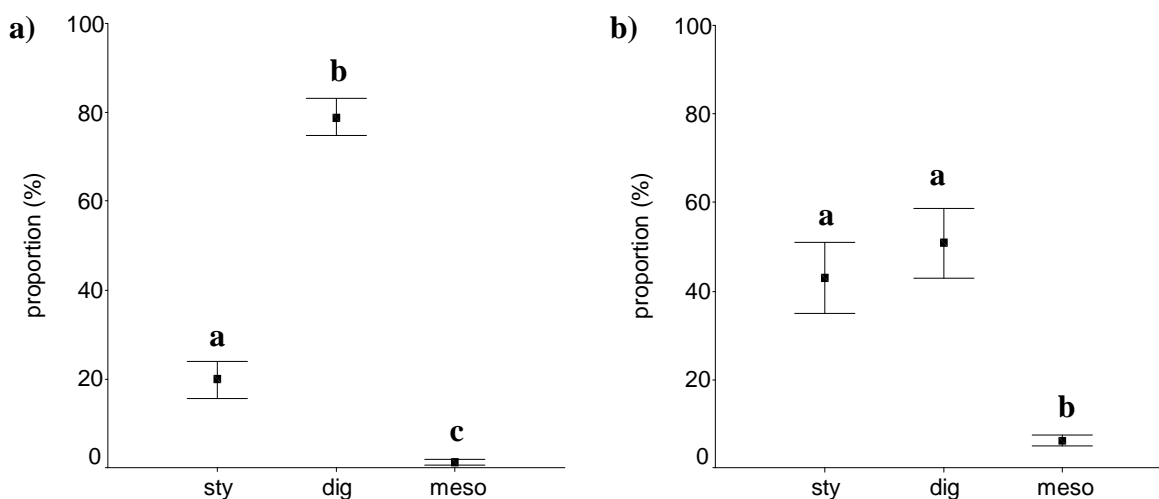


Figure 2.14 Proportions of parasitoids (%) emerged from mass samples collected in a) clover ($n = 14$) and b) camomile stands ($n = 11$); sty = *P. stygicus*; dig = *P. digoneutis*; meso = *M. curvulus* sp.; Wilcoxon signed ranks tests: a) sty-dig: $P = 0.001$; dig-meso: $P = 0.001$; sty-meso: $P = 0.003$ b) sty-dig: $P = 0.58$; dig-meso: $P = 0.003$; sty-meso: $P = 0.003$; vertical bars show standard errors and means with the same letter are not different.

In the second *Lygus* generation the proportion of *P. digoneutis* (mean: 79%, range: 52-100%, $d = 0.79$) in mass samples from clover fields was found to be significantly higher than that of *P. stygicus* (mean: 20%, range: 0-48%, $d = 0.2$), whereas the hyperparasitoid was almost absent (mean: 1%, range: 0-6%, $d = 0.01$) (Fig. 2.14 a; Appendix, Table 2). The dominance of *P. digoneutis* was also demonstrated by a high mean RC of 0.81 (Appendix, Table 6 a). In contrast, proportions of *P. digoneutis* (mean: 51%, range: 0-88%, $d = 0.51$) and *P. stygicus* (mean: 43%, range: 6-97%, $d = 0.43$) in camomile stands were observed to be similar, whereas proportions of *M. curvulus* were low but a little higher than in clover (mean: 6%, range: 0-13%, $d = 0.06$) (Fig. 2.14 b; Appendix, Table 3). The great variability of the dominance structures among parasitoid communities in camomile stands was also indicated by a relatively low mean RC of 0.65 (Appendix, Table 7 a). The absence of *M. curvulus* in many clover sites caused a relatively low mean JC (69) in comparison to camomile stands (JC = 85) (Appendix, Tables 6 a and 7 a). The combination of both factors (JC x RC) resulted in a similar mean K_W for clover (57) and camomile stands (56), suggesting that in both habitats parasitoid communities differed greatly (Appendix, Tables 6 b and 7 b).

The variation in species composition between different collection dates is shown in the results from mass collections at Lütjenbrode-Eichtal (Appendix, Table 4). Within 18 days the proportion of *P. stygicus* increased from 58% to 81%, whereas the degree of parasitization dropped from 24 to 9%.

2.3.4 Emergence pattern of *Lygus* parasitoids under laboratory and natural temperature conditions

Emergence of overwintered parasitoids in spring

In 2002, emergence of parasitoids reared from *Lygus* nymphs began in the laboratory within one day following transfer to 21°C (Fig. 2.15 c). The parasitoids emerged in a strict chronological order: the first parasitoid that emerged was *P. digoneutis*, followed by *P. stygicus*, and the last parasitoid emerging was the hyperparasitoid *M. curvulus*. *Peristenus digoneutis* emerged in 1-8 days while *P. stygicus* and *M. curvulus* took 4-34 days and 3-28 days to emerge, respectively. In both *Peristenus* species, the majority of the males emerged first, with females emerging shortly thereafter. After three days of incubation, 57% of *P. digoneutis* males emerged, compared to only 13% of *P. digoneutis* females. Similarly, 49% of *P. stygicus* males emerged after 8 days, whereas female emergence was only 19%.

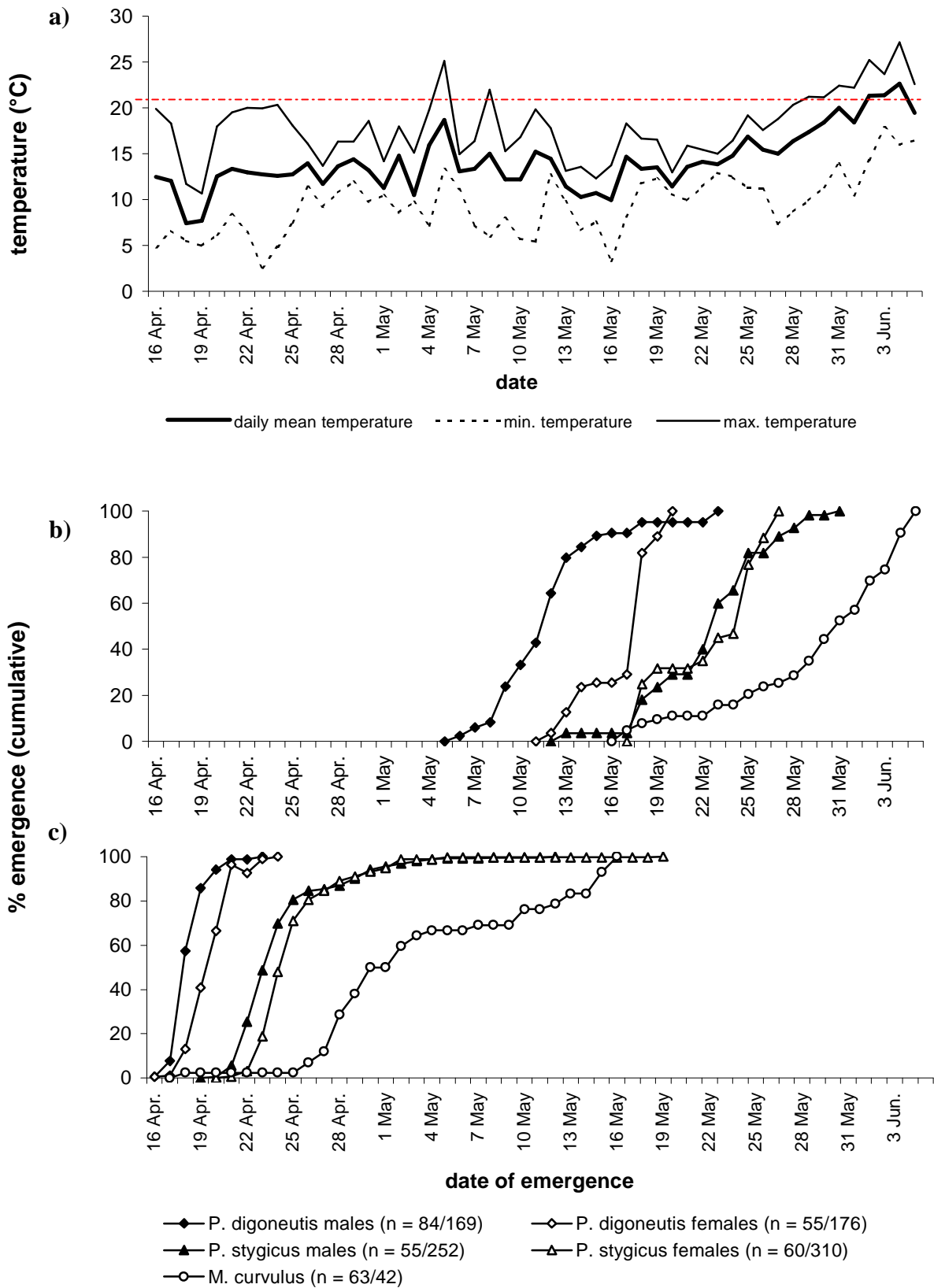


Figure 2.15 a) Daily mean, minimum and maximum temperatures in April – June 2003 in Kiel (red line = 21 °C), b) cumulative emergence of overwintered *Lygus* parasitoids at outside temperatures in 2003, and c) in the laboratory in 2002 at constant 21 °C; incubation started June 15th, 2002 (n = no. specimens em. 2002 / no. specimens em. 2003)

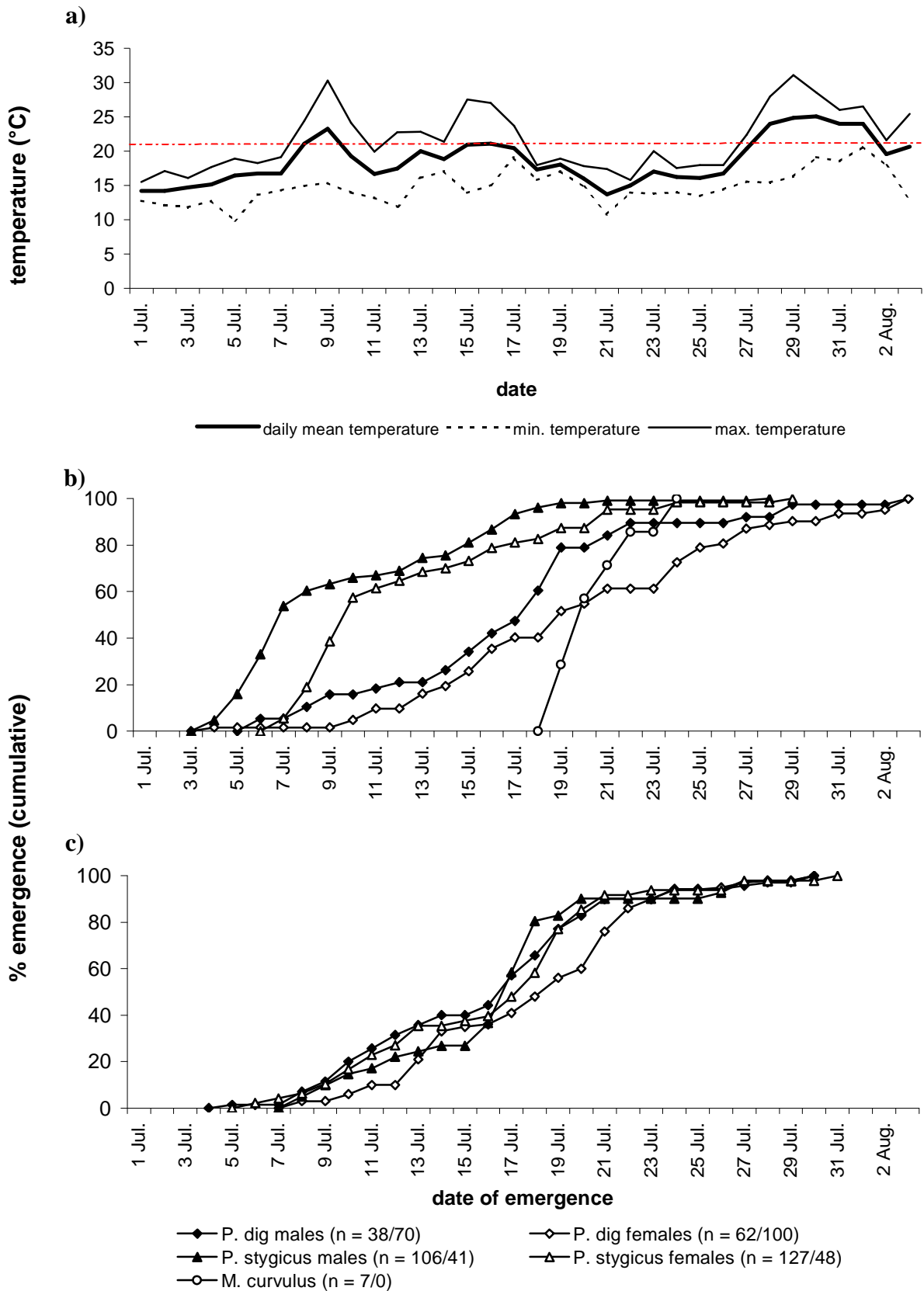


Figure 2.16 a) Daily mean, minimum and maximum temperatures in July/August 2002 in Kiel (red line = 21 °C), b) cumulative emergence of the 2nd generation of *Lygus* parasitoids at outside temperatures in 2002, and c) in the laboratory (21 °C) in 2003; incubation started July 5th, 2003 (n = no. specimens em. 2002 / no. specimens em. 2003)

In 2003, after parasitoids were stored outside for the entire winter, parasitoid emergence in spring started one day following the initial increase of average daily temperatures from 15 °C to 18 °C (May 5th; Figs. 2.15 a,b). The emergence of parasitoids followed the same chronological order as observed under laboratory temperature conditions. *Peristenus digoneutis* emerged over a period of 19 days (May 5th – May 23rd), whereas the emergence of *P. stygicus* lasted 20 days (May 12th – May 31st). The first hyperparasitoid was observed May 17th, but the majority emerged between May 30th and June 5th. Similar to the emergence in the laboratory, in *P. digoneutis* the majority of males came out before the females. In *P. stygicus*, however, males and females emerged almost simultaneously. Low parasitoid emergence was usually observed after outside temperatures had dropped down to a mean daily temperature between 10 and 11 °C (e.g. May 15th – 16th, Fig. 2.15 a) and thus, emergence curves were less uniform than observed under laboratory temperature conditions.

Emergence of the 2nd parasitoid generation in mid summer

After being stored outside, the majority of parasitoids obtained from the first nymphal generation of *L. rugulipennis* in 2002 started emerging in the same year (July 4th, Figs. 2.16 a, b). Although the emergence of both species started simultaneously, the majority of *P. stygicus* emerged before *P. digoneutis*. After one week 66% and 57.5% of *P. stygicus* males and females had emerged, respectively, whereas the emergence of *P. digoneutis* males and females was only 15.8% and 4.8% over the same time interval. The hyperparasitoids *M. curvulus* started to emerge two weeks after the first primary parasitoids were observed (July 19th, 2002). Surprisingly, varying proportions of all three parasitoid species reared from mass collections of the first nymphal generation of *L. rugulipennis* in June 2002, entered into an obligatory diapause and did not form a second generation the same year. Overall, 12.8% of *P. digoneutis* (n = 86), 31% of *P. stygicus* (n = 319) and 46.2% of *M. curvulus* (n = 13) did not emerge before spring 2003. However, the proportion of primary parasitoids entering diapause varied greatly between sites (*P. digoneutis*: 0-29%; *P. stygicus*: 0-64%; see Appendix, Table 8).

A different emergence pattern was observed in 2003, when parasitoid cocoons reared from the first nymphal generation of *L. rugulipennis* were transferred from outside to the laboratory (July 5th, 2003) and kept at a constant temperature of 21 °C (Fig. 2.15 c). In contrast to the emergence under natural temperature conditions, neither an obvious difference between the emergence of males and females nor between the two species was observed.

Emergence of parasitoids in late summer

Sporadic emergence of a 3rd parasitoid generation was observed in September (outside); however, the number of emerged adults was very small. Overall, in 2002 only four *P. digoneutis* and four *P. stygicus* emerged. In contrast to the small number of primary parasitoids, a surprisingly high number of 103 hyperparasitoids (*M. curvulus*) did emerge.

2.3.5 Nymphal parasitoids of *Lygus pratensis* L.

Lygus pratensis only occurred in low numbers at all sites, and therefore the seasonal occurrence was difficult to monitor. In red clover only single nymphs were found, whereas in camomile stands *L. pratensis* was more common. In Figure 2.17 a) numbers of *L. pratensis* found at two camomile sites are pooled. *Lygus pratensis* nymphs (Fig. 2.17 b) occurred in mid-June and were collected more frequently in July, when the second generation of *L. rugulipennis* began their development. The first nymphs found were nearly all non-parasitized; however, when the parasitization level in *L. rugulipennis* was increasing, this trend was also observed in *L. pratensis*. In total, 73 cocoons were reared from 446 *Lygus pratensis* nymphs collected in 2002/3. From these cocoons 65 parasitoids emerged. Of those that emerged, 34 specimens were *P. stygicus* (52%), 22 were *P. digoneutis* (34%) and 9 were the hyperparasitoid *M. curvulus* (14%).

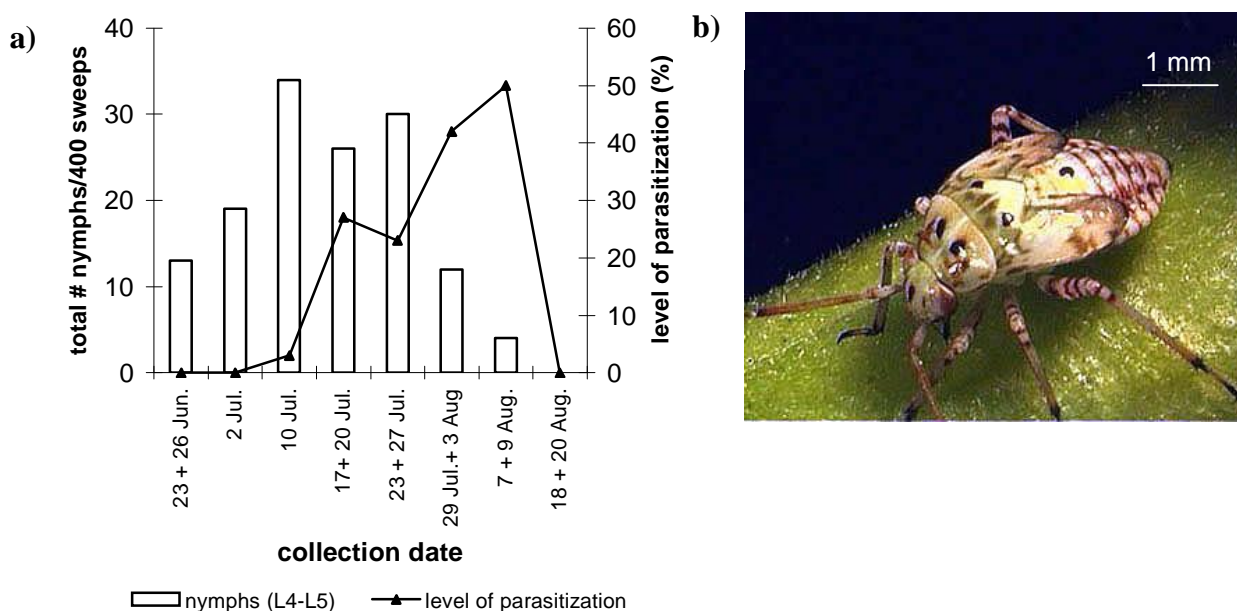


Figure 2.17 a) Total numbers of *Lygus pratensis* nymphs collected per 400 sweeps, and level of parasitization in nymphs by *Peristenus* spp. (estimated by rearing) from samples collected in camomile stands in Rastorf and Sophienhof (data are pooled); b) L5 of *Lygus pratensis*

2.3.6 Nymphal parasitoids of *Lygus maritimus* Wagner

The life cycle of *L. maritimus* is probably similar to that of *L. rugulipennis* (Boness, 1963); however, population development was not followed in detail throughout the season. During the three years of study high numbers of nymphs (Fig. 2.18) were only found in late August and early September at two sites along the North sea. Those populations, belonging to the second generation, were found at a natural site with *Atriplex lacinata* L. on the island Amrum and on a meadow with an abundance of *Matricaria* plants close to Dagebüll. At these sites *L. maritimus* was the dominating species, although *L. rugulipennis* and *L. pratensis* occasionally occurred. Fewer numbers of *L. maritimus* were also found close to Niebüll on *Matricaria* plants, at a parking lot with *Atriplex prostrata* Boucher in Bredtstedt and in St. Peter Ording on *Cakile maritime* Scopoli. Single specimens were also found on *Matricaria* plants at Lutterbek close to Kiel.



Figure 2.18 L5 of *L. maritimus*

The few nymphs of the first generation found in early summer ($n = 77$) were nearly all non-parasitized. Only three parasitoids were reared, all identified as *Peristenus stygicus*. The second generation started developing at the beginning of August. Parasitized nymphs were only recorded from Dagebüll, Bredtstedt, and Lutterbek. However, parasitization rates were low. In total, 33 cocoons were reared from 598 *L. maritimus* nymphs, and 29 parasitoid adult emerged; 25 specimens were identified as *P. stygicus* (86%), three as *P. digoneutis* (10%) and one as the hyperparasitoid *M. curvulus* (4%).

2.4 Discussion

2.4.1 Seasonal occurrence of *Lygus rugulipennis*

In Schleswig-Holstein, *L. rugulipennis* was found to have two generations per year. The temporal pattern of occurrence of nymphs and adults is consistent with the observations made by Afscharpour (1960) and Boness (1963). In April/May overwintering adults moved from their hibernation sites to newly grown plants (mainly *Matricaria*) and started oviposition. In spring, camomile stands seem to be the most favourable habitat for oviposition, and thus,

nymphal densities were much higher in camomile than in clover fields. The first nymphal generation was found from the end of May until the beginning of June. These field data are supported by Boness (1963), who stated that the nymphal development of the first generation lasts on average 28 days. As a result, newly emerged adults were dominant in July when nymphal densities in the field were low. At this time many adults dispersed from senescent *Matricaria* plants to new sites with more suitable host plants for oviposition, e.g. flowering clover fields. This may explain why densities of the second nymphal generation usually appeared to be much higher in clover than in camomile stands. According to Boness (1963) new adults start to oviposit after a feeding period of 10 to 14 days, and therefore, newly emerged nymphs of the second generation were first recorded at the end of July. At the end of August most nymphs had finished their development and the numbers of *Lygus* adults in the field increased. *Lygus* adults were observed feeding on their host plants until the end of October. Extremely high adult densities like at Lütjenbrode-Eichtal in 2001 were probably caused by cutting large areas of the clover field in September, which forced the adults to move to the remaining patches where fresh plants were still available.

High densities of nymphs found in late season (September) may indicate the occurrence of a third nymphal generation, as already suggested by Afscharpour (1960). At sites like Rastorf, located in a gravel pit, the warmer microclimate may support the development of a third generation. However, higher densities of nymphs were always found in stands of newly grown plants and thus, it seems more likely that adults that developed from the first nymphal generation move from areas where the host plant quality had deteriorated (see Rastorf, Lütjenbrode-Eichtal) to newly grown plants to finish their oviposition period.

In general, the number of generations of *L. rugulipennis* in Europe depends on the climatic conditions of each region. In England, France, Poland and northern Germany two generations can successfully develop (Southwood, 1956; Boness, 1963; Wagner & Weber, 1964; Bilewicz-Pawinska, 1982), whereas in Scandinavian countries, such as Norway, Finland and Sweden, *L. rugulipennis* is known to be univoltine (Sömermaa, 1962; Varis, 1972, 1997; Hellquist et al., 1989; Varis & van Achterberg, 2001).

2.4.2 Nymphal parasitization of *Lygus rugulipennis*

Peristenus spp. are solitary endoparasitoids of plant bugs and are able to oviposit in all

nymphal instars (van Steenwyk & Stern, 1976). The first two larval instars of the parasitoid feed inside the nymph, while the third emerges from the host, which is killed in the L5 stage. The mature larva spins a cocoon in the soil to pupate (Carnigan et al., 1995).

When females attack older instars (L4-L5), there is a strong defence reaction of the nymphs, and they are often capable of deterring the parasitoid (van Steenwyk & Stern, 1976). Thus, small instars are more likely to be attacked. According to Boness (1963), in Schleswig-Holstein *Lygus* females are able to oviposit over a period of several weeks depending on the weather. Small nymphs will therefore emerge over a period of several weeks, too. Each cohort of newly emerged nymphs needs approximately 14 days to reach the 4th instar. Consequently, the potential oviposition period for *Peristenus* parasitoids would be limited to the time when small nymphs are available in the field. In continuous collections in 2002, small nymphs were found over several weeks during the development of each *Lygus* generation, but periods with high densities were usually limited to three weeks. During this period, it is assumed that the parasitoid activity is most intense. The field data from collections conducted in 2001/2 largely correspond with these assumptions. Shortly after the first nymphs emerged in the field, the first parasitized nymphs were found, too, suggesting that the occurrence of the parasitoids is well synchronized with the occurrence of small instar nymphs. When the majority of *Lygus* nymphs had reached the L4 or L5 instar, parasitization rates stopped increasing. As soon as *Peristenus* larvae began to leave their hosts and densities of new *Lygus* adults significantly increased in the field, the degree of parasitization dropped immediately.

Parasitization of *Lygus* nymphs by *Peristenus* spp. was recorded at all clover and camomile sites studied. The degree of parasitization did not differ significantly between clover and camomile habitats, but varied greatly within each habitat. Broad ranges of parasitization were also found in various habitats, which have been studied in many other European countries. In Poland, parasitization of *Lygus* nymphs was monitored in six different crops (Bilewicz-Pawinska, 1977a). Among cereal crops (rye, barley, wheat, oats) the maximum degree of parasitization ranged from 13 to 49%, whereas parasitization in alfalfa and potatoes reached a maximum of 25%. In Switzerland, however, the degree of parasitization in alfalfa was occasionally found to be higher than 70% (White & Kuhlmann, unpublished). In Finland, the proportion of parasitized nymphs in *Lygus* collections was rarely over 10% (Varis & van Achterberg, 2001).

The degree of parasitization at each site is influenced by several factors that are virtually unpredictable; thus, data on parasitization have to be interpreted carefully. Besides temporal aspects of parasitization (see above), the character and history of the habitats may have an important influence on the abundance and occurrence of parasitoids. In the following, these aspects will be discussed referring to the collection sites of this study:

In April, overwintered *Lygus* adults move from their hibernation sites into habitats which provide suitable host plants for oviposition, e.g. clover or camomile. *Lygus* parasitoids which emerge from overwintered cocoons in May have to follow their hosts into these habitats. Thus, the degree of parasitization at a site infested with *Lygus* will be strongly dependent on the number of parasitoids invading. However, in the present study the degree of parasitization in the first nymphal generation of *Lygus* was relatively low (5-20%), suggesting that only small numbers of parasitoids had followed their hosts. In June, parasitoid larvae emerge from the first nymphal generation of *Lygus* and spin a cocoon in the soil. At the same time non-parasitized nymphs develop to new *Lygus* adults. If the site where the new *Lygus* adults developed as larvae continues to provide suitable host plants, then most adults of the new *Lygus* generation will stay at the site and start ovipositing instead of spreading out to search for new sites infested with *Lygus*. In July, the second nymphal generation of *Lygus* starts to develop, and at the same time the second parasitoid generation is emerging. Therefore, newly emerging parasitoids which have developed from the first nymphal generation of the same site will find suitable hosts immediately. In contrast to overwintered parasitoids emerging in spring, the parasitoids of the second generation emerge at a site where the host is already present and thus, they do not have to cover long distances to find habitats with suitable hosts. This advantage may explain why the degree of parasitization in stable habitats tends to be higher in the second nymphal generation of *Lygus*. These assumptions agree with observations made in Poland, where the highest level of parasitization was found in long-term habitats that were largely undisturbed and provided the opportunity for the parasitoids to complete the entire annual life cycle in the same location with the assurance of suitable hosts for subsequent generations (Bilewicz-Pawinska, 1977a).

In general, the host plant-*Lygus*-*Peristenus*-system can be regarded as a very flexible system. The preferred habitats of *Lygus* are *Matricaria* stands. *Matricaria* plants, however, are annual plants that initially colonize disturbed areas where soil has been removed and nearly no other plants are growing. They complete their complete life cycle within one year and are replaced

by a succession of different plant species afterwards. Therefore, temporary *Matricaria* habitats can be classified as *r*-selecting habitats and *Matricaria* plants as *r*-selected plants (Begon et al., 1998). Consequently, *Lygus* plant bugs and their parasitoids have to follow the *r*-strategy, too. Within a short period of time, *Lygus* bugs can produce large populations in *Matricaria* stands which provide an optimal resource for reproduction of *Lygus* parasitoids. However, in the long term the newly developed *Lygus* generation will be confronted with less optimal habitat conditions (poor plant quality) and hence, will disperse to more suitable habitats. Newly emerging parasitoids will have to use the remaining *Lygus* nymphs for reproduction or follow their hosts into new habitats. For temporary habitats infested with *Lygus*, this means that the degree of parasitization would essentially be dependent on the number of invading parasitoids.

2.4.3 Parasitoid species composition

In Schleswig-Holstein, *L. rugulipennis* nymphs were parasitized by two bivoltine parasitoids *Peristenus digoneutis* Loan and *P. stygicus* Loan. Furthermore, *M. curvulus*, a hyperparasitoid of *Peristenus* spp., was reared frequently. At nearly all clover sites visited (except Lütjenbrode-Eichtal), *P. digoneutis* was the dominant parasitoid, whereas in mass samples from camomile stands, the proportions of *P. digoneutis* and *P. stygicus* varied greatly with each site and thus, were not significantly different. Continuous collections throughout the season showed that the proportion of each parasitoid species varies greatly over time; this could explain the broad variations observed among mass collections, which were conducted at sites that were visited only once. The reason why *P. digoneutis* is clearly dominant in clover fields remains unexplained, but it is possible that clover plants may be attractive to the parasitoid. In North America, it has been shown that the native parasitoid *Peristenus pseudopallipes* was highly attracted to *Erigeron* plants, the preferred summer-host plant of *Lygus lineolaris* in eastern North America (Shahjahn & Streams, 1973; Shahjahan, 1974). It has been assumed that *Erigeron* flowers supply necessary nutritional resources for the adults of this species, and that perhaps it is a quest for food, rather than hosts, which initially brings the parasitoids to these plants. However, it is also possible that the olfactory response to plant odor has evolved as a means of finding both food and hosts. Clover plants are known to be an essential carbohydrate resource for many insects, but also a favorite host plant for *Lygus* bugs and thus, an attraction of *P. digoneutis* to clover flowers cannot be excluded.

Table 2.1 Species composition of *Peristenus* parasitoids associated with *Lygus rugulipennis* in Europe (modified after Bilewicz-Pawinska, 1977; Coutinot & Hoelmer, 1999; Rämert et al., unpublished; Varis & van Achterberg, 2001; Haye & Kuhlmann, unpublished)

Country	Locality	Latitude Longitude	Type of climate	Coll. Year	Crop	Species composition ²					
						P. dig.	P. sty.	P. rub.	P. pall.	P. var.	Mes.
Sweden	Umea	63.45N 20.15E	boreal	2002	clover, barley	0	0	0	****	0	+
	Uppsala ¹	59.49N 17.39E	suboceanic	2002	alfalfa, barley, oilseed rape	0	(+)	0	(+)	(+)	0
Finland	Helsinki	60.19N 24.58E	boreal	1978- 86	various	0	0	0	0	****	0
Northern Germany	Schleswig- Holstein	54N 10E	oceanic	2001- 03	clover camomile	*** *	* *	0 0	0 0	0 0	+ +
Poland	Warsaw	52.10N 20.58E	suboceanic	1971- 77	potato	****	+	0			+
					cereals	*	*	****	0	0	+
					alfalfa	****	*	*			+
Southern Germany	Rhine Valley	47N 07E	suboceanic	2000- 02	alfalfa	***	*	+	+		+
					red clover	***	*	*	+	0	+
					mustard (1 st)	****	+	+	0	+	
Austria	Vienna	48.13N 16.20E	suboceanic	1977- 85	alfalfa	***	*	*	0	0	*
France	Dinard	48.38N 2.04W	oceanic	1979- 83	alfalfa	***	+	+	0	0	*
	La Rochelle	46.10N 1.09W	oceanic	1983- 84	alfalfa	***	*	+	0	0	0
	Trappes	48.47N 2.00E	gradual oceanic	1977- 88	alfalfa	***	+	+	0	0	*
	Valences	44.56N 4.54E	temperate Mediterranean influence	1977- 86 1997	alfalfa	**	**	+			+
						*	*	*	0	0	***
	Nimes	43.50N 4.21E	Mediterranean	1997- 98	alfalfa	0	****	0	0	0	0
Montpellier	43.36N 3.53E	Mediterranean	1997- 98	alfalfa	+	****	0	0	0	0	
Greece	Thessalonica	40.38N 22.56E	Mediterranean	1982- 84 1997	alfalfa	0	****	0	0	0	0
Spain	Seville	37.21N 5.59W	Mediterranean	1981- 84	alfalfa	0	****	0	0	0	0
	Alicante	38.21N 0.29W	Mediterranean	1997	alfalfa	****	0	0	0	0	0
	Barcelona	41.24N 2.10E	Mediterranean	1998	alfalfa	*	***	0	0	0	0

**** species ≥ 90% *** species 70-90% **species 50-70% *species 10-50% + species present in low numbers
0 species absent

¹ results only based on 30 specimens ² P. dig. = *P. digoneutis*; P. sty. = *P. stygicus*; P. rub. = *P. rubricollis*;
P. pall. = *P. pallipes*; P. var. = *P. varisae*; Mes. = *Mesochorus* spp.

Both primary parasitoids found in Schleswig-Holstein are known to dominate in central and southern Europe, where *Lygus rugulipennis* completes at least two generations per year (Tab. 2.1). *Peristenus stygicus*, which is said to be more common in the south of Europe (Coutinot & Hoelmer, 1999; Broadbent et al., 2002), has also been reported from central Sweden (Rämert et al., unpublished), where it was found more frequently than expected. In some areas of Europe, the abundance of *P. stygicus* may have been underestimated because samples were mainly taken in alfalfa, a plant closely related to clover. To date, this study presents the most northern record of *P. digoneutis* in Europe. Thus, its northern distribution is assumed to be limited to Denmark or southern Sweden, as recent studies state its absence from central Sweden and Finland (Rämert et al., unpublished; Varis & van Achterberg, 2001). Surprisingly, univoltine *Lygus* parasitoids that appear to dominate in other regions of Europe, such as *P. varisae* in Finland, *P. pallipes* in northern Sweden or occasionally *P. rubricollis* in Poland, have not been recorded from Schleswig-Holstein. The absence of *P. rubricollis* in northern areas suggests that Poland represents the northern border of this species' geographic distribution.

2.4.4 Parasitoid emergence

In Schleswig-Holstein, most diapausing insects are reactivated by cold, allowing the continuation of development already in January; however, continuing low temperatures suppress the development until spring (thermal quiescence, see Braune, 1971). The parasitoids of *L. rugulipennis* started to emerge in the first week of May and emergence continued until the end of May, when the first *Lygus* nymphs normally appear in the field. Accordingly, the emergence of parasitoids is well synchronized with the occurrence of early instar nymphs. The emergence pattern found in Schleswig-Holstein mirrors field observations made in Poland, where both parasitoids first occurred in May as well (Bilewicz-Pawinska, 1976). Like in almost all species of parasitic wasps, males of *P. digoneutis* and *P. stygicus* emerged a day or more before females. This behaviour is referred to as protandry (Quicke, 1997). The advantage of protandry is that early-emerging males are likely to encounter predominantly unmated females.

In the laboratory, overwintered parasitoids emerged in the same sequence as under field conditions when transferred to 21 °C in April 2002. *P. digoneutis* emerged in 1-8 days while *P. stygicus* took 5-29 days to emerge. These results correspond with data from overwintering experiments conducted by Bilewicz-Pawinska (1978) in Poland: After winter diapause, when

moved to 21 °C (14 March), *P. digoneutis* emerged in 2-22 days, whereas *P. stygicus* emerged in 11-32 days. The immediate emergence in the laboratory suggests that the parasitoids had terminated their diapause and were already in a period of thermal quiescence when moved from outside into the laboratory. In this period parasitoid development can be initiated by high temperatures, whereas during the period of diapause, increasing temperatures would not result in parasitoid emergence. Bilewicz-Pawinska & Varis (1990) stated that *Peristenus* parasitoids need a minimum of 2.5 – 3.5 months at low temperatures to complete their diapause.

Under natural temperature conditions the second parasitoid generation emerged throughout July. Both parasitoid species started to emerge almost simultaneously. In contrast to spring emergence, the majority of *P. stygicus* emerged before *P. digoneutis*. The earlier occurrence of *P. stygicus* in samples collected at *Matricaria* sites in Rastorf and Sophienhof seem to confirm the observed emergence pattern. Although many aspects of the biology of *P. digoneutis* and *P. stygicus* have been studied before (Bilewicz-Pawinska, 1973, 1976, 1977a, 1978, 1982; van Steenwyk & Stern, 1976), it has never been reported that only a proportion of the parasitoids reared from the first nymphal generation emerge to build up a second generation in July and the remaining individuals behave like univoltine parasitoids. This behavior may be interpreted as an adaptation to spread the risk of survival, in case adult parasitoids, emerging in an unstable habitat, do not find suitable *Lygus* populations for reproduction in late summer.

The emergence of a small third parasitoid generation may explain why parasitized nymphs of *L. rugulipennis* were found until mid-September. Studies from Poland describe *P. digoneutis* and *P. stygicus* as bivoltine (Bilewicz-Pawinska, 1982), but similar studies in New Jersey (U.S.A.), where *P. digoneutis* was released in the early 1980s reported that this species has 2-3 generations per year (Day et al., 1998). The diapause of both parasitoids is induced by the length of the photoperiod (= facultative diapause), and, under laboratory conditions, it is known that both species can be reared continuously when kept under long-day conditions (16h light) and high temperatures. To induce diapause during larval development, an 11 hour photophase is required (Whistlecraft et al., 2000). The occurrence of a third *Peristenus* generation in some areas is likely related to latitude and climatologic conditions in late summer.

2.4.5 Nymphal parasitoids of *Lygus pratensis* and *L. maritimus*

The biology and seasonal occurrence of *Lygus pratensis* and *Lygus maritimus* in Schleswig-Holstein has been described in detail by Boness (1963). Adults of the univoltine species *L. pratensis* leave their hibernation sites 3-4 weeks after *L. rugulipennis* and as a result L5 nymphs of *L. pratensis* were found mainly during the period between the two nymphal peaks of *L. rugulipennis*. *Lygus maritimus* has two generations per year in the coastal areas of Schleswig-Holstein. The biology and life cycle is assumed to be similar to *L. rugulipennis* (Boness, 1963). Habitats with higher numbers of this species were rarely found. The record of *L. maritimus* at Lutterbek was unexpected, because this species was only known from the west coast of Schleswig-Holstein and was never before reported from coastal areas of the Baltic sea. *L. pratensis* and *L. maritimus* usually occur together with *L. rugulipennis* and consequently, it was not surprising that they were also attacked by the same nymphal parasitoids as *L. rugulipennis*. Furthermore, this overlap of the parasitoid complexes shows that *P. stygicus* and *P. digoneutis* are not species specific.

2.4.6 Consequences for biological control

Coutinot & Hoelmer (1999) suggested the exploration of northern regions such as Scandinavia in order to locate appropriate climatic strains of biological control agents for a potential introduction into North America. Thus far, parasitoids primarily collected in Spain, France, and Italy have been used for releases in California and New Jersey (Day, 1987; Pickett et al., 2002). For potential introductions into Canada, *P. digoneutis* strains from northern Germany might be more suitable based on latitudinal and climatic similarities. Since *P. digoneutis* does not occur in central Scandinavia, northern Germany is probably close to the northern limit of *P. digoneutis*. For releases of *P. stygicus*, northern German populations are promising, although populations can be found further north in coastal Central Sweden. However, the climatic conditions do not differ greatly between these two regions.

The present study shows that the two bivoltine nymphal parasitoids *P. digoneutis* and *P. stygicus* are well adapted to the temporal occurrence of the two *Lygus* generations in Schleswig-Holstein. Furthermore, the analysis of the distribution of both species within Europe indicates that the occurrence of *P. digoneutis* and *P. stygicus* is strongly dependent on the occurrence of two *Lygus* generations per year. In areas like Finland and northern Sweden,

where *Lygus* species are generally univoltine, these bivoltine parasitoid species are replaced by univoltine parasitoids (Rämert et al., unpublished; Varis & van Achterberg, 2001). For the biological control of *Lygus* spp. in Canada, this implies that a potential release of bivoltine parasitoids will only be successful in areas where *Lygus* spp. have at least two generations. Craig & Loan (1987) suggested that in the Canadian prairie provinces at latitudes below 50°N (a line through Winnipeg and Medicine Hat), 70% of the first generation adults will reproduce. They also stated that as the latitude increases, the percent of the population reproducing decreases until above 53°30' N, whereupon only a single generation is produced. Hence, in areas below 50°N a release of bivoltine parasitoids, as suggested by Braun et al. (2001), seems to be promising to control *Lygus* spp. in late season, when native parasitoids are absent. On the other hand, annual variation in the numbers of *Lygus* generations were observed by Braun et al. (2001) and Craig (1983). Braun et al. (2001) assumed that a delayed occurrence of the first generation population peak of *L. borealis* in Saskatoon resulted in a first generation of adults that entered directly into reproductive diapause without producing a second generation. This is likely to cause serious difficulties in establishing a bivoltine parasitoid. The presence of a native, univoltine *Lygus* parasitoid causing up to 70% parasitization in the first nymphal generation in these regions (Braun et al., 2001) and the absence of a native bivoltine or multivoltine parasitoid like *Leiophron uniformis*, which appears in areas where *Lygus* consistently has two generations (e.g. southern Ontario, Day & Saunders, 1990; Cermak & Walker, 1992; Broadbent et al., 1999) does support these concerns. Consequently, the factors influencing the numbers of *Lygus* generations in different prairie regions should be analyzed before releasing bivoltine parasitoids in order to estimate the potential for successful establishment.

The actual impact of parasitoids in crops like canola, where *Lygus* appears to be an increasing problem in Canada (Mason & Soroka, 1998), is difficult to predict. The present study as well as studies conducted in Poland (Bilewicz-Pawinska, 1977a), suggest that the highest impact of parasitoids is obtained in constant environments, where bivoltine parasitoids can complete their entire annual life cycle (two generations). However, in canola problems with *Lygus* only appear when adults of the first generation invade from other habitats to form a second generation. Consequently, released bivoltine European parasitoids would also have to follow their hosts into this crop, which may result in a low impact if they are unable to do so.

The introduction of European *Lygus* parasitoids into Canada would be an example of “new associations” in biological control. This strategy requires that European *Lygus* parasitoids

have more than one host – the “old” European host and the “new” North American target host. In Canada, the *Lygus* Hahn plant bug complex (Heteroptera: Miridae) contains 27 native species, 14 of which are recorded as agricultural field pests (Maw et al., 2000) and thus, these would be the potential “new” targets. It has been demonstrated that at least four of these “new” target species, e.g. *L. lineolaris*, *L. keltoni*, *L. elisius*, and *L. shulli*, are suitable hosts for *P. stygicus* and *P. digoneutis* (Day, 1996; Pickett et al., 2002; Mason et al., unpublished a). Furthermore, in the present study it has been shown that all *Lygus* species native to the parasitoid’s area of origin, e.g. *L. rugulipennis*, *L. pratensis*, and *L. maritimus*, were frequently attacked. Consequently, there is little doubt that all *Lygus* species native to Canada, including those with and without pest status, could serve as alternate hosts for the European parasitoids. The importance of these *Lygus* species in the ecosystem they belong to is poorly understood. For example, in western North America the holoartic species *L. rugulipennis* is not considered a pest and only occurs in habitats at high elevation and latitude in western North America. The ecological impact of invading European parasitoids attacking non-pestiferous *Lygus* species in these habitats would be difficult to predict. Consequently, the potential of *P. stygicus* and *P. digoneutis* to disperse and establish in high altitude habitats is currently the subject of ongoing research in alpine and sub-alpine habitats in the Swiss alps.

The introduction of a European *Peristenus* species into a guild of native species will certainly have an effect on the dynamics of host-parasitoid relationships. For euphorine parasitoids, only one species survives in the host when involved in interspecific competition. Ehler & Hall (1982) have assumed that in some cases, in-host competition of native and introduced parasitoids might result in the replacement of the native species. The in-host competition of *P. stygicus* and *P. digoneutis* with native North American *Lygus* parasitoids has been studied in detail by Lachance et al. (2001). In nearly all tests *P. digoneutis* and *P. stygicus* were better competitors than the native parasitoids. However, the risk of reducing a native parasitoid species by the introduction of exotic parasitoids had been estimated as low. One reason for this statement is the assumption that *P. stygicus* and *P. digoneutis* are more adapted to search in alfalfa than the North American species, *Peristenus pseudopallipes* and *Leiophron lygivorius*, which are commonly found in weedy areas characterized by the presence of *Erigeron* spp.. In the case of *P. digoneutis*, the results of the present study confirm the preference for habitats dominated by Fabaceae such as alfalfa and clover. However, *P. digoneutis* and *P. stygicus* were also frequently observed in weedy fields dominated by camomile. Camomile in Europe and *Erigeron* spp. in North America are both known to be

preferred host plants of *Lygus* spp.. Consequently, it is critical to relate the abundance of a parasitoid only to the presence of a given plant species. In the case of *Erigeron* spp., however, it has been demonstrated that *Erigeron* plants are highly attractive to *P. pseudopallipes* even when no *Lygus* nymphs are present. Nevertheless, the possibility that European parasitoids would not disperse to weedy areas with high *Lygus* densities cannot be excluded as long as the stimuli that guide the parasitoids to their hosts remain unknown. The impression that European parasitoids are more adapted to alfalfa may further have originated from the fact that most studies in Europe were conducted in alfalfa fields (see chapter 2), and weedy areas were largely neglected. Consequently, the dispersal of *P. digoneutis* and *P. stygicus* into weedy habitats and the encounter with native parasitoids is likely. However, long-term studies following the release of *P. digoneutis* in the United States indicate that native parasitoids have not been eliminated by the European parasitoid (Day, unpublished) and therefore, at least for *P. digoneutis*, the risk of replacing native parasitoids appears to be low.

3 Physiological host range of *Peristenus digoneutis* and *P. stygicus* (Hymenoptera: Braconidae), parasitoids of *Lygus* spp.

3.1 Introduction

Concerns about the introduction of arthropod biological control agents have recently increased because a lack of careful screening has resulted in the release of exotic generalist predators and parasitoids that have had negative environmental impacts (Howarth, 1983; Mannix, 2001). According to newly developed protocols for host range testing, a first essential step before releasing a natural enemy is to conduct laboratory tests in order to measure whether non-target species are attacked under a variety of tests conditions (van Driesche & Hoddle, 1997; van Lenteren et al., 2003). The set of species that can support development of a parasitoid or serve as prey for a predator - observed under *laboratory* conditions exclusively - can be defined as the *physiological host range* of a potential control agent (Onstad & McManus, 1996).

In the case of the biological control of *Lygus* spp. in Canada, European nymphal parasitoids such as *Peristenus stygicus* and *P. digoneutis* have been considered for introduction (Broadbent et al., 2002). In Europe, hosts other than *Lygus* spp. have rarely been reported for *P. digoneutis* and *P. stygicus* (Drea et al., 1973; Bilewicz-Pawinska, 1982) and consequently, these species have been regarded as having a narrow effective host range. Previous laboratory studies, however, indicated a much broader host range for *P. stygicus* compared to the somewhat limited field data previously reported (Porter, 1979; Condit & Cate, 1982). *Peristenus digoneutis* has been estimated as monophagous (Bilewicz-Pawinska, 1982), but recently it was also reared from two other non-target mirids in the United States (Day, 1999). To assess if it is justified to release *P. stygicus* into Canada or redistribute *P. digoneutis* that have already dispersed into eastern Canada after being released in the U.S., host range studies in Canada and Europe have been suggested as part of the pre-release evaluation program for European *Lygus* parasitoids.

In this chapter, results from laboratory tests on the host specificity of *P. digoneutis* and *P. stygicus* for seven European non-target mirids and two *Lygus* species are presented.

3.2 Material and methods

3.2.1 Selection criteria for non-target hosts

For the choice of non-target species in arthropod biological control, a procedure similar to the phylogenetic centrifugal method used for evaluation of weed biological control agents has been suggested (Wapshere, 1974; van Lenteren et al., 2003). This method involves the exposure of a sequence of non-target hosts to the biocontrol agent, starting with those most closely related and then progressing to successively more and more distantly related hosts until the host range has been adequately described. This method predicts that the likelihood of non-target and target hosts being attacked by the same natural enemy increases with their phylogenetic relatedness. In addition to species closely related to the target pest, non-targets occurring in the same habitat or time window as the target should be included in host range tests even if these species are distantly related (van Driesche & Hoddle, 1997; van Lenteren et al., 2003).

Species of the genus *Lygus* belong to the tribe Mirini Hahn (Subfamily: Mirinae Hahn) which includes 126 genera (949 species) in the palaeartic region (Aukema & Rieger, 1999). However, the phylogenetic relationship between species of the tribe Mirini is poorly described. At least 35 species belonging to the tribe Mirini are recorded from Schleswig-Holstein (e.g. Wagner, 1952; Weber, 1955; Remane, 1952, 1957; Afscharpour, 1960; Irmeler et al., 1994; Sioli, 1996; Heydemann, 1997). For practical reasons the number of non-targets to be tested had to be narrowed down. According to the maximum fit cladogram of *Lygus* and its outgroup taxa (Schwartz & Footitt, 1998), two Mirini species, *Lygocoris pabulinus* (L.) and *Liocoris tripustulatus* (Fabricius), were chosen as test candidates following a phylogenetic approach (Figs. 3.1 b, c). Both species are known to occur in Schleswig-Holstein and are considered closely related to the North American target pest *Lygus lineolaris*. Another candidate chosen from the tribe Mirini was *Closterotomus norwegicus* (Gmelin) (= “potato bug”, Fig. 3.1 d) which is the most abundant mirid found in spring in Schleswig-Holstein (Afscharpour, 1960) and occurs in the same time window and habitat as *Lygus* spp.. In addition, four grass bugs from the tribe Stenodemini were selected. These included *Leptopterna dolabrata* L. (= “meadow bug”), *Stenodema calcarata* (Fallén), *Notostira elongata* (Geoffroy), and *Megaloceraea recticornis* (Geoffroy) (Figs. 3.1 e-h). These grass bugs also occur at the same time as *Lygus* but primarily in grasslands, a habitat in which *Lygus* spp. are usually found in low numbers.

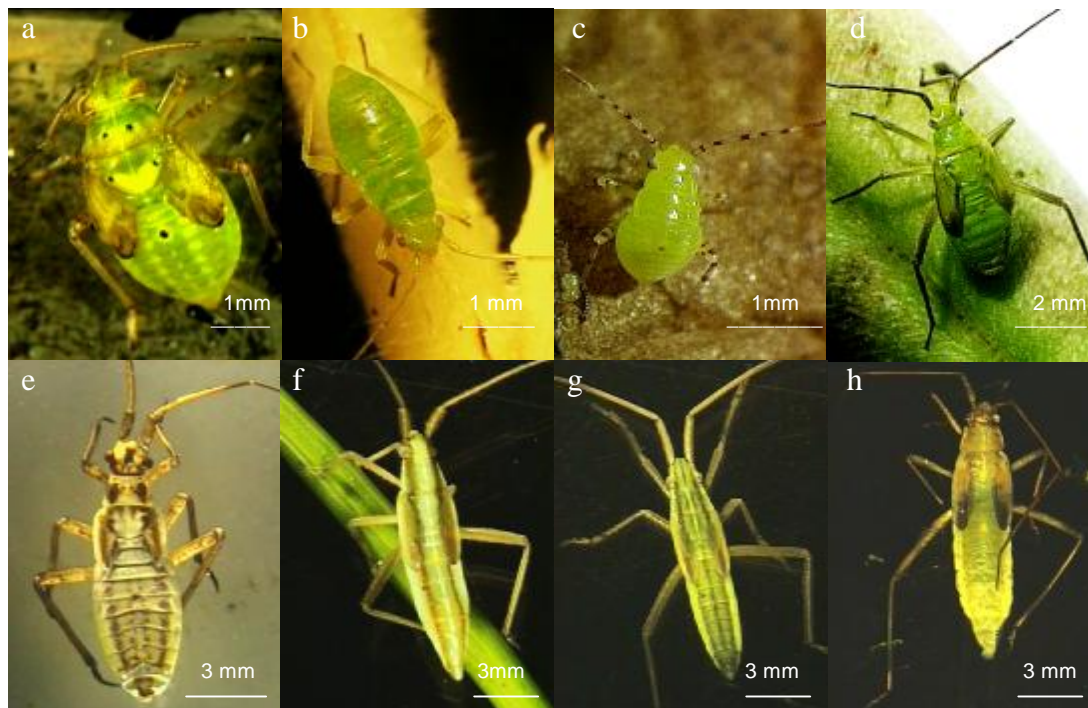


Figure 3.1: Target and non-target mirid nymphs selected for sequential non-choice tests : a) *Lygus maritimus* (“target”), b) *Lygocoris pabulinus*, c) *Liocoris tripustulatus*, d) *Closterotomus norwegicus*, e) *Leptopterna dolabrata*, f) *Stenodema calcarata*, g) *Notostira elongata* and h) *Megaloceraea recticornis*

In the present study, *L. rugulipennis* Poppius represents the target host (instead of the congeneric North American target *L. lineolaris*). In order to investigate if variations in acceptance and parasitoid development occur when different target hosts are offered, *Lygus maritimus* Wagner (Fig. 3.1 a) was included in the testing procedures as well.

3.2.1 Source of parasitoids and nymphs

Peristenus parasitoids used for experiments in spring were reared from overwintered cocoons obtained from *L. rugulipennis* mass collections from the previous year. For non-target tests performed in mid-summer, newly emerged parasitoids from samples collected in June were used instead. All parasitoids were collected in Schleswig-Holstein (Germany). Rearing methods for *Lygus* parasitoids have been described in detail in the second chapter of this thesis.

For rearing nymphs of *L. rugulipennis*, *L. maritimus*, *L. tripustulatus* overwintered adults, and for *L. pabulinus* newly emerged adults from the spring generation, were collected in camomile or stinging nettle stands in early season and brought to the laboratory for egg

laying. Following the rearing protocol for *L. lineolaris* developed by Whistlecraft et al. (2000), adults were kept at 25 °C and 16 hours photoperiod in cages with lettuce and sprouting potatoes as oviposition substrates.

To establish a rearing of *Stenodema calcarata*, grass ears (e.g. from *Dactylis glomerata*) in which overwintered adults had oviposited were brought to the laboratory to obtain freshly emerged nymphs.

To rear univoltine non-target mirids that overwinter in the egg stage, such as *C. norwegicus*, *L. dolobrata*, and *M. recticornis*, L1/L2 nymphs were field collected during early season (May/June). Small instar nymphs of the bivoltine species *N. elongata* were collected in early July when the second generation of nymphs started to emerge. In the early period of nymphal emergence, the risk that nymphs have already been parasitized in the field is generally low. As an additional control, a subsample of small nymphs was always taken into rearing and dissected for parasitization when nymphs had reached the L4 or L5 stage. Nevertheless, the risk of using nymphs that were already parasitized when collected in the field can only be reduced to a minimum, but it cannot be completely excluded.

3.2.2 Sequential non-choice tests

Sequential non-choice tests for *P. stygicus* and *P. digoneutis* were performed in the laboratory following a modified protocol based on Whistlecraft et al. (unpublished). The aim of the test was to answer the question of whether the parasitoids attack non-target nymphs consistently (“host acceptance”) and whether non-target nymphs are suitable hosts for parasitoid development. Three day old, mated *P. stygicus* and *P. digoneutis* females were individually placed into a clear plastic vial (diameter: 30 mm; height: 55 mm) each containing a single second or third instar nymph of *L. rugulipennis* (Fig. 3.2). At this time the parasitoids had no experience with target or non-target hosts. If oviposition ensued, the length of time to the first sting, the duration of sting, and the oviposition behavior were noted, and then the parasitoid female was removed. In the event that no stings were observed in a maximum time period of 20 minutes, the parasitoid female was removed.

The same procedure was repeated 24 hours later, but offering a non-target nymph. Another 24 hours later, the experiment was repeated by offering a *L. rugulipennis* nymph again. Females

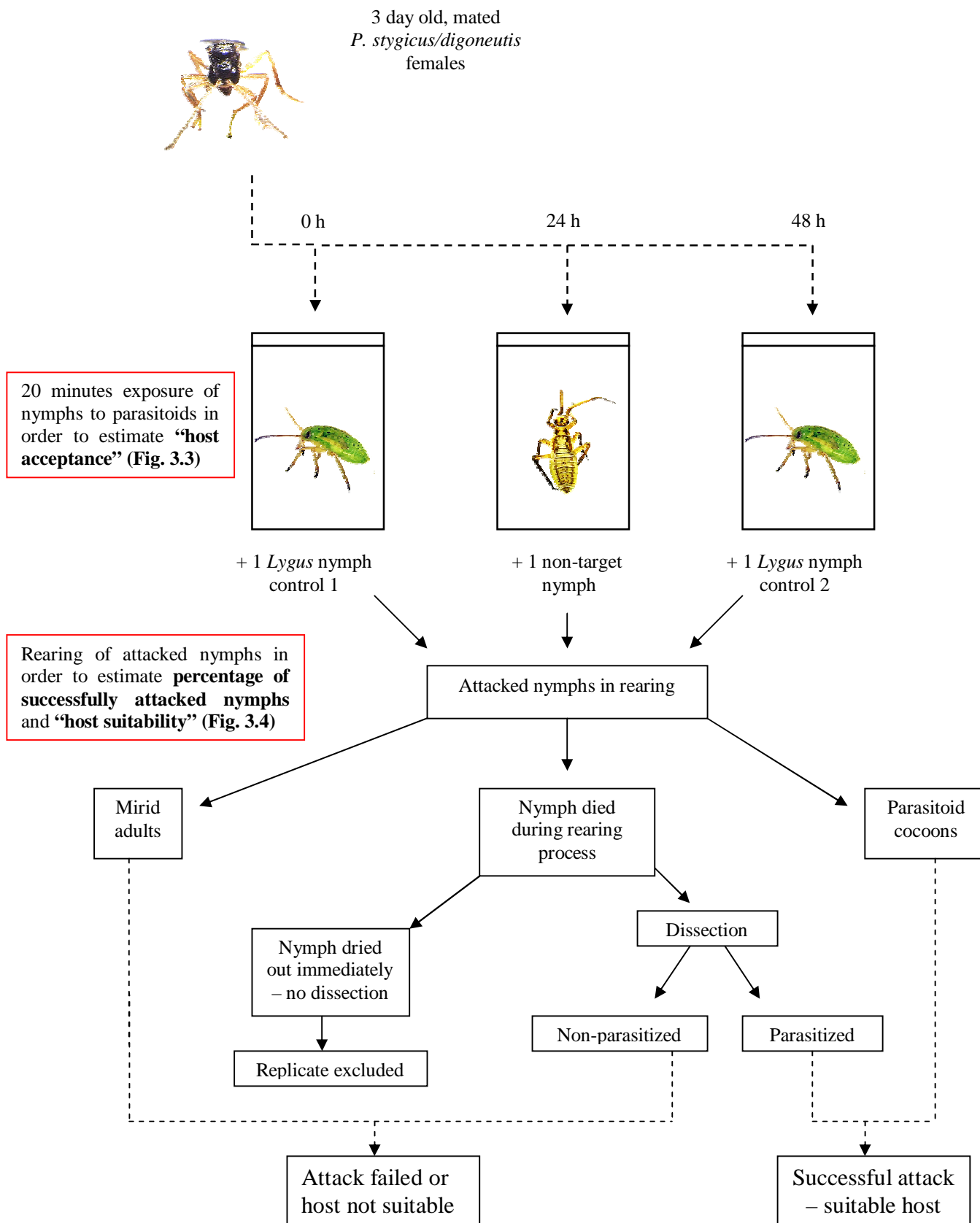


Figure 3.2 Evaluation method for sequential non-choice testing; data sets for *P. stygicus* and *P. digoneutis* are presented in the Appendix (Table 9).

that did not react to *Lygus* nymphs in the first run were presumably not ready for oviposition and thus, were excluded from the testing procedure. However, this rarely occurred during the experiment. In order to investigate whether any temporal effect appears during the course of the three day experiments, entire experimental runs were performed on *L. rugulipennis*, exclusively (in other words, parasitoids were offered *L. rugulipennis* for all three runs). In between each test, parasitoids were kept in a subterranean insectary at temperatures of 15 to 18 °C and provided with honey and water. Before each test, parasitoids were allowed to adapt to the laboratory temperature (25 °C) for at least one hour.

Potentially parasitized target and non-target nymphs were reared individually at 20 °C until they reached the adult stage or until a parasitoid larva emerged. When nymphs died prior to this point, they were dissected for parasitization using a microscope. An attack on a nymph was counted as successful when either a parasitoid cocoon was found in the rearing vial or a parasitoid larva was found in the dissection (Fig. 3.2). When parasitoid cocoons were obtained from non-target nymphs that had originally been field collected, cocoons were reared until adult emergence in order to determine whether the emerging parasitoid species was in fact the product of the laboratory experiment, or if parasitization by another *Peristenus* species occurred in the field prior to collection.

Nymphs of *L. rugulipennis*, *L. maritimus*, *C. norwegicus*, *L. pabulinus*, and *L. tripustulatus* were kept in small plastic vials (diameter: 40mm; heights: 55mm) and were provided with Romaine lettuce (or green beans) and potato sprouts as a food source. A thin layer of moistened vermiculite covered the bottom of the vials to offer emerging parasitoid larvae a pupation site. Small nymphs of grass bugs (*L. dolobrata*, *S. calcarata*, *M. recticornis*) were placed on grass ears which were stuck into moist foam (usually used by florists) and changed every two days. For rearing *N. elongata*, grass leaves were used instead of grass ears. In order to investigate whether parasitoid attacks result in a higher degree of unspecific nymphal mortality (= nymphs die before reaching the adult stage or before parasitoid larvae emerged from the host), control samples of non-attacked nymphs were reared for each mirid species under the same laboratory conditions. In these rearing controls, unspecific nymphal mortality describes the proportion of nymphs that die before reaching the adult stage.

The Cochran's Q test was used to analyze the data sets obtained from sequential non-choice tests. This test for paired samples can only be used when the same number of values is

received for each test replicate (in the present case the number of replicates is identical to the number of females tested). In the case of “host acceptance”, three values (one per run; the value can be negative (= no attack) or positive (= attack) were received for each individual parasitoid female and thus, test requirements were fulfilled for all females tested. “Host acceptance” in the first run (= 1st *Lygus* control) was always 100% (= all females attacked the offered *Lygus* nymph) because females ready for oviposition were used exclusively (see above).

For comparing the rates of attacks that resulted in successful parasitoid development, only data obtained from parasitoids which had attacked nymphs in *all* three runs of the experiments were used because of the test requirements. Consequently, the number of replicates was automatically reduced. The number of replicates was also highly dependent on the rearing success of nymphs. Some of the attacked nymphs died and dried out during the rearing process and thus, could not be dissected for parasitization. In these cases the complete test series had to be excluded from the analysis (Fig. 3.2) and consequently, the number of replicates was further reduced (see Appendix, Table 9).

3.2.3 Simultaneous choice tests

In order to investigate whether the behavioural threshold for acceptance of an alternative host can be changed in the presence of the target host, choice tests were conducted in which parasitoids were offered *Lygus* nymphs and non-target nymphs simultaneously in the laboratory at 25 °C. For these tests, 6 to 8 day old female parasitoids which had prior experience with both target and non-target hosts (from the sequential choice tests) were used. Individual female wasps were offered three 2nd or 3rd instar *Lygus* nymphs and three 2nd or 3rd instar non-target nymphs at the same time in a small Petri dish (5 cm diameter). Attacked nymphs were immediately removed with a mouth aspirator and replaced by new, non-parasitized ones to maintain a constant number of each mirid species at all times. Tests lasted for 5 minutes, and the number of attacks per mirid species was recorded. A total of 20 replicates for *P. digoneutis* and *P. stygicus* were done for each non-target mirid species of interest. Results were analyzed using the Wilcoxon paired-sample test.

3.3 Results

3.3.1 Parasitoid oviposition behavior

The oviposition behavior of *P. stygicus* closely resembles that described by Condit & Cate (1982). When *Lygus* nymphs were present, the females searched the vial with rapid but indirect motion. The nymphs were probably perceived at a very short distance from the wasp's antennae or at the moment of direct antennal contact. *Lygus* nymphs usually tried to escape when touched by the parasitoid, but the wasp followed the nymph and rapidly caught up. The wasp then held the nymph with its forelegs and, standing on its third pair of legs, lifted it off the ground (see Figure 2.10 a, chapter 2). The wasp then stung into the ventral side of the nymph, primarily in the thorax.

In general, *P. digoneutis* females were less active in searching for a host than *P. stygicus* females. When *Lygus* nymphs were present females started searching, slowly palpating the surface of the vials with their fast moving antennae. When a female perceived a suitable host at a short distance (2-3 mm) it slowed down its movements, and sneaked forward in a cat-like manner to the nymph. Then it put back its antennae and suddenly attacked the nymph. This behavior was sometimes observed even when no nymphs were within reach of the wasp. Similar to *P. stygicus*, it held the nymph with its forelegs while stinging in the ventral side of the host (see Figure 2.10 b, chapter 2). Most attacked nymphs remained paralyzed for several seconds.

In general, attacks on non-target mirids followed the same behavioral pattern but occasionally nymphs were rejected after a first contact with the parasitoid.

3.3.2 Sequential non-choice tests

When testing *P. stygicus*, no significant differences were observed when the alternative target *L. maritimus* and the non-targets, *L. tripustulatus*, *L. pabulinus*, and *C. norwegicus* were offered individually. Between 80 and 100% of the tested females attacked the non-target mirids as well as *Lygus* (Fig. 3.3 a), and more than 80% of the observed attacks on both target and non-target nymphs (except on *L. pabulinus*) resulted in successful parasitoid development (Fig. 3.4 a). Although attacks on *L. pabulinus* by *P. stygicus* were frequently observed, a large proportion of the test nymphs reached the adult stage and no sign of parasitization was found.

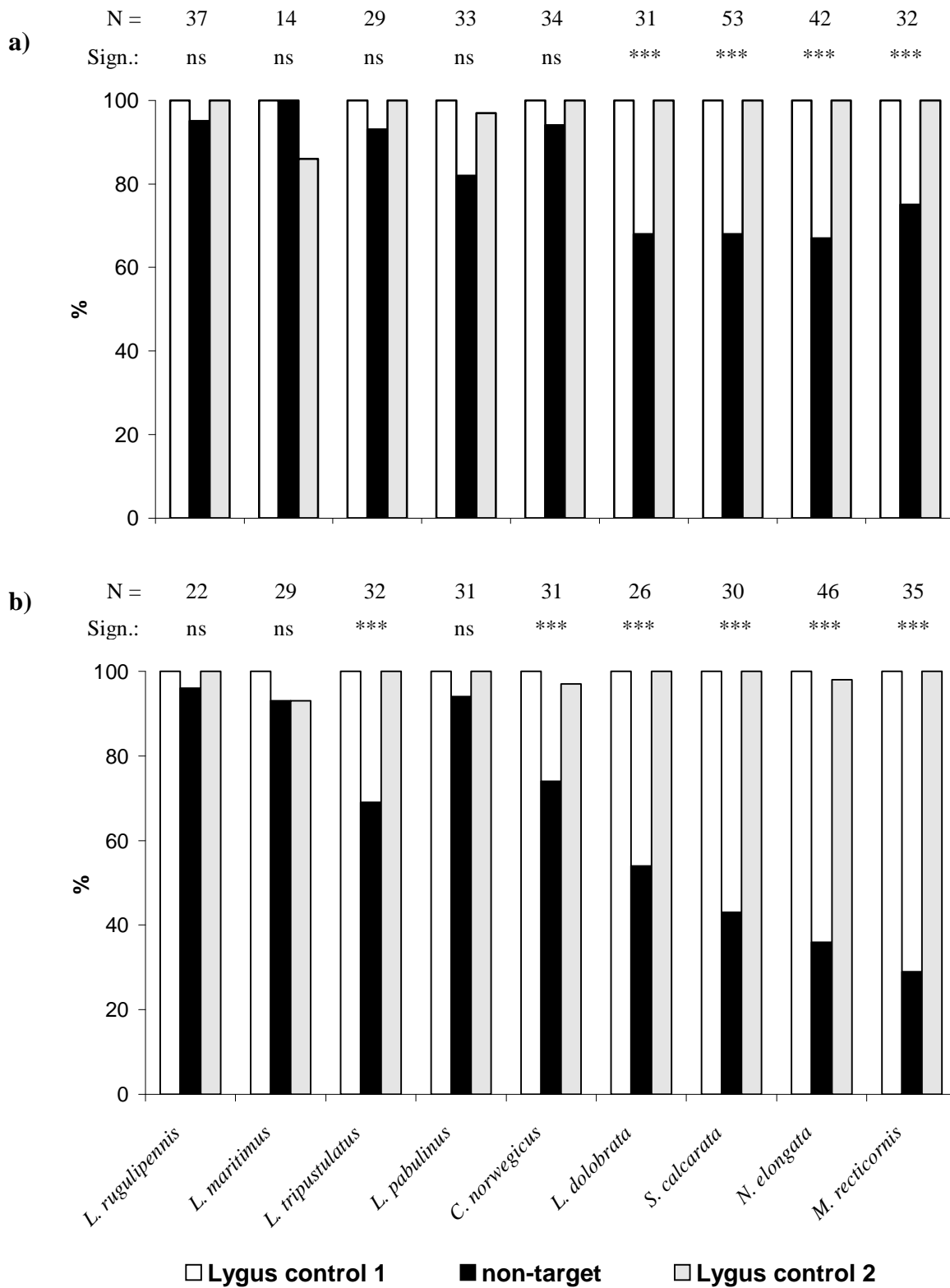


Figure 3.3 Percent acceptance of target and non-target hosts in sequential non-choice tests: a) *P. stygicus* and b) *P. digoneutis* (N = number of female parasitoids tested; test statistics: Cochran's Q test; Sign.: ns = not significant; --- = highly significant; group wise tests after McNemar are presented in the Appendix (Table 10); no significant difference was noticed between any *Lygus* control groups).

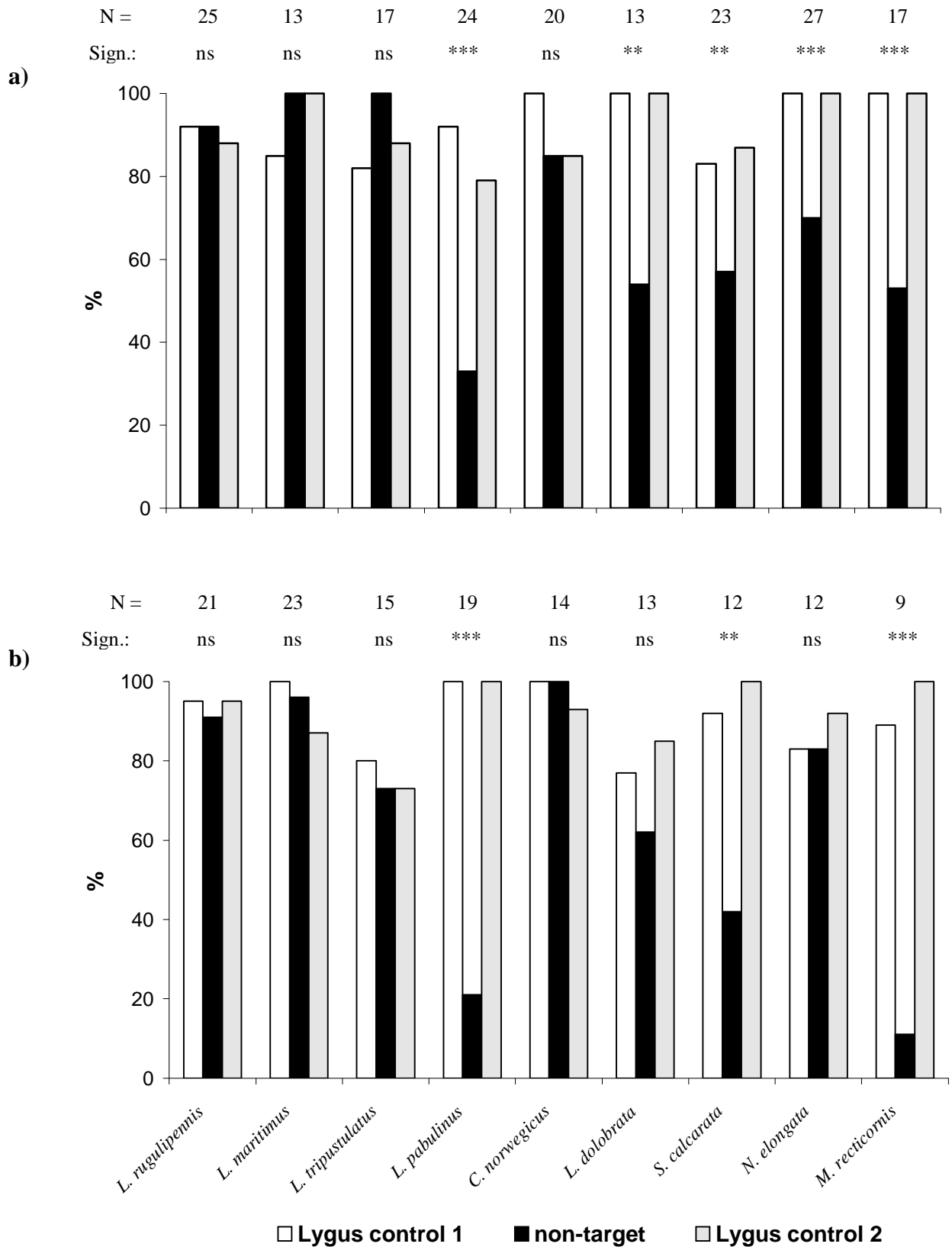


Figure 3.4 Percent successful attacks of *Peristenus* spp. on non-target and *Lygus* hosts a) *P. stygicus* and b) *P. digoneutis* (N = number of female parasitoids tested; test statistics: Cochran's Q test; Sign.: ns = not significant; ** = very significant; *** = highly significant; group wise tests after McNemar are presented in the Appendix (Table 11); no significant difference was noticed between any *Lygus* control groups).

The grass bugs *N. elongata*, *L. dolobrata*, *S. calcarata*, and *M. relicticornis* were significantly less accepted as hosts (68-75%)(Appendix, Table 10), and only 54% to 70% of the attacks observed on these species were successful in contrast to *Lygus* control samples (83 - 100%) (Appendix, Table 11).

Large variations were detected in the length of time until the first sting for *P. stygicus* (Appendix, Table 12). Attacks were observed within 2 seconds and up to 19:40 minutes following the start of the experiment. Nonetheless, the general trend was that the mean searching time decreased from the first to the third experimental run. However, results were significant in only four out of nine test groups (*L. pabulinus*, *L. tripustulatus*, *N. elongata*, and *M. relicticornis*) (Appendix, Table 12). The duration of stings ranged from 2 to 200 seconds (mean: 10 ± 0.5 (SE) sec., $n = 761$). However, the duration of stings on *Lygus* nymphs was not consistent and varied greatly within each test group and thus, the results were less evident and difficult to interpret. When nymphs of *L. tripustulatus* were attacked, stinging took significantly less time than in *Lygus* control groups, whereas oviposition in *Notostira* nymphs lasted significantly longer (see Appendix, Table 12). In the case of *L. dolobrata*, attacks within the first *Lygus* control group were shorter than attacks on non-target nymphs in the second and *Lygus* nymphs in the third experimental run. In the remaining test groups no differences were found in the duration of sting.

In non-choice tests, *P. digoneutis* females only attacked the alternative target *L. maritimus* and the non-target *L. pabulinus* with the same frequency as *L. rugulipennis* (90-100%) (Fig. 3.3 b; Appendix, Table 10). All other test mirids were significantly less attacked (29-70%). Similar to the results obtained for *P. stygicus*, *P. digoneutis* attacked *L. pabulinus* frequently but parasitoids rarely developed (Fig. 3.4 b; Appendix, Table 11). The potato bug *C. norwegicus* and the nettle bug *L. tripustulatus* were suitable hosts, and attacks were as successful as those occurring on *Lygus* nymphs. Mirids living on grass were accepted less often (29-53%). Among the grass bugs the highest acceptance was observed for *L. dolobrata* (54%), whereas the lowest was found for *M. relicticornis* (29%). Although attacked less frequently, the two grass bugs *L. dolobrata* and *N. elongata* were suitable hosts, as parasitoids developed in 64% and 71% of the attacked nymphs, respectively (Fig. 3.4 b). In contrast, less than 40% of attacks on the grass bugs *M. relicticornis* and *S. calcarata* resulted in parasitoid development. In the case of *M. relicticornis*, only one out of ten attacked nymphs was actually

parasitized. This nymph, however, died before the parasitoid larva had left its host to form a cocoon (Appendix, Table 9).

For *P. digoneutis*, no significant differences were found in the length of time until the first sting among the test groups. *Peristenus digoneutis* females needed between 2 seconds and 19:52 minutes to find their hosts (Appendix, Table 13). The duration of sting lasted between 1 and 273 seconds (mean: 11 ± 1 (SE) sec., $n = 735$). Like in *P. stygicus* the results were not consistent among *Lygus* control groups. In three test groups (*L. rugulipennis*, *L. tripustulatus*, and *S. calcarata*) oviposition lasted on average significantly longer in the first experimental run (= 1st *Lygus* control) than in the second (= exposure to non-target) and third (2nd *Lygus* control) (see Appendix, Table 13). In general, longer stinging times occurred more often when parasitoids were exposed to *Lygus* nymphs for the first time.

For many of the non-target species no rearing protocols were available and thus, nymphal mortality in rearing control samples was relatively high, varying between 7 and 60 % depending on the species (Appendix, Table 15). However, in five non-target species an increased unspecific nymphal mortality was observed after nymphs were attacked by *P. stygicus*. Especially high proportions of *L. pabulinus*, *N. elongata* and *S. calcarata* nymphs died before they had reached the adult stage or before a parasitoid larva had emerged. In one case (*L. maritimus*), the unspecific nymphal mortality was lower after attacks by *P. stygicus*; however, the sample size for this species was low ($n = 14$). An increased unspecific mortality was also observed when nymphs of *L. rugulipennis*, *L. maritimus*, *M. recticornis*, and *L. pabulinus* were attacked by *P. digoneutis* (Appendix, Table 15).

3.3.3 Simultaneous choice tests

When nymphs of the grass bugs *L. dolobrata*, *N. elongata*, *S. calcarata*, and *M. recticornis* were offered simultaneously with *Lygus* nymphs, *P. digoneutis* showed a significant preference for the target nymphs (*L. dolobrata*, *S. calcarata*: $P \leq 0.001$; *N. elongata*, *M. recticornis*: $P < 0.01$) (Fig. 3.5 b; Appendix, Table 14). In all cases more than 75% of attacks observed were on *Lygus* nymphs. A lower, but still significant preference for *Lygus* was noticed when *Lygus* nymphs were offered together with the species *L. tripustulatus* or *C. norwegicus* ($P < 0.05$). However, *P. digoneutis* showed no preference for the target or non-target hosts when nymphs of the closely related species *L. pabulinus* were offered together

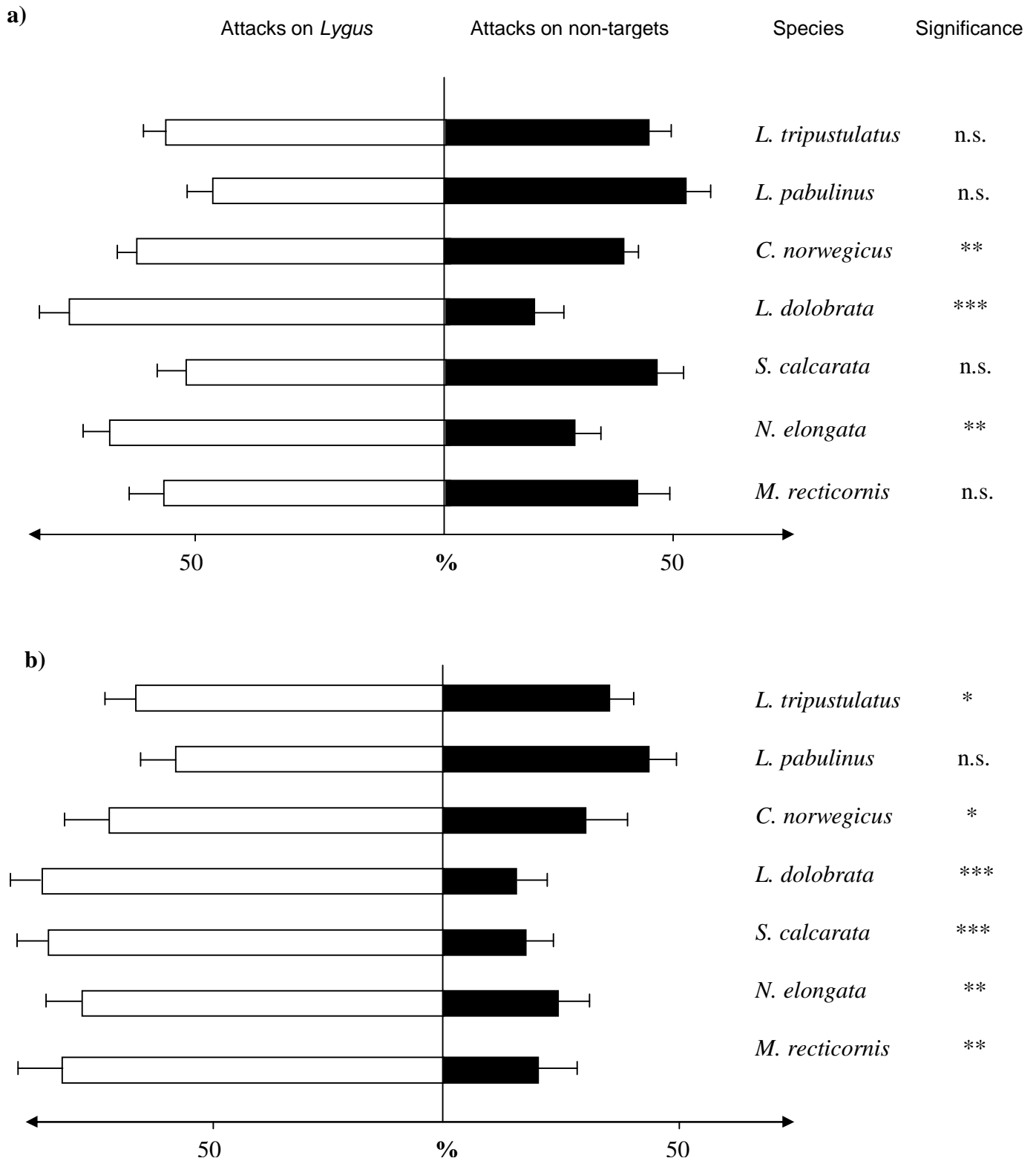


Figure 3.5 Mean percentage of attacks / 5min on target and non-target nymphs when offered simultaneously to a) *P. stygicus* and b) *P. digoneutis* (n = 20 females tested / non-target species; test-statistic: Wilcoxon paired-sample test, see Appendix, Table 14, ns = non significant; * = significant; ** = very significant; *** = highly significant).

with *Lygus* nymphs ($P = 0.31$). In comparison to *P. digoneutis*, *P. stygicus* always showed a lower preference for *Lygus* nymphs (Fig. 3.5 a). Nymphs of the non-target species *L. tripustulatus*, *L. pabulinus*, *S. calcarata*, and *M. recticornis* were attacked with nearly the same frequency as *Lygus* (P values: see Appendix, Table 14). When nymphs of the potato bug *C. norwegicus* or either of the two grass bugs *M. recticornis* and *L. dolobrata* were exposed simultaneously with *Lygus* nymphs, *P. stygicus* attacked the target significantly more often than the non-target. The lowest rate of attacks on a non-target species was observed when nymphs of the meadow bug *L. dolobrata* and *Lygus* were offered simultaneously (20%; $P \leq 0.001$).

Within the 5 minutes duration of the test, *P. stygicus* attacked on average five nymphs (range: 1-14) and *P. digoneutis* three nymphs (range: 1-7) (Fig. 3.6).

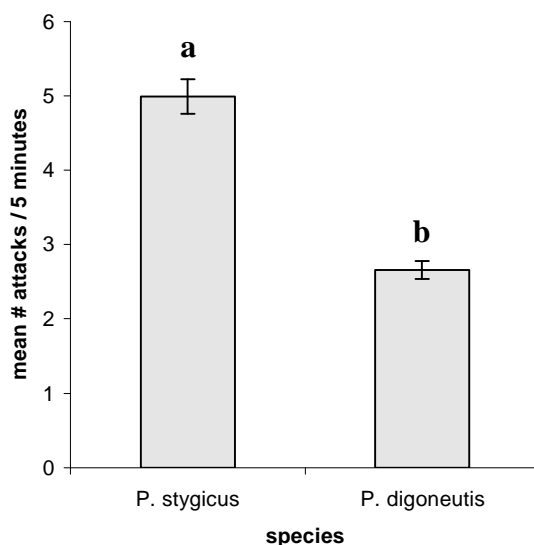


Figure 3.6 Average number of attacks / 5min on mirid nymphs by *P. stygicus* and *P. digoneutis* observed in simultaneous choice tests (Mann-Whitney test: $n = 140$, $Z = -7.965$, $P < 0.001$; mean *P. stygicus*: 4.99 ± 0.23 SE, range: 1-14; mean *P. digoneutis*: 2.66 ± 0.12 SE, range: 1-7).

3.4 Discussion

3.4.1 Host specificity with regard to the experimental design

In sequential choice tests, *P. stygicus* and *P. digoneutis* showed a remarkable acceptance of non-target hosts. More than 50% of *P. stygicus* females attacked all species selected as

alternative hosts, and all non-target species tested were suitable for parasitoid development. However, mirids from the tribe Stenodemini were significantly less attacked and likely less suitable for parasitoid development than those from the tribe Mirini. This indicates that the phylogenetic relatedness of non-target hosts with *Lygus* targets may have an influence on *P. stygicus*; however, this does not prevent the parasitoids from attacking non-target hosts in general. The results of the present study are consistent with the observations made by Condit & Cate (1982). They found that *P. stygicus* develops in several North American mirids, e.g. in the *Mirinae* species *Lygus hesperus*, *L. lineolaris* and *Polymerus basalis*, the *Orthotylinae* species *Labopidicola geminata*, *Phylinae* species *Pseudoatomoscelis seriatus*, and incomplete development occurs in the mirine species *Dichrooscytus* sp. In the present study non targets phylogenetically close to *Lygus* were attacked with the same frequency as *Lygus* suggesting that these species stimulate *P. stygicus* to oviposit in the same way as *Lygus* nymphs. However, potential chemical cues responsible for the parasitoid's reaction are not known, and it is possible that closely related species such as *L. tripustulatus* and *L. pabulinus* were only attacked because of their morphological similarity to *Lygus*. However, Condit & Cate (1982) postulated that factors like host colour, mobility and size are rejected as host selection criteria for *Peristenus* species. Surprisingly, the closely related mirid *L. pabulinus* seems to be a less suitable host for *P. stygicus*. Although attacks were frequently observed and oviposition behavior did not differ remarkably, one third of the test nymphs reached the adult stage, and in the majority of dissected nymphs no sign of parasitization was found. However, it remains questionable if parasitoid females actually oviposited into the nymphs or if the eggs were destroyed by the host's immune response. When *P. stygicus* was exposed to *Lygus* and non-target nymphs simultaneously, non-target nymphs were attacked consistently, and a preference for *Lygus* nymphs was only observed when offered together with three out of the seven non-targets. These results confirm the observations made in the non-choice tests and suggest that *P. stygicus* has a high potential to attack non-target nymphs as already pointed out by Condit & Cate (1982). Surprisingly, the results from non-choice tests indicated a trend regarding the amount of time needed to locate a host; these data suggest that search time decreases with each experimental run. It seems possible that naive *P. stygicus* females learned to associate certain stimuli with the host following their first oviposition experience, resulting in the ability to respond to host cues quicker and locate nymphs faster in subsequent runs. Studies on other parasitoid-host associations have shown that oviposition experience tends to be the most efficient factor changing a parasitoid's behavior and that the changes that result are slowest to decay (Turlings et al., 1992; Godfray, 1994). Variations in the length of attacks

did not follow a uniform pattern and thus, differences remain unexplained. In some mirid species, an increased unspecific nymphal mortality was observed after nymphs were attacked by *P. stygicus*. This indicates that the venom injected by the parasitoid during stinging or the injury by the sting itself, probably had a negative impact on the nymph's immune system (Quicke, 1997). For example, Kyeipoku & Kunimi (1996) showed that parasitization can increase susceptibility to viral infections. However, nymphal mortality was also high among non-parasitized nymphs and in some cases the sample size was small and thus, data have to be interpreted carefully.

Similar to *P. stygicus*, all non-target hosts offered in non-choice tests were attacked by *P. digoneutis*. However, for three of the selected non-target species less than 50% of the parasitoid females reacted positively when offered nymphs of these species. Results from non-choice tests strongly agree with those from choice tests. In both test situations *L. pabulinus* was the only non-target host that was accepted by *P. digoneutis* with the same frequency as *Lygus* hosts. In particular, mirids from the tribe Stenodemini were attacked less often. This suggests that *P. digoneutis* is potentially more selective than *P. stygicus* when choosing hosts for oviposition. However, there are good reasons to question the assumption by Bilewicz-Pawinska (1982) that *P. digoneutis* is likely monophagous because most non-targets, although attacked less frequently than *Lygus* hosts, were suitable for parasitoid development. The only species in which *P. digoneutis* failed to complete development was *M. recticornis*; no parasitoid cocoons were obtained in tests with this non-target species. Despite the frequent attacks on *L. pabulinus* in non-choice and choice tests, almost no parasitoids developed from this mirid. This indicates that *L. pabulinus* is a less suitable host, as was already mentioned for *P. stygicus*. This assumption is supported by the increased unspecific nymphal mortality rate following attack by *P. digoneutis*. However, in the dissected *L. pabulinus* nymphs and adults obtained from the tests, no encapsulated parasitoid eggs or larvae were found. In many cases oviposition lasted remarkably longer when naive females were exposed to nymphs in the first run of sequential non-choice tests. As explained for *P. stygicus*, it is possible that naive females learn to associate certain stimuli with the host during their first encounter; consequently, the following attacks would be shorter. However, this observation cannot be generalized, as results were not consistent.

None of the parasitoid species showed a difference in their acceptance of the alternate target species, *L. maritimus*, nor in their development from this host when compared with the target species *L. rugulipennis*. These laboratory results confirm field observations which

demonstrated that both *Lygus* species were frequently attacked by *P. stygicus* and *P. digoneutis* (see chapter 2).

According to Godfray (1994), parasitoids with high egg loads will oviposit in nearly all hosts they encounter, including hosts in which the probability of offspring survival is small. As demonstrated in chapter 5 of this thesis, *P. stygicus* produces on average 50% more eggs over its lifetime than *P. digoneutis*; this may explain why *P. stygicus* oviposits more readily into non-target hosts and significantly more often within the same time interval than *P. digoneutis*. On the other hand, *P. digoneutis* may be more selective when choosing suitable hosts than *P. stygicus* in order to use its limited egg resources more efficiently. The more distinct oviposition behaviour exhibited by *P. digoneutis* may be an indication of this, and may be another means of host discrimination in order to prevent oviposition in unsuitable hosts.

Assuming that the laboratory conditions had no influence on the parasitoids' behavior, the present study indicates that both parasitoids actually have a broad host range and may attack non-target hosts from at least two tribes as an alternative resource for reproduction. The fact that parasitoids were also able to develop successfully from the non-target hosts supports this assumption. On the other hand, it has been shown that under laboratory conditions (e.g. restricted space) parasitoids attack non-suitable hosts when given no other choice for oviposition and thus, parasitoid's host range can be easily overestimated (Loan & Holdaway, 1961; Sand & Papacek, 1993; Godfray, 1994; Strand & Obrycki, 1996). In the case of *P. stygicus* and *P. digoneutis*, the pressure to oviposit during the tests is likely high, as it has been shown that both species reach their highest daily oviposition rates within the first five days after emergence (see chapter 5). The inconsistency of high acceptance of *L. pabulinus* nymphs but poor parasitoid development and high unspecific nymphal mortality may be an example of abnormal behavior under laboratory conditions. However, when parasitoids were given the choice between *L. pabulinus* and *Lygus* nymphs, both species were accepted at the same level. There has also been concern that parasitoids may oviposit into species outside their normal host ranges when presented with hosts and non-hosts in choice tests (van Driesche & Hoddle, 1997). This is assumed to occur because specific volatiles from the hosts stimulate oviposition behaviors which are unleashed against both species present ("spill-over effects") (Sands, 1993). If this was the case, *P. stygicus* and *P. digoneutis* should not have attacked non-targets in the sequential non-choice tests; however, the results significantly demonstrate that both species did attack the non-targets in this scenario.

3.4.2 Consequences for biological control

Investigations clearly showed that *P. stygicus* and *P. digoneutis* do attack non-target mirids consistently under laboratory conditions and that both parasitoids are able to develop in hosts other than *Lygus*. Thus, the risk of direct effects on non-target species may be considerable, and both species have to be classified as potentially risky for introduction. However, these assumptions were obtained solely from laboratory tests, and may not reflect parasitoid behaviour under natural conditions (Kuhlmann et al., 2000). In order to prevent an overestimation of parasitoid's potential host range, the next step in host specificity assessment should be to conduct choice tests in large cages, simulating semi-natural conditions, or field tests following the protocol developed by van Lenteren et al. (2003). This method should be the preferred approach when host range is tested in the area of introduction. On the other hand, if host specificity is investigated in the area of the parasitoid's origin (like in the present study), collections of non-target mirids in the field in order to determine the parasitoid's host range in a natural situation may represent a good alternative to large-scale cage experiments.

4 Ecological host range of *Peristenus digoneutis* and *P. stygicus* (Hymenoptera: Braconidae) in Schleswig-Holstein

4.1 Introduction

In order to prevent undesirable non-target effects of biological control agents, an increasing number of countries nowadays apply risk assessment procedures (e.g. laboratory tests on host specificity) before an exotic natural enemy can be imported or released. However, in the laboratory it is difficult to accurately reproduce the factors that influence host searching and assessment behavior of a parasitoid in its natural environment (Sands, 1993). Thus, laboratory observations must be combined with field studies of a parasitoid's host range in the area of origin to provide the basis for correctly interpreting physiological host range estimations made via laboratory testing (Onstad & McManus, 1996; Kuhlmann et al., 2000).

In contrast to the *physiological host range*, the current and evolving set of host species actually used for successful reproduction in nature is defined as *realized* or *ecological host range* (Nechols et al., 1992; Onstad & McManus, 1996); it is constrained by environmental influences including physical (e.g. geographical barriers, climatic tolerance) and biological (e.g. competition, predation) factors (Futuma & Moreno, 1988).

European *Lygus* parasitoids considered for biological control of *Lygus* spp. in North America are assumed to be specific and probably restricted to the family Miridae (Heteroptera) (Kuhlmann et al., 2000). According to Wagner (1952), approximately 2000 species belonging to the family Miridae occur in the palaeartic region (307 in Germany) and thus, the number of potential alternative host for *Lygus* parasitoids is immense. Limited studies on the ecological host range of European *Lygus* parasitoids were conducted in Poland in the 1970s (Bilewicz-Pawinska, 1982). However, investigations were mainly made in agricultural crops, where species-richness is poor and thus, the study included only five potential non-target mirid species. Interestingly, in Poland a higher level of parasitization of *Lygus* was found in cereal crops adjacent to grassland. Furthermore, observations made in Great Britain showed that at least 51 mirid species collected in non-crop habitats were parasitized by non-identified parasitoid species, most likely belonging to the same subfamily (Euphorinae) as *Lygus* parasitoids (Leston, 1957, 1959, 1961).

For that reason, the question arises whether *Lygus* parasitoids are using alternative hosts in natural habitats as a potential resource for reproduction. To answer this question it is essential to extend investigations on the ecological host range of *Lygus* parasitoids to potential non-target hosts living in non-crop habitats.

This chapter reports field data from a three-year study on the ecological host range of the European *Lygus* parasitoids *Peristenus stygicus* and *P. digoneutis* in Schleswig-Holstein (northern Germany).

4.2 Material and methods

4.2.1 Sampling

Collections were mainly conducted in natural habitats rich in mirid species, such as stinging nettle stands (*Urtica* spp.) or grasslands which are frequently found adjacent to agricultural land. Other host plants sampled were camomile (*Matricaria recutita* (L.) and *Tripleurospermum perforatum* (Mér.)), black medick (*Medicago lupulina* L.), red clover (*Trifolium pratense* L.), mugwort (*Artemisia vulgaris* L.), tansy (*Chrysanthemum vulgare* (L.) Bernhardi), tansy Phacelia (*Phacelia tanacetifolia* Benth), bladder campion (*Silene vulgaris* (Moench) Garcke), winter oilseed rape (*Brassica* spp.), hedge nettle (*Stachys silvatica* L.), *Atriplex lacinata* L., various *Apiaceae*, and oak trees (*Quercus* spp.).

The aim of collections in various habitats was to obtain samples from a broad range of common and rare mirid species considered as potential alternative hosts of the *Lygus* parasitoids *P. stygicus* and *P. digoneutis*. In order to investigate whether these parasitoids are actually specific to the subfamily *Mirinae* (the subfamily to which *Lygus* spp. belongs), collections were also focused on other mirid subfamilies, such as *Bryocorinae*, *Deraeocorinae*, *Orthotylinae* and *Phylinae*. For each mirid species used in laboratory tests (see chapter 3), large samples were collected from at least ten to thirty sites, if possible, to provide a reliable data basis for comparing results from laboratory host range tests with field data. The occurrence of most non-target mirids was documented throughout the field season (April-September) to examine if parasitoid emergence (under natural conditions) was adapted to the time of appearance of their hosts in the field.

Heavy duty sweep nets (38 cm diameter, BioQuip) were used for all collections. After sweeping a site, samples were transferred into white plastic trays to separate mirid nymphs from other insects and spiders. To reduce the time consuming rearing process, mirid nymphs were collected exclusively in the 4th or 5th instar. This method is acceptable because the mature, parasitoid larvae are known to emerge from late nymphal instars or teneral adults, but not from early instars (Loan, 1980). Collected nymphs were maintained on host plant foliage in ventilated, clear plastic containers and transported in a cooler to the laboratory. In the laboratory, mirid samples were separated by species. Since immature stages of hymenopteran parasitoids of mirids are morphologically indistinguishable (Bilewicz-Pawinska & Pankanin, 1974), the nymphs were reared until parasitoid adults emerged in the same or the following year. When high numbers of non-target mirids were found, subsamples (20-50 nymphs) were taken randomly and dissected to establish the level of parasitization. Otherwise, rare mirid species were only used for rearing out the parasitoids. Voucher specimens of non-target mirids collected from each site were sent to Dr. Albert Melber (University of Hanover, Germany) for identification. *Peristenus* parasitoids were identified by Dr. Henri Goulet (Agriculture and Agri-Food Canada, E.C.O.R.C., Ottawa).

4.2.2 Rearing, overwintering and parasitoid emergence

For non-target mirids, the same three rearing systems described for *Lygus rugulipennis* (see chapter 2) were used. However, for rearing grass bugs, the container size for samples of 30 to 50 nymphs had to be slightly modified because of the size of the host plants and thus, two stacked 1.2 L plastic containers were used instead of one (Fig. 4.1). Mirids belonging to the genus *Adelphocoris*, *Closterotomus* and *Calocoris* were fed with organically grown beans and lettuce. For all other mirid species the host plants they were collected from were added to the rearing cages, because these species would not accept any other food.



Figure 4.1 Rearing container for grass bugs

Grass bugs were mainly kept on grass ears and nettle bugs on flowering stinging nettles. Freshly cut host plants were stuck into moist foam to keep plants fresh; these were changed after three to four days to provide a continuous supply of fresh plant material. For the nettle bugs *L. tripustulatus* and *L. pabulinus* rearing was successful when both sprouting potatoes and stinging nettles were provided.

For overwintering parasitoid cocoons and monitoring of adult emergence, the same procedure was followed as described for *Lygus* parasitoids in chapter 2 (2.2.5 and 2.2.6), except that parasitoid emergence was monitored daily over a period of 2.5 months.

4.2.3 Estimating the impact of *Lygus* parasitoids on non-target mirids

First, the number of parasitoids (primary parasitoids and hyperparasitoids) emerging from each non-target mirid species was summed up. Then, the proportion of *P. digoneutis* and *P. stygicus* to the total number of emerged parasitoids was calculated. These proportions show the general importance of *P. digoneutis* and *P. stygicus* among nymphal parasitoids of mirids but do not reflect the impact on non-target mirids at single collection sites. Consequently, the proportions of *P. stygicus* and *P. digoneutis* relative to the total number of parasitoids emerged from single collection sites were analyzed. The impact of *P. stygicus* and *P. digoneutis* on non-target mirids at single collection sites was calculated by a 2-step process: 1) the percentage of parasitized nymphs on the day of sampling was determined by dissections (or rearing). Then, 2) the proportion parasitized by each parasitoid species was calculated using data from duplicate samples that had been reared until parasitoid adults emergence (e.g. if 40% of dissected specimens were parasitized, and 75% of the reared (= emerged) parasitoids were species A, then the level of parasitization for species A was 30%, and the level for species B was 10%, assuming there were only two parasitoid species in the sample).

$$\frac{\text{level of parasitization}}{\text{total no. of parasitoids emerged}^*} \times \text{no. of parasitoids (species A) emerged} = \text{level of parasitization caused by species A}$$

* total no. of parasitoids emerged: this includes all specimens that emerged from a sample, primary parasitoids as well as hyperparasitoids.

For the evaluation of single sites, only samples were used from which more than ten parasitoids had emerged. This way, it was prevented to greatly overestimate the impact of *Lygus* parasitoids. For example, if from 500 nymphs (level of parasitization 1%) five

parasitoids emerged and two of them were *P. stygicus*, the proportion of *P. stygicus* relative to the total number of parasitoids emerged was relatively high (40%). However, in relation to the sample size and the level of parasitization, the impact of *P. stygicus* would be negligible. If hyperparasitoids emerged, the actual level of parasitization caused by a primary parasitoid could not be estimated exactly, because it was not possible to associate each hyperparasitoid with the primary parasitoid it had killed before (especially when more than one primary parasitoid emerged from a sample).

Occasionally, *Lygus* nymphs and other non-target mirid nymphs occurred simultaneously at the same site. The proportions of parasitoid species emerging from target and non-target hosts were then analyzed in order to investigate the impact of *Lygus* parasitoids in the field when the potential to encounter more than one host species is high.

4.3 Results

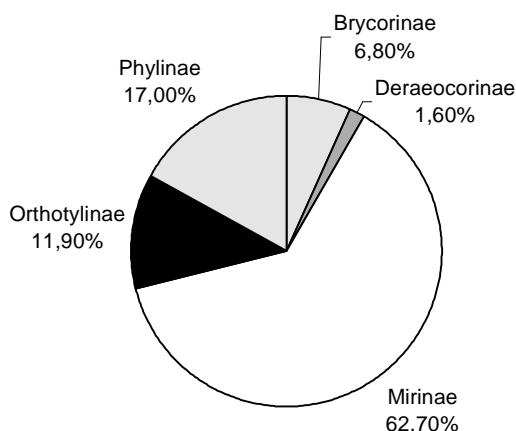
4.3.1 Records of mirid species in Schleswig-Holstein

During the 2001-2003 seasons, adults of 59 non-target mirid species were recorded in the area of investigation (Appendix, Table 16). The subfamily Mirinae was represented by 37 species (62.7%); ten species (17%) belonged to the subfamily Phylinae, seven to Orthotylinae (11.9%), four to Bryocorinae (6.8%) and one to Deraeocorinae (1.6%) (Fig. 4.2). Species belonging to the subfamilies Isometopinae, Psallopinae, and Cylapinae were not recorded. One species – *Creontiades pallidus* (Ramour, 1839) - was not known to appear in Schleswig-Holstein and has probably been introduced from Mediterranean countries, the Near East or eastern Asia (A. Melber, personal communication). However, the nymphs collected (in total 50,603) only represented 39 out of the 59 non-target mirid species (Appendix, Table 16).

The highest numbers of non-target mirid nymphs were found on *Gramineae* (17 species) and *Urtica* spp. (10 species) (Appendix, Table 17). Some mirid species were collected only from one host plant, e.g. *Oncotylus punctipes* on *Chrysanthemum vulgare* or *Macrotylus solitarius* on *Stachys sivatika*. Other species like *Closterotomus norwegicus* were collected from a broad spectrum of host plants.

Figure 4.2

Proportions of species belonging to different mirid subfamilies obtained by the non-target mirid collections conducted in Schleswig-Holstein (2001-2003).



4.3.2 Ecological host range of *P. digoneutis* and *P. stygicus*

Peristenus digoneutis has been reared from seven non-target hosts belonging to the subfamily Mirinae (Table 4.1, yellow column). In stinging nettle habitats *Liocoris tripustulatus* (6 specimens), *Lygocoris pabulinus* (2), *Calocoris affinis* (1) and *Apolygus lucorum* (1) were found to be suitable hosts. Furthermore, *P. digoneutis* developed from the grass bugs *Stenodema calcarata* (1) and *Stenodema holsata* (1). More frequently, *P. digoneutis* was reared from *Closterotomus norwegicus* (51). It was recorded from nine out of 29 sites (31%), where *C. norwegicus* nymphs were collected (Appendix, Table 17).

In contrast to the former species, *P. stygicus* has been reared from 16 non-target hosts (Table 4.1, red column). Twelve hosts belonged to the subfamily Mirinae, three to Phylinae and one species to Brycorinae. *Peristenus stygicus* developed in nymphs of the phylogenetically closely related mirid species *Liocoris tripustulatus* (3 specimens) and *Lygocoris pabulinus* (2), both occurring on stinging nettles. In grassland habitats, *P. stygicus* was occasionally found to develop from seven non-target hosts [*Stenodema calcarata* (24), *Megaloceraea recticornis* (24), *Amblytylus nasutus* (30), *Leptopterna dolobrata/ferrugata* (10), *Notostira elongata* (4), and *Trigonotylus caelestialium* (20)]. Furthermore, *P. stygicus* emerged from *Closterotomus norwegicus* (58), collected from various host plants, e.g. camomile, clover, tansy *Phacelia*, stinging nettles, and oilseed rape. Other hosts were *Lopus decolor* (1), *Orthops kalmi* (1), *Plagiognathus chrysanthemi* (10), *Calocoris roseomaculatus* (1), *Calocoris affinis* (1), and *Dicyphus globulifer* (2). *Peristenus stygicus* was most regularly recorded from *T. caelestialium*. It occurred at 56% of the sites where *T. caelestialium* was

Table 4.1 Number of parasitoids emerged from non-target mirids collected in Schleswig-Holstein (total number of parasitoids: 4123; asterisks indicate bivoltine parasitoids).

Mirid subfamily	Parasitoid species		<i>F. digoneuris</i> *	<i>F. nigricans</i> *	<i>F. laeviventris</i> *	<i>F. grandiceps</i>	<i>F. pictipes</i>	<i>F. facialis</i>	<i>F. orthoglypti</i>	<i>F. adelphocoridis</i>	<i>F. ns. "small eyes"</i>	<i>F. near pallipes</i> « « <i>Clasterotomus</i> »	<i>P. near pallipes</i> « « <i>Liochorts</i> »	<i>F. near pallipes</i> « « <i>Notostira</i> »	<i>F. near pallipes</i> « « <i>Leptopterna</i> »	<i>F. near pallipes</i> « « <i>Plagiognathus</i> »	<i>F. near pallipes</i> « « <i>Dicyphus</i> »	<i>F. ns. "pseudometopes"</i>	<i>F. near pallipes</i> « « <i>Amblystus</i> »	<i>F. near pallipes</i> « « <i>Stenodema</i> »	<i>F. near pallipes</i> « « <i>Stenotus</i> »	<i>Leptohron deficiens</i>	<i>Leptohron nsp. near deficiens</i>	<i>Mesochorus curvulus</i> (hyperparasitoid)			
	Mirid species	<i>Dicyphus globulifer</i>																									
Bryococcinae	<i>Adelphocoris lineolaris</i>			2						34								36							1		
	<i>Apolytus lucorum</i>		1		3			59															1		22		
	<i>Calocoris affinis</i>		1		1							17													1		
	<i>Calocoris roseomaculata</i>			1								1														93	
	<i>Clasterotomus norvegicus</i>		51		58	2		2					1143			6				1						82	
	<i>Liochorts tripustulatus</i>		6		3	14								304												4	
	<i>Lygocoris pabulinus</i> (1st gen.)					5																				27	
	<i>Lygocoris pabulinus</i> (2nd gen.)		2		2	31														3							
	<i>Orthops kalmi</i>				1																						
	<i>Rhabdomeris strassellus</i>							8																			
	<i>Stenotus binotatus</i>																										
	<i>Leptopterna albobrata</i>				9							2	2			251											31
	<i>Leptopterna ferrugata</i>				1											3											110
	<i>Megaloceraa recticornis</i>				24											15											4
	<i>Noxanthra elongata</i> (1 st gen.)				1											7											19
	<i>Noxanthra elongata</i> (2 nd gen.)				3							9															90
	<i>Stenodema calcitrans</i> (1st gen.)		1		15																						82
<i>Stenodema calcitrans</i> (2nd gen.)				9																							
<i>Stenodema isolata</i>		1																								5	
<i>Stenodema laevigata</i>																											
<i>Stenodema tripustata</i>																											
<i>Trigonophylus caelestislinus</i> (1 st gen.)				4																							
<i>Trigonophylus caelestislinus</i> (2 nd gen.)				16											23												
<i>Orthopsylus moneragiffi</i>																											
<i>Orthopsylus marghalla</i>									5																		
<i>Eurospidula arthenicaria</i>																											
<i>Lopus decolor</i>																											
<i>Plagiognathus chrysanthemi</i>					1																						
<i>Plagiognathus arbustorum</i>					10																			14		1	
<i>Amblypsylus rannatus</i>																								303		72	
					30																					1	

collected (n = 9). Furthermore, *P. stygicus* was regularly found at sites where *S. calcarata* (at 40% of sampled sites, n = 25), *Amblytylus nasutus* (50%, n = 8), and *C. norvegicus* (45%, n = 29) were sampled (Appendix, Table 17).

4.3.3 Ecological host range of further nymphal parasitoids of mirids

In total, 19 species of *Peristenus*, two species of *Leiophron*, and one species of *Mesochorus* (hyperparasitoid of *Peristenus* spp.) were reared from nymphs of 27 non-target species (20 genera) (Table 4.1). Four out of the eight subfamilies of Miridae (Aukema & Rieger, 1999) were found parasitized. The subfamily Mirinae contained 70% (19) of the parasitized species, followed by the subfamilies Phylinae 19% (5), Orthotylinae 7% (2), and Bryocorinae 4% (1). Among the 21 recorded primary parasitoids, 12 species were previously unknown. Most of the new species belong to the species complex of *Peristenus pallipes*, which likely contains more than ten independent species. The material collected in the present study has served as the basis for a revision of the genus *Peristenus* in Europe; this is currently being done by Dr. Henri Goulet, Ottawa. Until a new species-identification key is published, the new species will be named after the host genus the parasitoids were most frequently reared from (e.g. *Peristenus near pallipes* “*Leptopterna*”).

The ecological host range of primary parasitoids, others than *P. stygicus* and *P. digoneutis*, contained between one and six hosts (Table 4.1). The majority of the parasitoids were related to one to three hosts (15 species), whereas four parasitoids were associated with four to six mirid hosts. Parasitoids that were reared from more than one host were usually related to one mirid genus or to mirid hosts that belonged to different genera but occurred simultaneously within the same habitat. For example, *P. grandiceps* and *P. near pallipes* “*Stenodema*” developed only from hosts belonging to the genus *Stenodema*; and *P. near pallipes* “*Plagiognathus*” was recorded exclusively from *Plagiognathus* species. On the other hand, *P. laeviventris* attacked five hosts (five genera) living on stinging nettles (*Urtica* spp.); and *P. near pallipes* “*Leptopterna*” was recorded from six hosts (five genera) occurring in grasslands in early summer.

Nymphal parasitoids belonging to the genus *Leiophron* were found only sporadically. Single specimens emerged from *Apolygus lucorum* and *Orthotylylus moncreaffi*. Although the present study was focused on nymphal parasitoids of mirids, *Leiophron reclinator* was identified as a parasitoid of mirid adults. Larvae of this species emerged from *L. pabulinus* adults of the first

generation, as well as from overwintered adults of *L. tripustulatus* which were collected in July for rearing nymphs in the laboratory (see chapter 3.2.2). In order to confirm that *L. reclinator* is actually an adult parasitoid, the parasitization behavior was documented in the laboratory (see Appendix, Fig. 2).

The hyperparasitoid *Mesochorus curvulus* was reared from 18 mirid hosts (15 genera), which were parasitized by 16 *Peristenus* and one *Leiophron* species. However, it was not possible to exactly clarify which primary parasitoids *M. curvulus* was related to.

4.3.4 Proportions of *P. stygicus* and *P. digoneutis* emerging from non-target mirids

Although *P. stygicus* and *P. digoneutis* were found in many field-collected non-target mirids, their occurrence was generally low. Among non-target hosts from the tribe Mirini (subfamily Mirinae) the proportions of *P. digoneutis* and *P. stygicus* relative to the total number of parasitoids emerging from each species were less than 5% (Fig. 4.4.). In comparison, in the two common target hosts *L. rugulipennis* and *L. pratensis*, the proportions of *P. digoneutis* were 34% and 58.43%, and the proportions of *P. stygicus* were 36.96% and 52%, respectively (Fig. 4.3). The data presented in Figure 4.3 are based on parasitoid emergence from *Lygus* mass collections in clover and camomile (compare chapter 2 and Appendix, Tables 1 to 3).

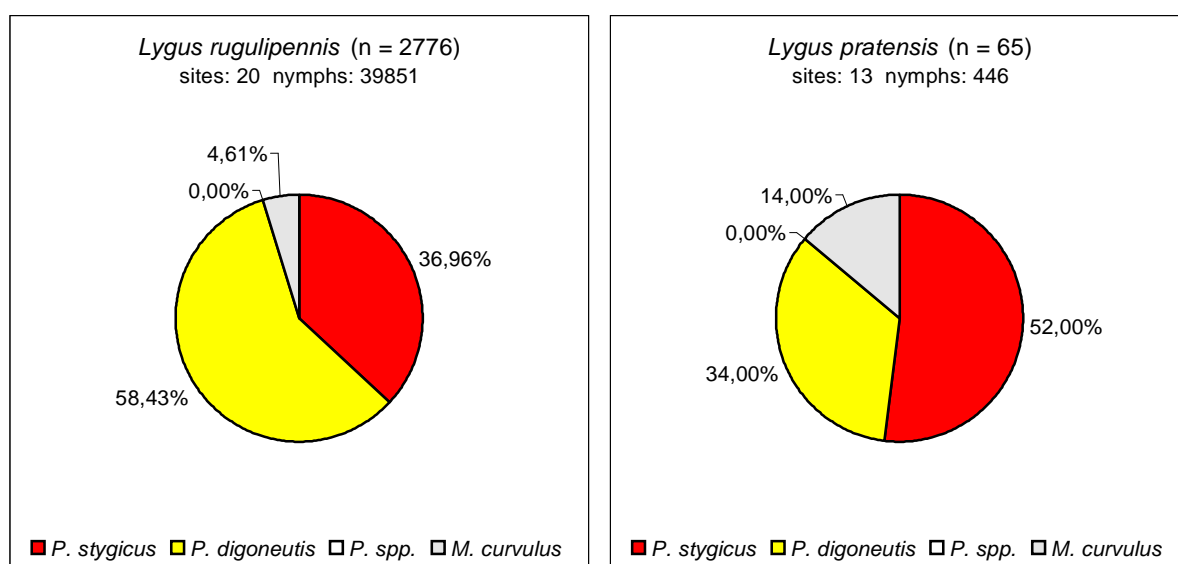


Figure 4.3 Proportions of *P. stygicus* and *P. digoneutis* relative to the total number of parasitoids that emerged (n) from *Lygus rugulipennis* and *L. pratensis*; *M. curvulus* represents the proportion of hyperparasitoids. Further, the number of sites sampled and the total number of nymphs collected are presented.

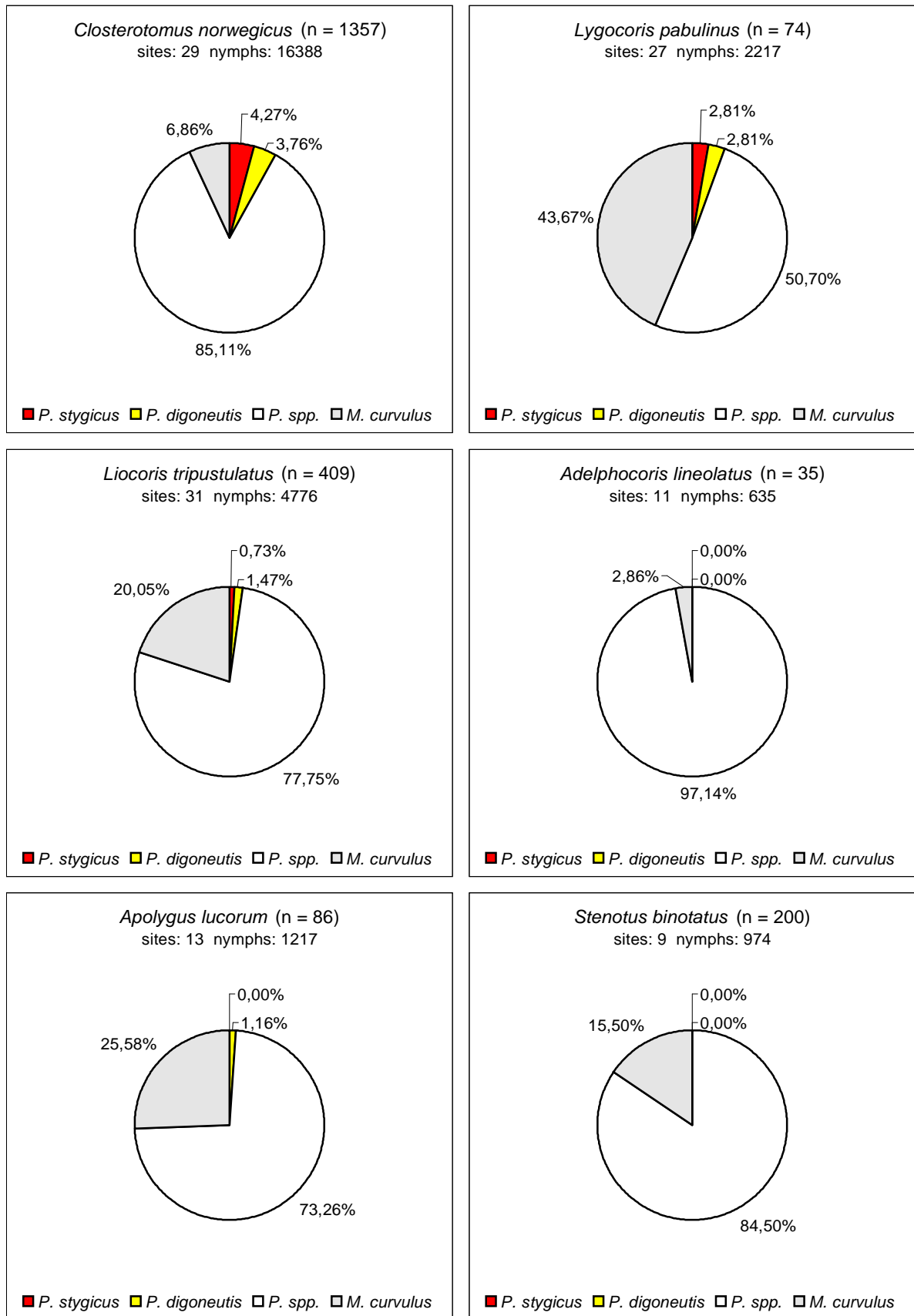


Figure 4.4 Proportions of *P. stygicus* and *P. digoneutis* relative to the total number of parasitoids that emerged (n) from non-target mirids belonging to the tribe Mirini (subfamily Mirinae); *M. curvulus* represents the proportion of hyperparasitoids. Further, the number of sites sampled and the total number of nymphs collected are presented.

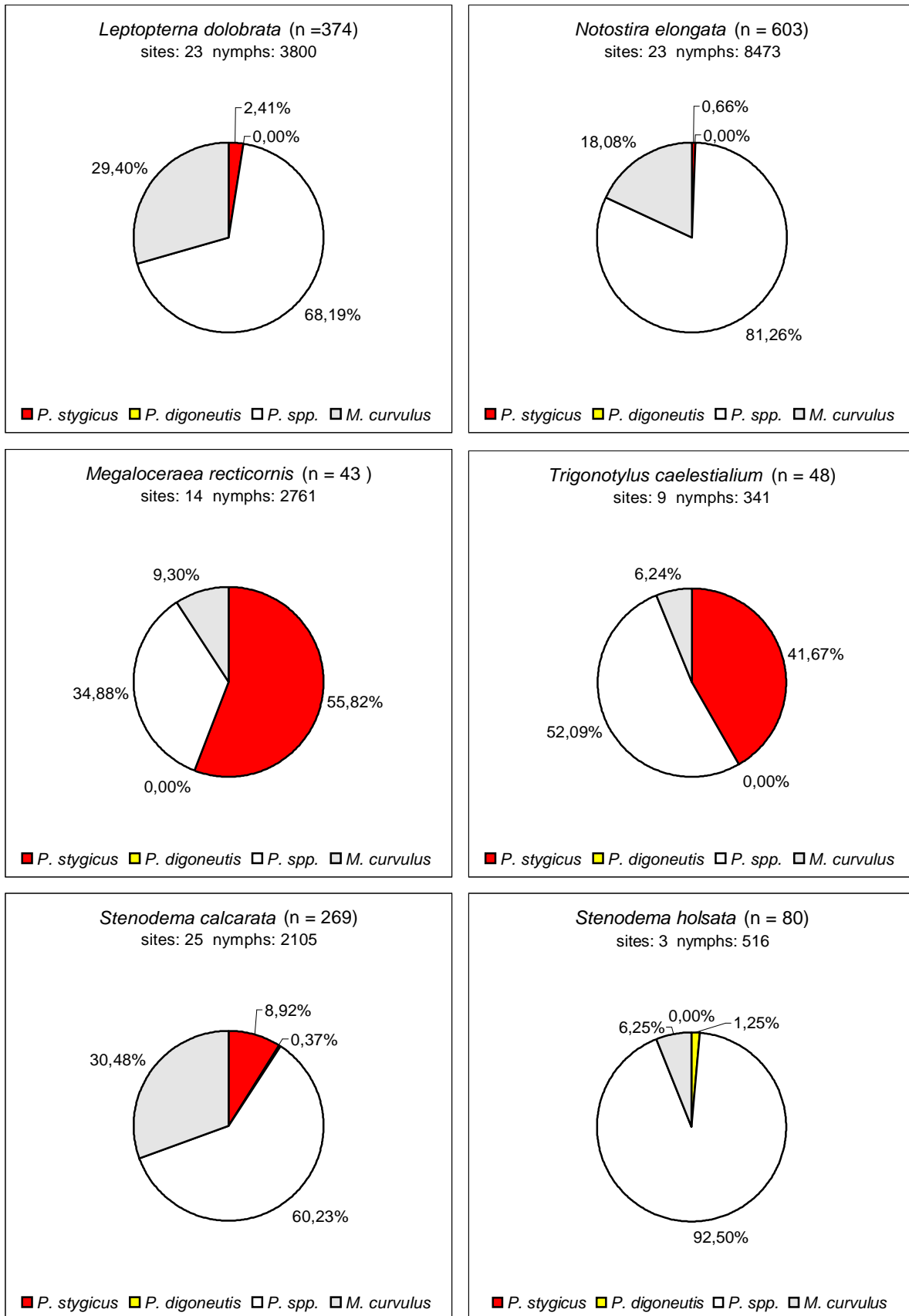
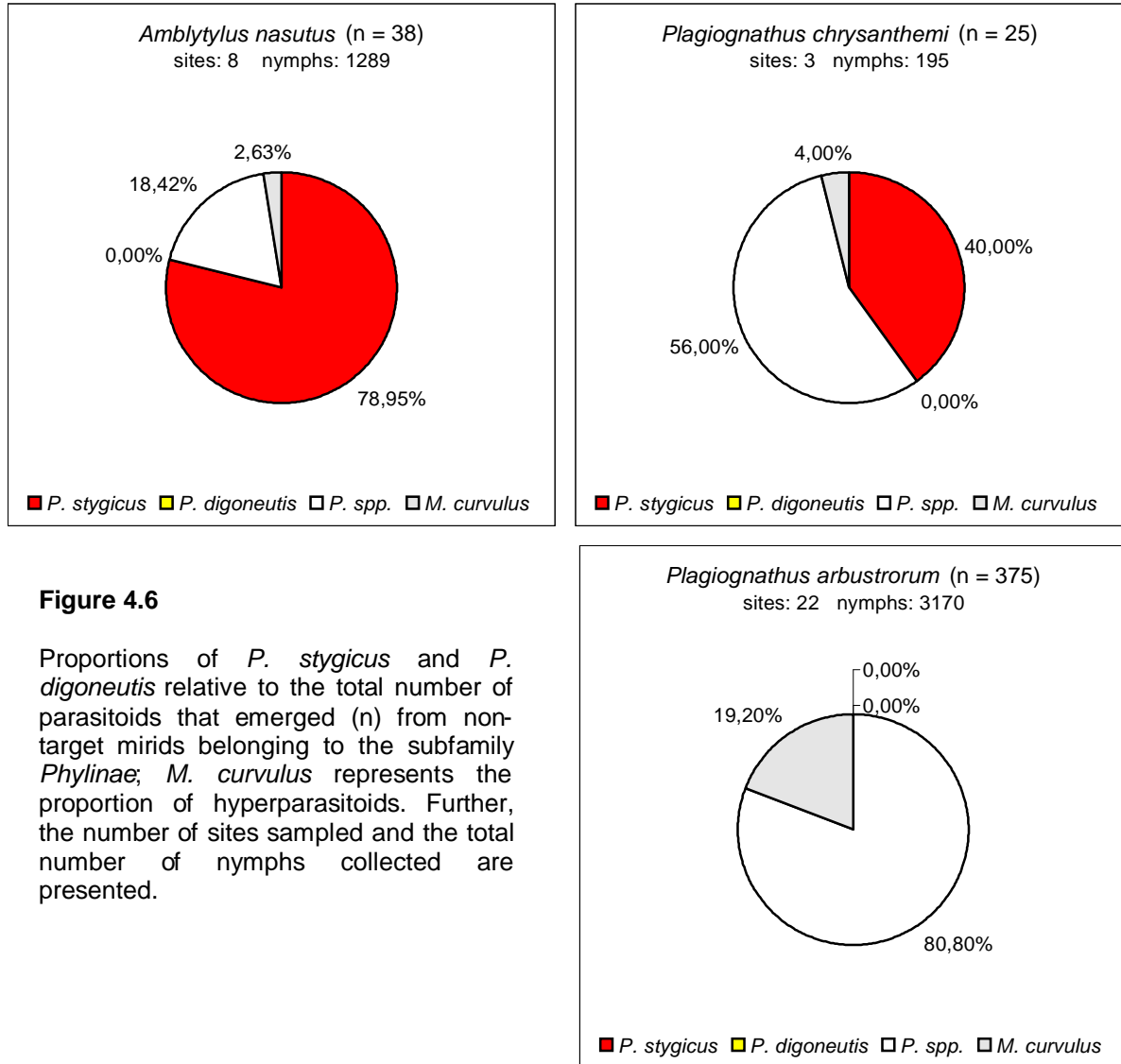


Figure 4.5 Proportions of *P. stygicus* and *P. digoneutis* relative to the total number of parasitoids that emerged (n) from non-target mirids belonging to the tribe Stenodemini (subfamily Mirinae); *M. curvulus* represents the proportion of hyperparasitoids. Further, the number of sites sampled and the total number of nymphs collected are presented.



Of the parasitoids that emerged from Stenodemini (e.g. *L. dolobrata*, *S. calcarata*, and *N. elongata*), the proportion of *P. stygicus* was less than 9% (Fig. 4.5); however for *T. caelestialium* and *M. recticornis* it was 41.67% and 55.82%, respectively. *Peristenus digoneutis* was only recorded from *S. calcarata* and *S. holsata* with proportions of 0.37% and 1.25%, respectively.

The relative proportion of *P. stygicus* to the total number of parasitoids that emerged from the Phylinae members, *P. chrysanthemii* and *A. nasutus*, was 40% and 78.95%, respectively (Fig. 4.6). However, these data are based on a limited number of emerged parasitoids and a relatively low number of sampled sites. In the case of *P. chrysanthemii*, *P. stygicus* was only reared from nymphs collected from one of three sampled sites.

The proportions of *P. stygicus* and *P. digoneutis* obtained from mirid hosts which were only collected at one site or from which less than 25 parasitoids emerged are not presented in pie charts, but detailed information is given in Table 4.1.

4.3.5 Impact of *P. stygicus* and *P. digoneutis* on non-target mirids at single collection sites

Overall, *P. digoneutis* emerged from 28 out of 499 non-target mirid samples; however, in 13 collections the total number of parasitoids (including *P. digoneutis*, *Peristenus* spp. and *M. curvulus*) that emerged was below ten individuals and thus, for statistical reasons, these samples were excluded from the analysis. The remaining 15 samples (Appendix, Table 18) were used to estimate the impact of *P. digoneutis* on non-target mirids at single collection sites. In 13 out of 15 collections, the proportion of *P. digoneutis* relative to the total number of parasitoids emerged was below 10%. Only in two samples of *C. norwegicus* nymphs the proportions were 10.5% and 26.5%. However, when these proportions were related to the actual levels of parasitization in the samples, the level of parasitization caused by *P. digoneutis* was below 6%.

Peristenus stygicus emerged from 69 out of 499 non-target mirid samples. Twenty-seven samples were used to estimate the impact of *P. stygicus* (Appendix, Table 18). In 19 samples, the proportion of *P. stygicus* relative to the total number of parasitoids emerged was below 10%, and in four samples it was between 10 and 20%. Furthermore, in four samples the proportion of *P. stygicus* ranged from 40 to 95%. These samples were obtained from the hosts *Stenodema calcarata* (proportion of *P. stygicus*: 66.7%), *Megaloceraea recticornis* (95.2%), *Amblytylus nasutus* (95.5%), and *Plagiognathus chrysanthemi* (40%). However, even in samples with a high proportion of *P. stygicus*, the level of parasitization actually caused by *P. stygicus* was maximum 20.05%.

4.3.6 Simultaneous collections of *Lygus* and non-target hosts

At four sites where nymphs of *L. rugulipennis* and *C. norwegicus* occurred simultaneously, *P. digoneutis* and *P. stygicus* were the dominant parasitoids in *Lygus* (Fig. 4.7 a). However, the mean proportions of *P. digoneutis* and *P. stygicus* emerging from *C. norwegicus* were below 5%. Furthermore, the dominant parasitoid in *C. norwegicus* was *P. near pallipes*

“*Closterotomus*” (mean = 83,7%). For comparing the mean proportions of *P. stygicus* and *P. digoneutis* per site, the number of sites where *Lygus* and non-target mirids occurred simultaneously (n = 4), was statistically too low. However, when the proportions of *P. stygicus* and *P. digoneutis* emerging from *L. rugulipennis* (57% and 38%; n = 427) and *C. norwegicus* (2% and 2%; n = 154) were compared, proportions were significantly different (Binominal test; $P < 0.001$; as test proportions, the proportions of *P. digoneutis* (38%) and *P. stygicus* (57%) emerging from *Lygus* were used, respectively, instead of the usual test proportion of 50%).

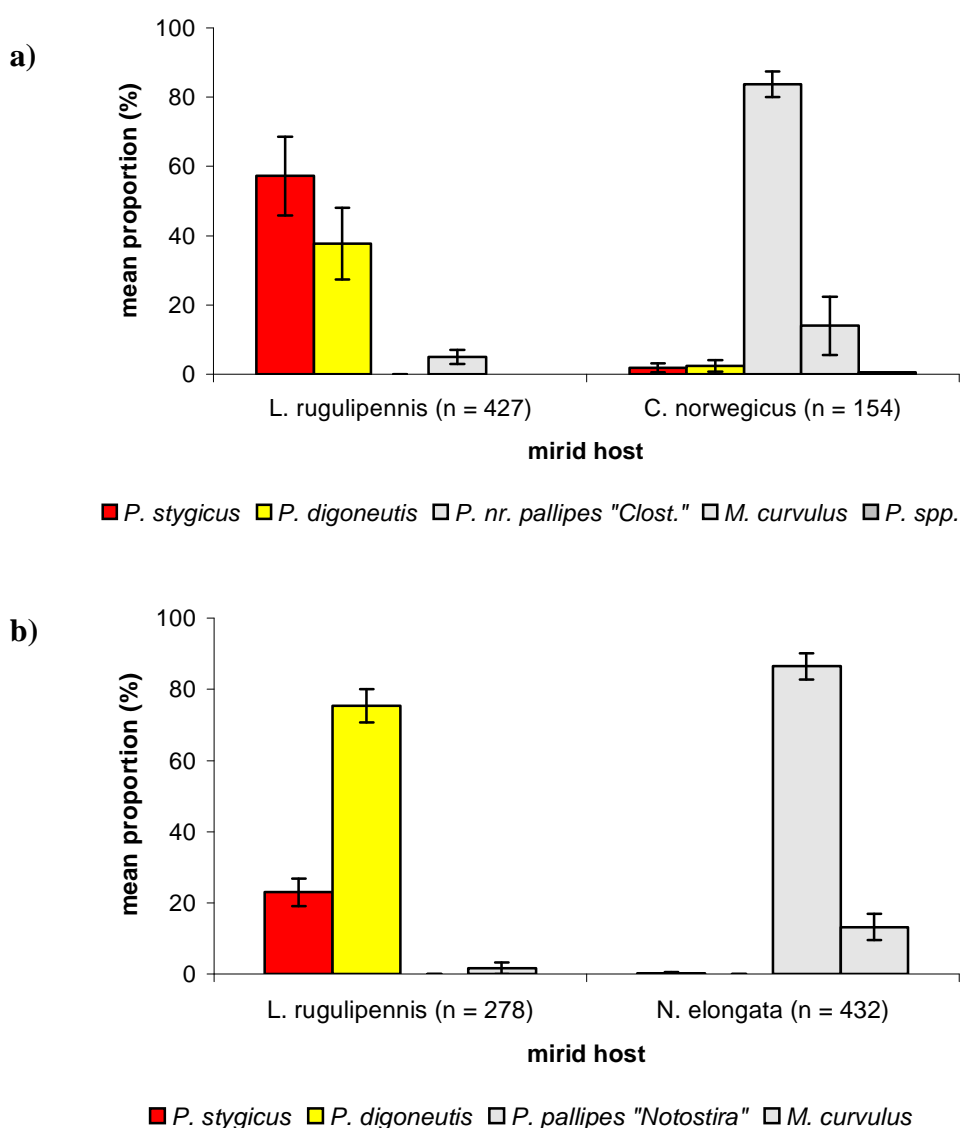


Figure 4.7 Mean proportions of nymphal parasitoids emerging from a) *Lygus rugulipennis* and *Notostira elongata* at five sites, and b) *Lygus rugulipennis* and *Closterotomus norwegicus* at four sites where both hosts occurred simultaneously (n = number of parasitoids reared, vertical bars show standard errors).

At sites where the second nymphal generations of *Notostira elongata* and *L. rugulipennis* occurred simultaneously, only one nymph of the non-target host was parasitized by *P. stygicus* and none by *P. digoneutis* (Fig. 4.7 b). In contrast, *N. elongata* was dominantly parasitized by *P. near pallipes* “*Notostira*”. The proportions of *P. stygicus* and *P. digoneutis* emerging from *Lygus* (23% and 75%; $n = 278$) and *Notostira* (0.3% and 0%; $n = 432$) differed significantly (Binominal test; $P < 0.001$).

4.3.7 Occurrence of mirid plant bugs and emergence pattern of their associated parasitoids under natural temperature conditions

In 2003, after the parasitoids were stored outside for the entire winter, parasitoid emergence in spring started April 29th, following a period of maximum daily temperatures of up to 20°C (Fig. 4.8). Among the parasitoids reared from plant bugs collected at camomile and clover sites, the first species that emerged in early May were *P. nr. pallipes* (“*Closterotomus*”) and *P. digoneutis*, followed by *P. stygicus* (Fig. 4.9 a). At the same time, the first early instar nymphs of their respective hosts, *C. norwegicus* and *L. rugulipennis*, were observed in camomile and clover stands (Fig. 4.10; see also chapter 2). In contrast, the majority of *P. adelphocoridis* adults emerged later in mid-June when small nymphs of *Adelphocoris lineolatus* occurred more frequently.

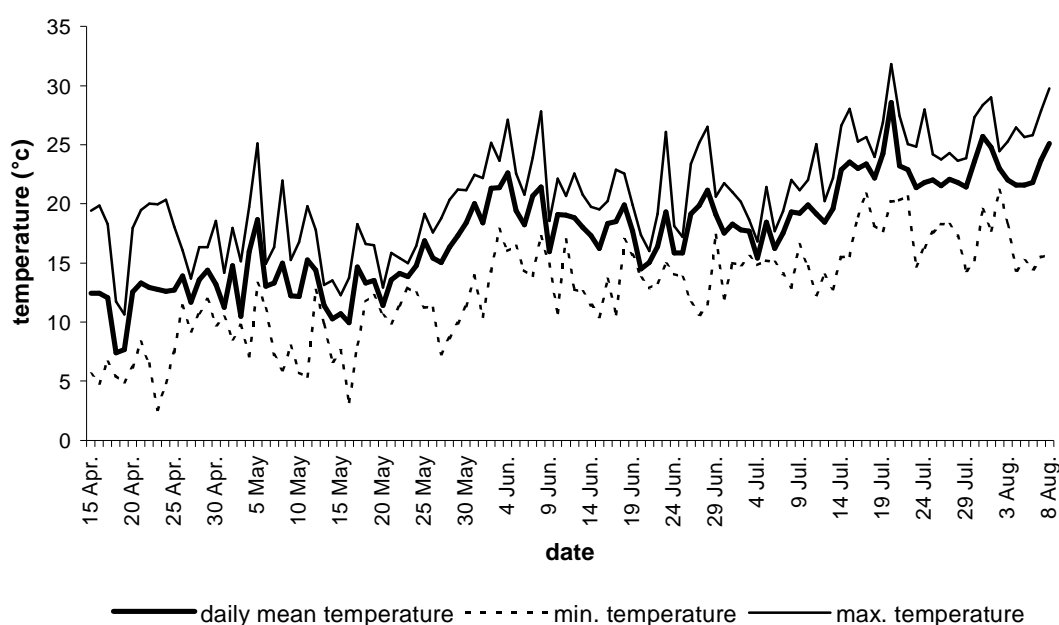


Figure 4.8 Daily mean, minimum and maximum temperatures in April – August 2003 in Kiel (measured in an open wooden shelter, 30 cm above the soil).

The emergence of parasitoids reared from grass bugs followed the same chronological order as their hosts occurred in grasslands (Fig. 4.9 b and Fig. 4.10). Almost parallel to parasitoids reared from *L. rugulipennis*, parasitoids of the grass bugs *Leptopterna dolabrata*, *Amblytylus nasutus*, and *Megaloceraea recticornis* emerged during the first weeks of May. Corresponding with the late occurrence of their mirid hosts, parasitoids of *Stenotus binotatus* and *Stenodema* spp. emerged between the end of May and mid-June. *Peristenus* nr. *pallipes* (“*Notostira*”) started to emerge not before the second week of July when the second nymphal generation of its host, *Notostira elongata*, occurs in the field. This is remarkable, because the total absence of *P.* nr. *pallipes* (“*Notostira*”) in the first nymphal generation of *N. elongata* occurring from mid-May to the end of June indicates that this parasitoid species is indeed attacking the second nymphal generation of its host, exclusively.

In stinging nettle stands, the first mirid nymphs observed in May belonged to the species *Orthotylus marginalis* and *Lygocoris pabulinus* (Fig. 4.10). In correspondence with the occurrence of their hosts, the parasitoids *P. orthotyli* and *P. laeviventris* emerged between the last week of April and the second week of May (Fig. 4.9 c). In the second half of May, mainly parasitoids of *Plagiognathus arbustrorum* emerged. *Peristenus facialis*, reared from *Apolygus lucorum*, emerged over a long period extending from May 18th to June 25th. *Peristenus* nr. *pallipes* reared from the nettle bug *Liocoris tripustulatus* (a species in which nymphs do not occur prior to mid-June) started to emerge in the second week of June, and emergence was completed in the third week of July. In 2002, after overwintered parasitoids were incubated at 21°C in April, the emergence of parasitoids followed the same chronological order as observed under natural temperature conditions (Appendix, Fig. 3).

4.4 Discussion

4.4.1 Ecological host range of *P. digoneutis* and *P. stygicus*

Prior to the present study, *Peristenus digoneutis* was only known to parasitize *Lygus rugulipennis* and *Adelphocoris lineolatus* in Europe (Loan & Bilewicz-Pawinska, 1973; Carl & Mason, 1996; Varis & van Achterberg, 2001). The seven non-target hosts and the two additional *Lygus* hosts (*L. pratensis* and *L. maritimus*; see chapter 2) examined in the present study were previously not known to be associated with *P. digoneutis* (Table 4.2). The assumption of Bilewicz-Pawinska (1982) that *P. digoneutis* is monophagous cannot be confirmed by the results of the present study. In the eastern United States, where *P. digoneutis*

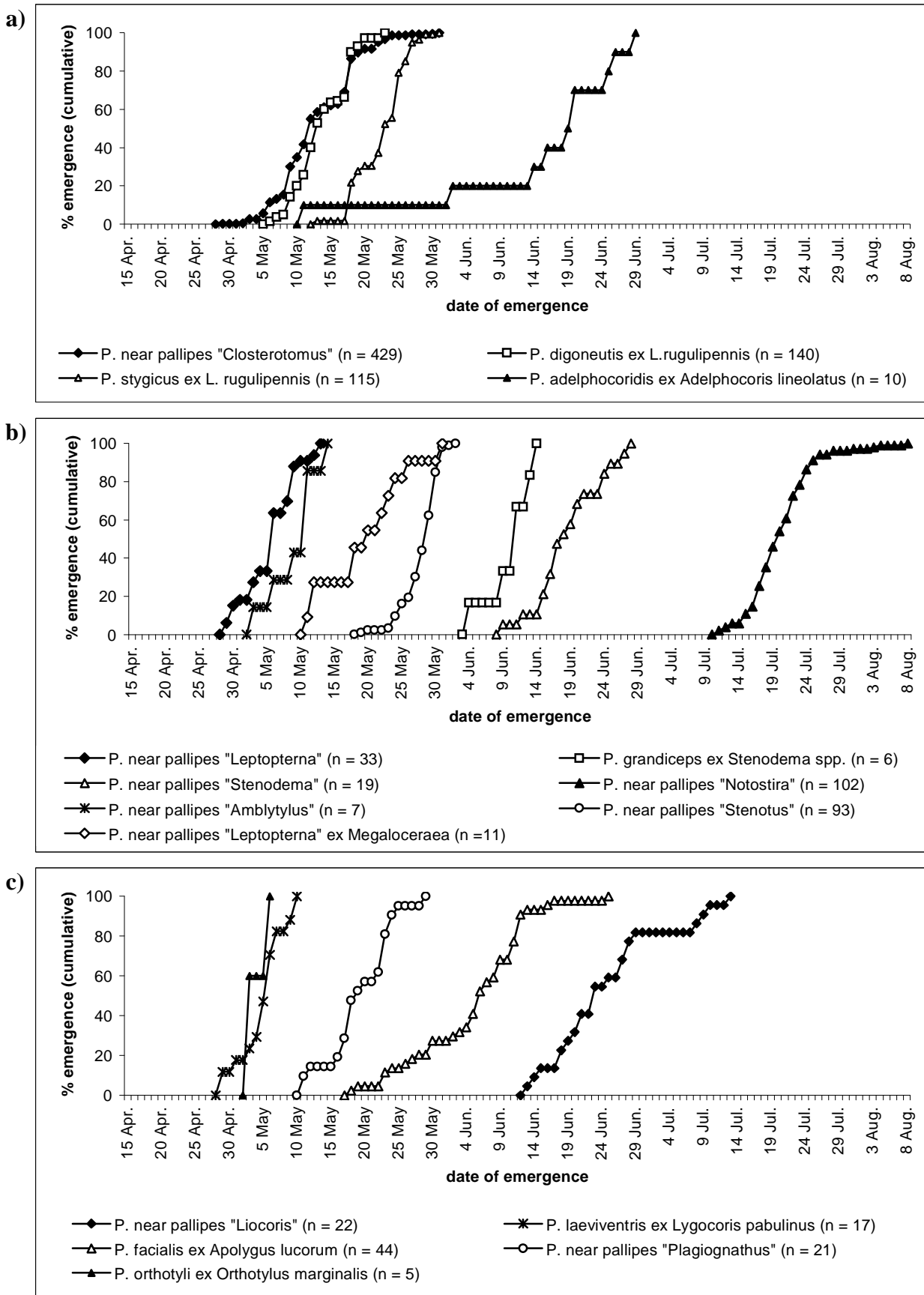


Figure 4.9 Cumulative emergence of overwintered parasitoids at outside temperatures in 2003; parasitoids were reared from mirid hosts collected in a) camomile or clover stands, b) grasslands, and c) stinging nettle stands (n = number of specimens emerged).

Mirid species	May	June	July	August	September
<i>Lygus rugulipennis</i> (a)		██████████		██████████	██████████
<i>Lygus pratensis</i> (a)		██████████	██████████	██████████	
<i>Lygus maritimus</i> (a)		██████████	██████████	██████████	
<i>Closterotomus norwegicus</i> (e)	██████████	██████████			
<i>Adelphocoris lineolatus</i> (e)		██████████	██████████		
<i>Leptopterna dolabrata</i> (e)	██████████	██████████			
<i>Amblytulus nasutus</i> (e)	██████████	██████████			
<i>Megaloceraea recticornis</i> (e)		██████████			
<i>Stenotus binotatus</i> (e)		██████████	██████████		
<i>Stenodema laevigata</i> (a)		██████████	██████████	██████████	
<i>Stenodema calcarata</i> (a)		██████████	██████████	██████████	██████████
<i>Stenodema holsata</i> (a)		██████████	██████████		
<i>Notostira elongata</i> (a)	██████████	██████████		██████████	
<i>Trigonotylus caelestialium</i> (a)	██████████	██████████		██████████	
<i>Orthotylus marginalis</i> (e)	██████████	██████████			
<i>Lygocoris pabulinus</i> (e)	██████████	██████████		██████████	
<i>Plagiognathus arbustrorum</i> (e)	██████████	██████████	██████████		
<i>Apolygus lucorum</i> (e)		██████████	██████████	██████████	
<i>Liocoris tripustulatus</i> (a)		██████████	██████████	██████████	
<i>Calocoris affinis</i> (e)	██████████	██████████	██████████		

Figure 4.10 Occurrence of mirid nymphs in Schleswig-Holstein; each black bar represents the period when nymphs of each species occurred in the field in 2001-2003; the habitats in which the mirid species were mainly found are indicated by a colored background: yellow = chamomile or clover stands; green = grasslands; blue = stinging nettle stands (overwintering stage: a = adult; e = egg).

has been introduced against *Lygus lineolaris*, the development of *P. digoneutis* from the invasive mirid *Leptopterna dolobrata* and the holoartic mirid *Trigonotylus caelestialium* has occasionally been reported (Day, 1999). However, in Schleswig-Holstein, these two mirid species are not parasitized by *P. digoneutis*. In general, the ecological host range of *P. digoneutis* is most likely restricted to the subfamily Mirinae. Among mirids of the subfamily Mirinae, *P. digoneutis* develops more frequently from hosts belonging to the tribe Mirini (5 hosts), whereas it has been only sporadically recorded from the tribe Stenodemini (2). The proportion of *P. digoneutis* relative to the total number of parasitoids emerged from each recorded alternative host did not exceed 5%. Furthermore, when compared to the total number of parasitoids emerging from non-target hosts collected at single sites, the proportion of *P. digoneutis* was rarely above 10%; and the level of parasitization was always below 6%. Therefore, although recorded from seven non-target hosts, the impact of *P. digoneutis* on non-target mirids can be regarded as negligible.

Peristenus stygicus has been previously reported from four hosts which belong to the subfamily Mirinae, exclusively (Drea et al. 1973; Bilewicz-Pawinska, 1977b, 1982; Carl & Mason, 1996). In the present study, 16 non-target hosts and the three *Lygus* hosts (see chapter 2) are recorded for *P. stygicus*; however, of these 16 hosts, only *Trigonotylus caelestialium* and *Lygus rugulipennis* were previously known to be associated with *P. stygicus* (Table 4.2). The ecological host range of *P. stygicus* in Schleswig-Holstein primarily includes hosts belonging to the subfamily Mirinae, of which the tribes Mirini and Stenodemini are equally represented with six hosts each. Furthermore, it has been shown for the first time that the ecological host range of *P. stygicus* is not only restricted to hosts of the subfamily Mirinae, but also contains hosts from the subfamilies Bryocorinae (1) and Phylinae (3). Similar to *P. digoneutis*, the proportion of *P. stygicus* relative to the total number of parasitoids that emerged from non-target hosts belonging to the tribe Mirini was below 5%, and at single sites it was maximum 12%. The highest level of parasitization caused by *P. stygicus* in a Mirini host (*C. norwegicus*) was 6.7%. These fairly low values are remarkable, because the tribe Mirini contains not only the genus *Lygus* but also the most closely related non-target mirids (e.g. *Liocoris tripustulatus* and *Lygocoris pabulinus*). Of the total number of parasitoids that emerged from common grass bugs (e.g. *Leptopterna dolobrata*, *Notostira elongata*, and *Stenodema calcarata*), the proportion of *P. stygicus* was below 9%. At a single site the proportion of *P. stygicus* relative to the total number of parasitoids emerging from *S. calcarata* (1st gen.) was 66.7%, resulting in a parasitization level of 16.7%. However, this was

Table 4.2 European *Peristenus* and *Leiophron* species and their associated hosts based on published information.

Genus	Parasitoid species	Mirid hosts	Reference
Peristenus	<i>P. digoneutis</i>	<i>Lygus rugulipennis</i> ^{1,3} <i>Adelphocoris lineolatus</i> ^{2,3}	¹ Loan & Bilewicz-Pawinska (1973) ² Varis & van Achterberg (2001) ³ Carl & Mason (1996)
	<i>P. stygicus</i> *	<i>Lygus rugulipennis</i> ^{1,2} <i>Trigonotylus caelestialium</i> ¹ <i>Polymerus unifasciatus</i> ³ <i>Adelphocoris lineolatus</i> ²	¹ Bilewicz-Pawinska (1977b, 1982) ² Carl & Mason (1996) ³ Drea et al. (1973)
	<i>P. rubricollis</i>	<i>Lygus rugulipennis</i> ^{1,2} <i>Adelphocoris lineolatus</i> ^{1,2,3}	¹ Bilewicz-Pawinska (1973, 1977b) ² Carl & Mason (1996) ³ Craig & Loan (1987)
	<i>P. varisae</i>	<i>Lygus rugulipennis</i>	Varis & van Achterberg (2001)
	<i>P. pallipes</i> **	<i>Closterotomus norwegicus</i> ¹ <i>Leptopterna dolobrata</i> ^{2,3,4} <i>Stenotus binotatus</i> ² <i>Orthotylus virescens</i> ² <i>Notostira erratica</i> ³ <i>Trigonotylus caelestialium</i> ³ <i>Adelphocoris lineolatus</i> ⁵ <i>Lygus</i> spp. ⁶	¹ Brindley (1939) ² Waloff (1967) ³ Bilewicz-Pawinska (1982) ⁴ Loan (1965) (in the U.S.) ⁵ Bilewicz-Pawinska (1977b, 1982) ⁶ Rämert et al. (unpublished)
	<i>P. picipes</i>	unknown	Richards (1967); Loan (1974)
	<i>P. accinatus</i>	(<i>Lygus</i> sp.)	Brindley (1939)
	<i>P. laeviventris</i>	<i>Lygocoris pabulinus</i>	Blommers et al. (1997)
	<i>P. nitidus</i>	unknown	
	<i>P. orthotyli</i>	<i>Orthotylus virescens</i> <i>Orthotylus concolor</i> <i>Asciodema obsoletum</i> <i>Orthotylus adenocarpi</i>	Waloff (1967)
	<i>P. fascialis</i>	<i>Orthotylus marginalis</i>	Richards (1967)
	<i>P. obscuripes</i>	<i>Notostira erratica</i> <i>Trigonotylus caelestialium</i> <i>Leptopterna dolobrata</i>	Bilewicz-Pawinska (1982)
	<i>P. orchesiae</i>	<i>Orchesia micans</i>	Loan (1974)
	<i>P. grandiceps</i> ***	<i>Stenodema virens</i> <i>Stenodema</i> sp.	Bilewicz-Pawinska (1982)
	<i>P. adelphocoridis</i>	<i>Adelphocoris</i> sp. ¹ <i>Adelphocoris lineolatus</i> ^{2,3}	¹ Loan (1979) ² Carl & Mason (1996) ³ Craig & Loan (1987)
	<i>P. malatus</i>	<i>Blepharidopterus angulatus</i>	Loan (1976)
	Leiophron	<i>L. microcercus</i>	<i>Psallus varians</i>
<i>L. reclinator</i>		unknown	Loan (1974)
<i>L. deficiens</i>		unknown	Loan (1974)
<i>L. heterocordyli</i>		<i>Heterocordylus tibialis</i> <i>Asciodema obsoletum</i> <i>Orthotylus adenocarpi</i>	Waloff (1967)
<i>L. truncator</i>		unknown	Loan (1974)
<i>L. ruthei</i>		unknown	Loan (1974)
<i>L. basalis</i>		unknown	Loan (1974)
<i>L. fascipennis</i>		unknown	Loan (1974)
<i>L. apicalis</i>	<i>Orthotylus adenocarpi</i> <i>Orthotylus virescens</i> <i>Orthotylus concolor</i> <i>Asciodema obsoletum</i> <i>Pachylops bicolor</i>	Waloff (1967)	

* In Varis & van Achterberg (2001) *P. stygicus* Loan is synonymous with *P. relictus* (Ruthe).** According to the data of the present study *P. pallipes* (Curtis) is a species complex*** *P. grandiceps* (Thomson) is synonymous with *P. stenodemae* Loan (personal comment H. Goulet)

an exception. Remarkably, *P. stygicus* was the only parasitoid reared from the 2nd generation of *S. calcarata* (9 specimens out of 274 nymphs). Nevertheless, the impact of *P. stygicus* on *S. calcarata* populations is assumed to be relatively low. In contrast to the previous Stenodemini, the proportions of *P. stygicus* relative to the total number of parasitoids emerging from the Stenodemini *Megaloceraea recticornis* (56%) and *Trigonotylus caelestialium* (42%) were surprisingly high. Although *M. recticornis* occurred occasionally in high densities, the level of parasitization in this species was low (median: 4%). Therefore, only 75 parasitoid cocoons were obtained, from which 48 adults emerged. The only other primary parasitoid reared from *M. recticornis* was *Peristenus* near *pallipes* “*Leptopterna*”, which is the dominant parasitoid of *Leptopterna dolobrata* occurring in grasslands in spring. In contrast to most other mirids, *M. recticornis* has no characteristic parasitoid. The generally low level of parasitization indicates that *P. stygicus* and *P. near pallipes* “*Leptopterna*” parasitize *M. recticornis* only fortuitously. Therefore, *P. stygicus* is assumed to be of minor importance for regulating populations of *M. recticornis*. In comparison to other grass bugs, the densities of the bivoltine species *Trigonotylus caelestialium* in the field were generally low and thus, only a small number of this mirid species was collected per site (max. 70 nymphs). Of the total number of parasitoids emerging from the two largest *Trigonotylus* samples, the proportions of *P. stygicus* were 19% and 8%, respectively (level of parasitization caused by *P. stygicus*: 8% and 3%). Similar to *M. recticornis*, *T. caelestialium* was parasitized by *Peristenus* species (*P. near pallipes* “*Leptopterna*”, *P. near pallipes* “*Notostira*”, *P. stygicus*) that are mainly characteristic for mirids that occur simultaneously with *Trigonotylus* in grasslands. Bilewicz-Pawinska (1982) reported that in Poland the first generation of *T. caelestialium* on wild grasses was parasitized by *P. stygicus*, *P. pallipes*, and *P. obscuripes* (level of parasitization: maximum 62%), whereas the second generation on barley was parasitized by *P. stygicus*, exclusively, but the maximum level of parasitization was only 7%. Furthermore, in the community of parasitoids attacking the first generation of *T. caelestialium*, Bilewicz-Pawinska (1982) stated that the proportion of *P. stygicus* was approximately 60%. Unfortunately, neither the total number of parasitoids that emerged from *T. caelestialium* nor the number of samples was given and thus, it is difficult to estimate the actual impact of *P. stygicus* on *T. caelestialium* in Poland. The corresponding high proportions of *P. stygicus* that emerged from *T. caelestialium* in Poland and Schleswig-Holstein suggest that besides *Lygus* spp., *T. caelestialium* is the most suitable host for *P. stygicus*. However, it is difficult to estimate the impact of *P. stygicus* on non-target hosts that only occur at relatively low densities. For example, the Phylinae species *Plagiognathus*

chrysanthemi was only found at three sites, but at one of these sites, *P. stygicus* amounted to 40% of all the parasitoids that emerged (level of parasitization 16%). These results may show the potential of *P. stygicus* to heavily parasitize non-target hosts, but general conclusions are impossible. The impact of *P. stygicus* on *Amblytylus nasutus* is probably low, too. The proportion of *P. stygicus* relative to the total number of parasitoids that emerged from this species was nearly 80%, but on the other hand more than 1000 nymphs had to be reared to obtain the total number of 38 parasitoids. Nevertheless, at one site *P. stygicus* caused a remarkable 20% parasitization in *A. nasutus*.

The extremely low numbers of *P. stygicus* and *P. digoneutis* specimens reared from field collected *C. norwegicus* and *N. elongata* nymphs, both of which occur simultaneously with *Lygus* nymphs in the field, indicate that both parasitoids species have a strong preference for *Lygus* nymphs.

An important aspect influencing the ecological host range of *P. digoneutis* and *P. stygicus* is the seasonal availability of mirid hosts and the time of activity of their associated parasitoids in the field. The ecological host range of *P. digoneutis* and *P. stygicus* will only contain non-target hosts which are in a suitable stage for parasitization when *Lygus* parasitoids are abundant in the field. In spring, the larvae of mirids that overwinter in the egg stage, such as *L. dolobrata*, *C. norwegicus*, and *L. pabulinus*, occur earlier in the field than the first *Lygus* nymphs and their associated parasitoids. Consequently, the majority of nymphs belonging to the former non-target mirid species are already in a late, less suitable developmental instar when *Lygus* parasitoids are searching for hosts in the field. Therefore, the likelihood that non-target nymphs are attacked by *Lygus* parasitoids is decreased. Furthermore, nymphs of non-target mirid species that primarily occur in July when *Lygus* parasitoids are almost absent in the field (see chapter 2) would rarely be attacked, despite the fact that they may be suitable hosts in laboratory tests. For example, the occurrence of the nettle bug, *Liocoris tripustulatus*, only partly overlaps with the occurrence of *Lygus rugulipennis*, and the highest densities of *Liocoris* nymphs are observed in July. Thus, the encounter of *L. tripustulatus* and *Lygus* parasitoids is unlikely; this may explain why the number of *Lygus* parasitoids reared from *L. tripustulatus* was low. Another example is likely *Adelphocoris lineolatus*, which was - in contradiction to former investigations (Carl & Mason, 1996; Varis & van Achterberg, 2001) - not recorded as host of *P. stygicus* and *P. digoneutis*. In northern Germany, *A. lineolatus* is strictly univoltine (Wagner, 1952) and nymphs primarily occur in July. However, in regions

where *A. lineolatus* has been recorded as a host of *Lygus* parasitoids (southern Germany, Switzerland), *A. lineolatus* is at least partly bivoltine, and *Adelphocoris* and *Lygus* nymphs occur simultaneously in lucerne fields (Carl & Mason, 1996). Thus, the encounter of *Adelphocoris* nymphs and *Lygus* parasitoids in these regions is more likely.

4.4.2 Parasitoid associations of other mirids

In the past, parasitization of mirid nymphs has been frequently reported, but in many studies the rearing of adult parasitoids failed and consequently, parasitoids responsible for the observed parasitization were not identified (Butler, 1923; Hey, 1933; Brindley, 1939; Southwood & Scudder, 1956; Leston, 1957, 1959, 1961). In Table 4.2, host associations of *Peristenus* spp. and *Leiophron* spp. determined by analysis of reliable published information are presented. Besides the host parasitoid associations described for *P. stygicus* and *P. digoneutis*, the present study identifies numerous new mirid-parasitoid associations. For *P. laeviventris*, three new mirid hosts were found (*Liocoris tripustulatus*, *Apolygus lucorum*, and *C. norwegicus*). *Peristenus facialis*, previously only known from *Orthotylus marginalis*, was frequently reared from *Apolygus lucorum* and twice from *C. norwegicus*. *Peristenus grandiceps* (Syn. *P. stenodemae*, H. Goulet, personal communication), a common parasitoid of *Stenodema virens* in Poland (Loan & Bilewicz-Pawinska, 1973), was also reared from *S. calcarata*, *S. laevigata*, and *S. holsata*. For *P. orthotyli*, named after the host genus it was most frequently reared from (Waloff, 1967), one new host, *Orthotylus marginalis*, was identified. For *P. picipes*, parasitoid of *Rhabdomiris striatellus*, and *L. deficiens*, parasitoid of *Orthotylus moncreaffi*, no associated hosts had been described before. Surprisingly, *Leiophron* (subgenus *Mama*) *reclinator* has been identified as *adult* parasitoid of *Lygocoris pabulinus* and *Liocoris tripustulatus* (C. van Achterberg, personal communication). To date, *L. reclinator* represents the only *adult* parasitoid of Miridae that is known from the family Braconidae.

The numerous records of “*P. pallipes*” from a wide variety of mirid hosts was confirmed by the present study. The distinct differences in the emergence patterns of the univoltine “*P. pallipes*” species reared from different mirid hosts indicate that “*P. pallipes*” is most likely a complex of more than ten species. After overwintering, the first “*P. pallipes*” emerged from *Leptopterna dolobrata* at the end of April; in contrast, “*P. pallipes*” reared from *Notostira elongata* emerged more than two months later in the first week of July. Furthermore, “*P.*

pallipes” specimens reared from different hosts show morphological differences (H. Goulet, personal communication). In order to clarify, whether “*P. pallipes*” is actually a species complex, it will be necessary to compare the DNA sequences of “*P. pallipes*” specimens reared from different hosts.

Day (2002) reported that in the U.S., the holoartic *Mesochorus curvulus* was the only hyperparasitoid obtained from six primary parasitoids (five *Peristenus* spp. and one *Leiophron* sp.), of which *P. pallipes* was the preferred host. The primary parasitoids were reared from four different mirid hosts. In the present study, the large number of records for *M. curvulus* from numerous mirids associated with many primary parasitoids corresponds with the observations made in North America. Although it was not possible to exactly relate *M. curvulus* to its hosts, it is obvious that *M. curvulus* was reared more frequently from mirid species that were mainly parasitized by primary parasitoids other than *P. stygicus* and *P. digoneutis*.

In general, the results of the present study suggest that almost each non-target mirid has a dominant parasitoid species, with *P. digoneutis* and *P. stygicus* being mainly dominant in *Lygus*. Parasitoids that develop from more than one host are usually restricted to one mirid genus or to mirid hosts that belong to different genera but occur simultaneously within the same habitat.

4.4.3 Consequences for biological control

A broad ecological host range in the area of origin suggests that *P. stygicus* is a generalist, whereas *P. digoneutis* is likely oligophagous. In general, both parasitoids are most likely of minor importance for regulating non-target mirid populations in the area of origin. However, when comparing host specificity, *P. stygicus* has a higher potential to cause non-target effects because its ecological host range includes far more alternative hosts than that of *P. digoneutis*, and occasionally it achieves relatively high levels of parasitization in non-target hosts. In contrast, *P. digoneutis* has never been recorded to cause high levels of parasitization in hosts others than *Lygus* spp.. Consequently, if only host specificity (estimated by the ecological host range) is taken into consideration, *P. digoneutis* is more suitable for the biological control of Canadian *Lygus* populations than *P. stygicus*.

The results of the studies on the ecological host range of *P. digoneutis* and *P. stygicus* in the area of origin will aid in selecting potential North American non-target mirids (see Maw et al., 2000) for evaluation of the physiological host range of *Peristenus* in quarantine laboratories in Canada. In the case of *P. digoneutis*, laboratory tests should be primarily focused on mirids belonging to the tribe Mirini, since attacks on Stenodemini are rare. However, *P. stygicus* has to be tested on a much larger scale, including not only hosts from the tribes Mirini and Stenodemini (e.g. native *Trigonotylus* spp.) but also from the subfamilies Phylinae and Bryocorinae. A major concern regarding the introduction of exotic biological control agents is the potential harm to beneficial insects, such as the predatory mirid *Dicyphus hesperus* (subfamily Bryocorinae). In the present study, two specimens of *P. stygicus* have been reared from *Dicyphus globulifer*; however recent laboratory tests conducted in the U.S. and Canada (Day & Fuester, 2003; Mason et al., unpublished a) indicate that *P. stygicus* does not represent a threat to beneficial *Dicyphus* species.

In Schleswig-Holstein, hyperparasitization by *M. curvulus* in *Lygus* hosts was rarely higher than 15% (see chapter 2). In North America, the highest level observed (from various mirid hosts) was 11% and consequently, Day (2002) suggested that *M. curvulus* does not have a strong negative effect on beneficial *Peristenus* species. Nevertheless, the possibility that the holarctic hyperparasitoid *M. curvulus* in North America may negatively influence populations of *P. stygicus* and *P. digoneutis* when introduced into Canada cannot be excluded.

The numerous associations between parasitoids and mirids identified in the present study may further contribute to present and future biological control programs against mirid pests other than *Lygus* spp.. Seventeen out of 54 mirids obtained in northern Germany are known to be invasive species in Canada (Maw et al., 2000). So far, six of them have reached pest status (Appendix, Table 16). Mirids are pests not only in Canada, but also cause problems in Europe and New Zealand. For example in Holland, the native *Liocoris tripustulatus* and *Lygocoris pabulinus* are pests of glasshouse cucumber and sweet pepper crops (Hunter, 2002; Jacobsen, 1999). Furthermore, *L. tripustulatus* is an emergent pest of *Cannabis* plants (McPartland, 1996). Of particular interest is the adult parasitoid *Leiophron reclinator*, which could be an effective biological control agent for *L. pabulinus* and *L. tripustulatus* in greenhouses because it kills the mirid adult females before they start to oviposit and thus, the damage caused by nymphs can be prevented. The nymphal parasitoid complex of *C. norwegicus* was investigated intensively in the present study; the knowledge acquired in this research may be

applicable in New Zealand, where this mirid has been accidentally introduced and causes serious damage in white clover seed production (Schroeder & Chapman, 1998).

5 Reproductive biology of *Peristenus digoneutis* and *P. stygicus* (Hymenoptera: Braconidae), parasitoids of *Lygus* spp.

5.1 Introduction

Neither the characteristics of a potential biocontrol agent observed in the laboratory nor its behavior in the area of origin are an entirely reliable index of its effectiveness in the area of introduction (Doutt & DeBach, 1964). However, when searching for effective biocontrol agents, certain promising attributes are obvious prior to introduction. Among other characters like high searching efficiency, high larval survival, balanced sex ratio, host specificity, and short handling time, high lifetime fecundity of adult parasitoids has frequently been postulated as a desirable attribute of an ideal biological control agent (Waage & Hassell, 1982; Waage, 1990; Ehler, 1995; Sagarra et al., 2001).

Fecundity is defined as an animal's reproductive output in terms of the total number of eggs produced or laid (Jervis & Copland, 1996). It can be distinguished between potential and realized fecundity; however, these terms have been used in slightly different contexts (Godfray, 1994; Jervis & Copland, 1996; Mills & Kuhlmann, 2000). According to Mills & Kuhlmann (2000), potential fecundity is defined as the average number of progeny that a specific population of mature parasitoid females can produce under optimal conditions (usually estimated in laboratory studies), whereas realized fecundity is the corresponding average progeny production achieved by the population under field conditions.

Parasitoids with a high fecundity are regarded as favorable because they are usually able to reproduce more rapidly than the pest population and respond to changes in the abundance of the pest (Doutt & DeBach, 1964; Waage & Hassell, 1982; Waage, 1990; Ceballo & Walter, 2004). Furthermore, it is assumed that fecundity is an important factor for successful establishment of biological control agents (Stilling, 1990; Sagarra et al., 2001) and therefore, the evaluation of parasitoids considered for biological control requires detailed knowledge on the reproductive biology (Zhang et al., 2004; Ceballo & Walter, 2004).

The objective of this study was to examine the reproductive biology of *Peristenus digoneutis* and *P. stygicus*. Both species frequently occur in Schleswig-Holstein and occasionally cause high levels of parasitization (see chapter 2). The reproductive potential of both agents was

compared under laboratory conditions in order to estimate their potential as biological control agents for *Lygus* populations in Canada.

All experiments were conducted at Agriculture and Agri-Food Canada, Southern Crop Protection and Food Research Center (S.C.P.F.R.C.) at London, Ontario, Canada.

5.2 Material and methods

5.2.1 Timing of egg production in adult parasitoids

Twenty *P. digoneutis* and *P. stygicus* females were dissected with insect needles in a drop of water on a microscope slide to determine the number of mature eggs in newly emerged parasitoids. The abdomen was carefully dissected and the ovaries removed. Acetocarmine staining of the eggs permitted the distinction between mature and immature eggs, as the follicle surrounding the mature eggs picks up the stain (Jervis & Copland, 1996).

5.2.2 Age specific and potential lifetime fecundity

In order to estimate the lifetime fecundity of *P. stygicus* and *P. digoneutis*, individual mated females were exposed to thirty *Lygus lineolaris* nymphs (2nd and 3rd instar) daily throughout their lifetime. Because it has been shown that *Peristenus* parasitoids oviposit more successfully into early instars (van Steenyk & Stern, 1976), 2nd and 3rd instar nymphs were used for these experiments. The initial exposure to *Lygus* nymphs took place immediately after emergence when female parasitoids were 1 to 24 hours old. Female parasitoids were transferred into clear, ventilated 1L plastic cylinders containing *Lygus* nymphs placed on a leaf of Romaine lettuce (Fig. 5.1). For transfer, parasitoids were guided into small glass pipettes which were used instead of mouth aspirators to reduce the risk of damaging wings, legs or antennae. Single parasitoid males were added to each container during the first two days of the experiment to ensure females were properly mated. Males were then removed, because females refuse to mate once they are older than two days (Hormchan, 1977). Furthermore, it is likely that continued male presence would disturb ovipositing females and reduce the oviposition rate, as was shown by Mehrnejad (2003). Parasitoids and nymphs were kept at 73 ± 5 % RH, 16 hours light, and a temperature of 25 ± 1 °C during the day and 15 ± 1 °C at night. Each parasitoid was provided daily with one cotton wick saturated in water and a

little honey placed inside of the lid of the container. Exposed nymphs were removed every 24 hours and replaced with non-parasitized nymphs until the parasitoids died.

Figure 5.1

Container used for fecundity experiments.



Potentially parasitized nymphs were reared in 1L plastic cups on organically grown Romaine lettuce and kept at 23 ± 1 °C, 60% RH and a photoperiod of 16:8 (L:D). Samples from each female and date of exposure were kept individually. Five days after exposure, when the parasitoid larvae had reached the late 1st instar (Fig. 5.2), the nymphs were removed from the rearing containers and dissected for parasitization (approximately 37,500 nymphs in total). The mortality of *Lygus* nymphs during the 5 day rearing process was generally low and thus, on average 28 out of 30 nymphs (per sample) were recovered. Although *Peristenus* species are known to be solitary parasitoids, self-superparasitized nymphs were frequently found in the samples. Self-superparasitism is defined as the deposition of a clutch of eggs (or a single egg) on a host that has already been parasitized by the same female (Godfray, 1994). Therefore, the total number of eggs laid per female per day was calculated based on the number of parasitoid larvae found in the recovered nymphs, including fully developed L1 larvae (1/host) as well as supernumerary larvae (Fig. 5.2) that perished due to intraspecific competition (Fisher, 1971). The oviposition period, longevity, the daily and total number of eggs laid over lifetime was documented for each female studied.

Figure 5.2

Fully developed 1st instar larva and supernumerary larva (small individual) of *P. stygicus* (5 days after oviposition).

(Photo: T. Haye)



After the female parasitoids had died, their body size was estimated in terms of the length of hind tibia and wing nerves as well as width of head capsules, because a positive correlation between body size and egg load or body size and longevity has been found in many parasitoid species (Jervis & Copland, 1996).

5.2.2 Source of hosts and parasitoids

All material used in the experiments was provided by the insect research laboratory at the S.C.P.F.R.C., London. Rearing methods for *Lygus* hosts and parasitoids have been described in detail by Whistlecraft et al. (2000). For establishing a non-diapausing strain of *Lygus lineolaris* at the S.C.P.F.R.C., adults were collected in alfalfa fields in the areas surrounding the research center in London. In order to obtain *Peristenus stygicus*, surveys were conducted in collaboration with CABI Bioscience, Switzerland, in agricultural habitats in the Rhine Valley in southern Germany. Parasitized nymphs of *Lygus rugulipennis* were collected in August 2001 from mustard fields and parasitoid cocoons obtained from the rearing were shipped to the laboratory in London to establish a continuous rearing of *P. stygicus*. *Peristenus digoneutis* colonies were established from *Lygus* mass collections made in Albany, New York State, where *P. digoneutis* has established following previous releases by the United States Department of Agriculture (USDA) in the North Eastern United States to control *Lygus* spp.. *Peristenus* females used for dissections were obtained from *Lygus* mass collections in Schleswig-Holstein (Germany).

5.3 Results

5.3.1 Timing of egg production in parasitoid adults

In addition to numerous developing eggs, the ovarioles of newly emerged *P. digoneutis* females (Fig. 5.3) contained between 12 and 38 mature eggs (mean: 23 ± 2 SE; $n = 20$). For *P. stygicus* ($n = 20$), a significantly higher average number of 48 ± 3 (SE) mature eggs was observed, with a range between 29 and 62 mature eggs (Kruskal-Wallis test: $\text{Chi}^2 = 22.4$; $\text{df} = 1$; $P < 0.001$).

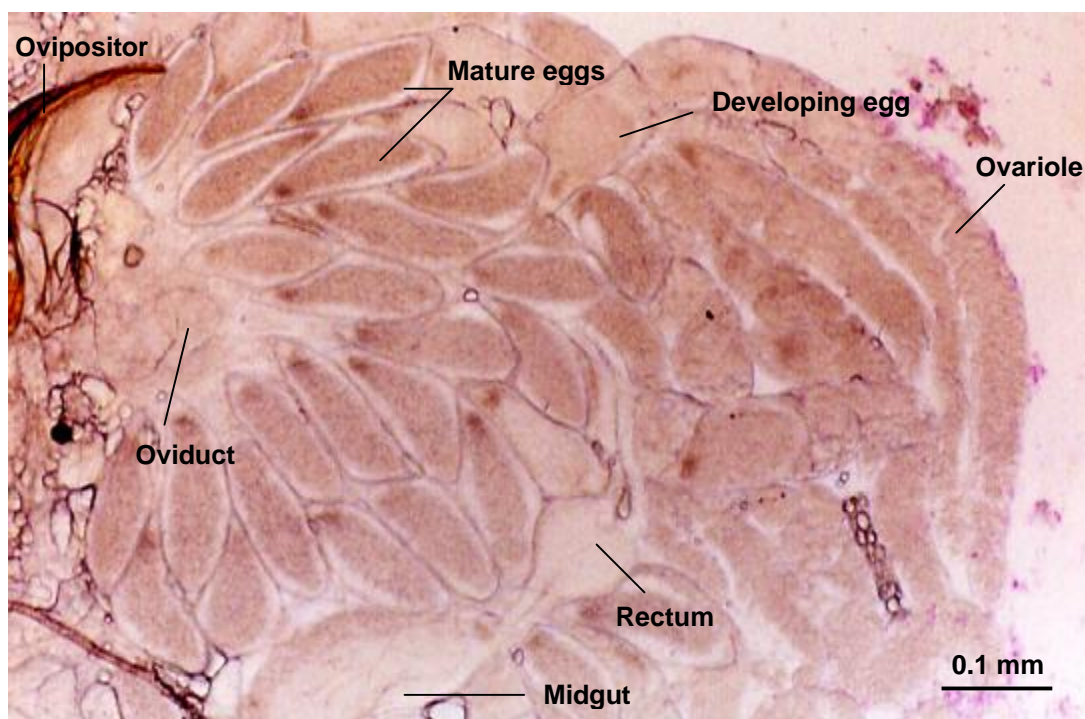


Figure 5.3 Ovary of a newly emerged *P. digoneutis* female containing mature eggs (photo: G. König & T. Haye).

5.3.2 Age-specific fecundity

The age-specific fecundity of a parasitoid is defined as the number of eggs laid per female per day (Jervis & Copland, 1996). Both parasitoid species began to oviposit immediately following exposure to nymphs. The mean number of eggs laid by newly emerged females on the first day of oviposition was 13.2 ± 1.1 (SE) for *P. digoneutis*, and 38.5 ± 3.2 (SE) for *P. stygicus* (Fig. 5.4 a). During the first five days, daily oviposition rates for *P. digoneutis*

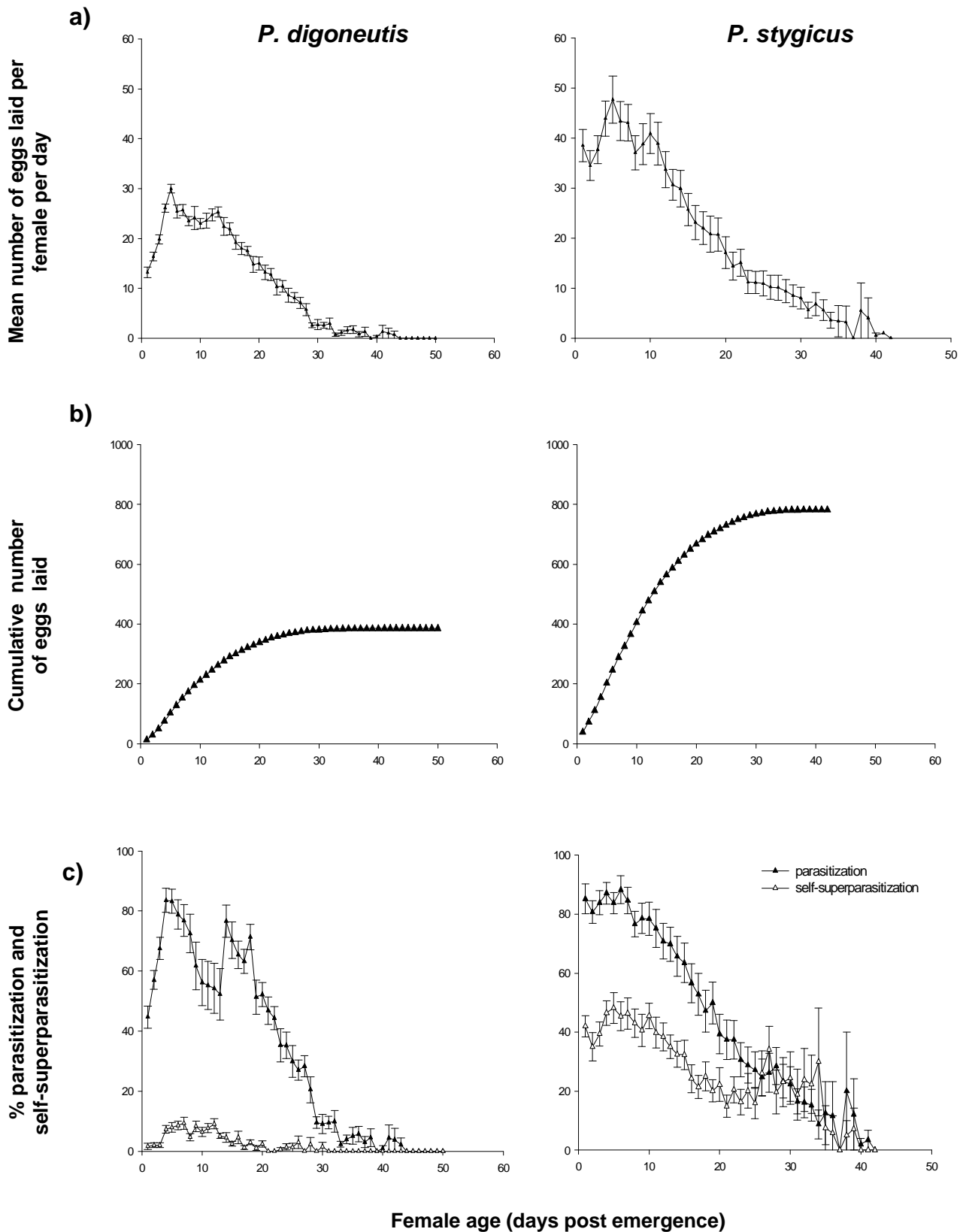


Figure 5.4 Age-specific profiles of (a) the daily mean number of eggs laid per female over life time (30 hosts offered daily), b) the mean cumulative fecundity, and c) daily average parasitization and self-superparasitization rates per female per sample (*P. digoneutis*: n = 23 and *P. stygicus*: n = 21; standard errors are indicated by vertical bars).

increased, and the highest age-specific fecundity was observed on day five (30 ± 0.9 (SE) eggs). The mean age-specific fecundity of *P. stygicus* females decreased slightly from 38.5 ± 3.2 (SE) to 34.5 ± 3.0 (SE) eggs/day after the first day of exposure. The following days the age specific fecundity increased again, and similar to *P. digoneutis*, the peak was reached on the 5th day after emergence (47.7 ± 4.7 (SE) eggs). Following this peak, daily oviposition rates of *P. digoneutis* decreased slightly and varied between 23 and 25 eggs per day between day 6 and day 13. After two weeks, a consistent decline in daily oviposition rates was observed until the death of all females after 50 days. In contrast to *P. digoneutis*, the age-specific fecundity of *P. stygicus* started decreasing immediately after the maximum had been reached on day five and only at day nine and ten a slight increase was noticed. The maximum number of eggs laid per day was 83 eggs for *P. stygicus*, and 36 eggs for *P. digoneutis*.

5.3.3 Potential lifetime fecundity

The potential lifetime fecundity of *P. digoneutis* females was on average 385.4 ± 35.2 (SE) eggs, ranging from 90 to 605 eggs ($n = 23$) (Fig. 5.4 b). The oviposition period of *P. digoneutis* lasted for a maximum of 43 days, and the mean oviposition period was 22.4 ± 2.6 (SE) days. For *P. stygicus*, a significantly higher mean potential lifetime fecundity of 782.1 ± 65.1 (SE) eggs was observed (Student's *t*-test; $t = -5.36$; $df = 31$; $P < 0.0001$), ranging from 99 to 1185 eggs ($n = 21$). The oviposition period lasted for a maximum of 41 days, and the mean oviposition period (28.3 ± 1.3 (SE) days) was significantly longer than that of *P. digoneutis* (Student's *t*-test; $t = 2.05$; $df = 32$; $P = 0.049$). The maximum fecundity period of both species, defined as the period when the females had oviposited at least 60% of their total egg load (Rodríguez-Leyva et al., 2000), lasted from the day of parasitoid emergence until the females were 12 days old.

5.3.4 Level of parasitization and self-superparasitization

Parasitization of nymphs by *P. digoneutis* increased during the first four days, averaging from 44.7 to 83.5% (100% = all nymphs in each sample were parasitized) (Fig. 5.4 c). In the following days, parasitization rates decreased until a second smaller peak was reached after 14 days (76.6%). Twenty days after the parasitoids emerged, parasitization rates dropped below 50% and continued to decrease until the females died. Superparasitization of nymphs by *P. digoneutis* was infrequent. However, the highest rates were measured during the

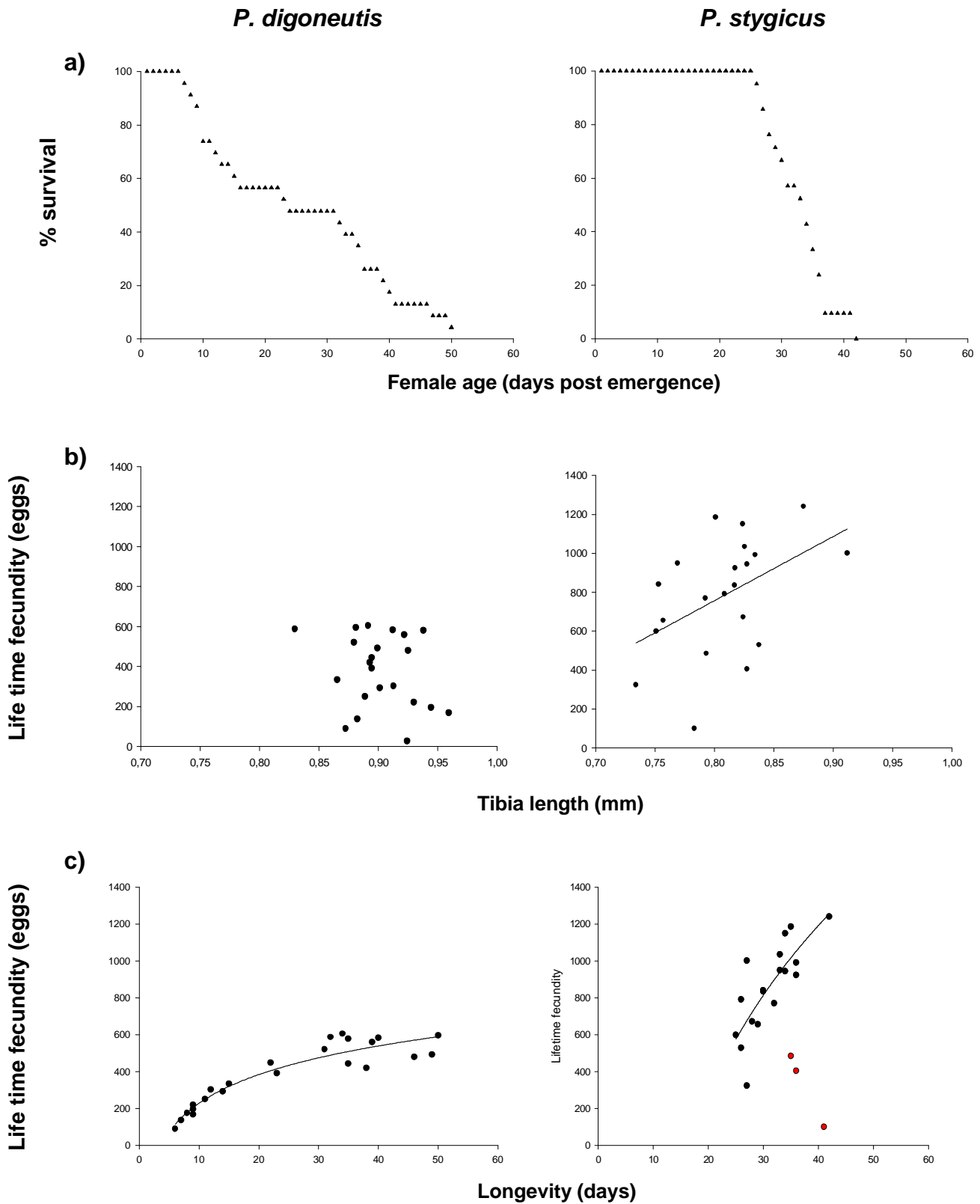


Figure 5.5 Reproductive parameters of *P. digoneutis* and *P. stygicus*: a) Adult longevity (percentage survival of female parasitoids), b) relationship between lifetime fecundity and body size, and c) relationship between lifetime fecundity and longevity (red marked data excluded, see explanation in the text) (*P. digoneutis*: n = 23 and *P. stygicus*: n = 21).

maximum oviposition period, with superparasitization rates that varied between 1 and 9%. The majority of superparasitized hosts contained two parasitoid larvae (93.7% of 431 nymphs), while 6.3% contained three to five larvae (1 fully developed L1 + supernumeraries; see Figure 5.2).

In contrast to *P. digoneutis*, mean parasitization rates obtained from *P. stygicus* were high from the first day onwards (85.2%). The highest rate (88.6%) was observed after 6 days (Fig. 5.4. c). Following this period, the daily mean parasitization rate decreased as age of females increased, and declined below 50% after 17 days. Self-superparasitized nymphs were observed frequently, and self-superparasitization occurred even when several unparasitized hosts were available for parasitization in the same sample. Similar to *P. digoneutis*, superparasitization was observed more frequently during the maximum fecundity period. The degree of superparasitization ranged from 32 to 48%, with 94.7% of 3980 superparasitized nymphs containing two to four parasitoid larvae, and 5.3% containing five to fourteen larvae. As parasitoids aged and parasitization rates decreased, superparasitization rates decreased as well. However, between day 26 and 34 an additional increase in superparasitization was observed, ranging from 18 to 34%.

5.3.5 Adult longevity

A comparison of female survival demonstrated that adult mortality in *P. stygicus* first started after 25 days, whereas in *P. digoneutis* a constant number of adults died per unit of time after six days (Fig. 5.5 a). A significant difference was found between the mean adult longevity of both species (Student's *t*-test; $t = -11.62$; $df = 20$; $P < 0.0001$). Mean adult longevity of *P. digoneutis* females was 25 ± 3.1 (SE) days (min. = 6, max. = 50, $n = 23$); for *P. stygicus* it was 32.1 ± 1.1 (SE) days (min. = 25, max. = 42, $n = 21$). In both species most females displayed post-reproductive survival. *Peristenus digoneutis* females continued living on average 2.6 ± 0.8 (SE) days (max. 12 days) and *P. stygicus* females 3.3 ± 0.9 (SE) days (max. 14 days) following the end of the oviposition period.

5.3.6 Relationship between lifetime fecundity and body size, longevity, and oviposition period

A positive correlation between lifetime fecundity and body size was found for *P. stygicus*

females when body size was measured in terms of hind tibia length (Fig. 5.5 b) (Pearson Correlation: 0.466*, $n = 21$, $P = 0.033$). For *P. digoneutis*, there was no correlation between body size (length of tibia) and fecundity. In addition, no correlations were found when body size was measured in terms of wing nerve length and head capsule width for either species. Furthermore, adult longevity was not correlated with any of the measurements for female body size.

For *P. digoneutis*, a significant relationship was found between lifetime fecundity (dependent variable) and longevity (independent variable) (Logarithmic regression: $y = -286.97 + 224.054 \ln(x)$; $R^2 = 0.89$; $F = 173.06$, $df = 21$; $P < 0.0001$). As such, lifetime fecundity increased with increasing longevity (Fig. 5.5 c). However, no significant relationship was found between the lifetime fecundity and longevity of *P. stygicus* when data from all females used in the experiment were included (ANOVA; $F = 0.074$; $df = 1, 19$; $P = 0.79$; for log transformed data; $n = 21$). A significant relationship was only observed when data from three females that had an extremely short oviposition period compared to their life span were excluded (Logarithmic regression: $y = -3655.4 + 1314.44 \ln(x)$; $R^2 = 0.60$; $F = 24.22$, $df = 16$; $P < 0.0001$; $n = 18$; data from excluded females are marked with red color in Figure 5.5 c).

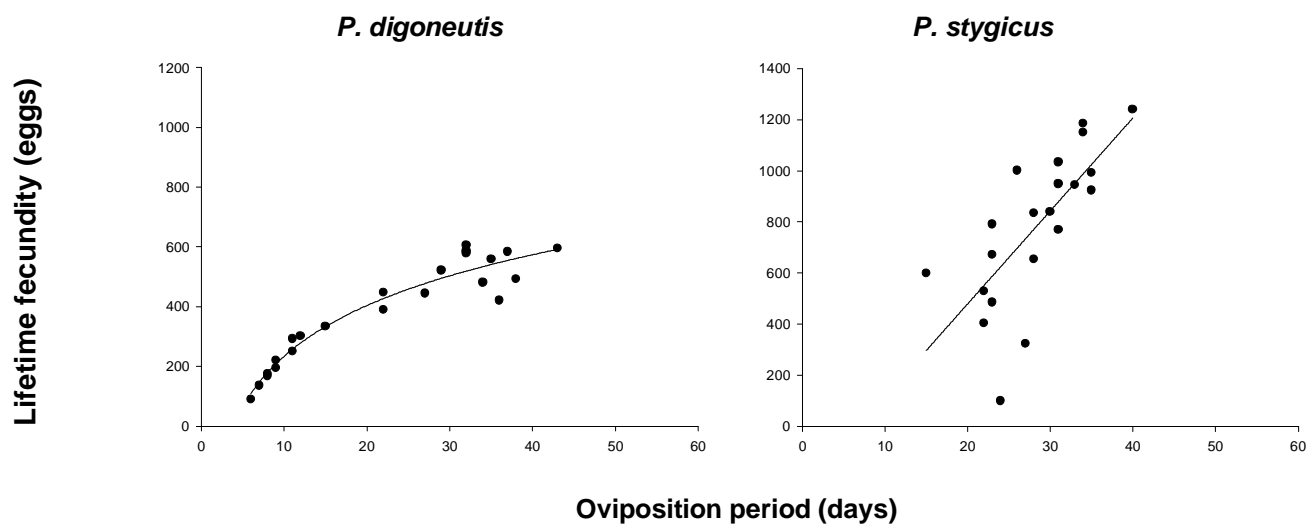


Figure 5.6 Relationship between lifetime fecundity and oviposition period for *P. digoneutis* ($n = 23$) and *P. stygicus* ($n = 21$).

For both species there was a significant relationship between lifetime fecundity (dependent variable) and actual oviposition period (independent variable). Similar to the previously described relationship, lifetime fecundity increased with increasing oviposition period (Fig. 5.6). For *P. digoneutis*, a logarithmic model was fitted to describe the relationship of both parameters ($y = -330.85 + 245.167 \ln(x)$; $R^2 = 0.93$; $F = 258.83$, $df = 21$; $P < 0.0001$), while

for *P. stygicus* a linear relationship was found to fit best (Linear Regression: $y = 36.41x - 249.5$; $R^2 = 0.53$; ANOVA; $F = 21.03$, $df = 19$; $P < 0.0001$; R^2 for logarithmic regression = 0.46).

5.4 Discussion

5.4.1 Reproductive biology of *Peristenus* spp.

Flanders (1950) distinguished between synovigenic and pro-ovigenic parasitoids. Pro-ovigenic parasitoids emerge with their full lifetime supply of mature eggs, while synovigenic species emerge with mainly immature eggs or only a few mature eggs but continue developing eggs during adult life (Jervis & Copland, 1996; Quicke, 1997). Pro-ovigenic parasitoids have no pre-oviposition period, a short lifetime and may feed only for maintenance purposes (Flanders, 1950). Synovigenic species often show a pre-oviposition period, live longer and need to feed (nectar, pollen or host fluids) to reach their full reproductive potential (Jervis & Copland, 1996; Jervis et al., 2001). However, these two types represent the two extremes, and many parasitoids with intermediate strategies are known (Jervis & Copland, 1996; Quicke, 1997; Jervis et al., 2001).

The ovarioles of newly emerged *P. digoneutis* and *P. stygicus* females contain mainly immature eggs, and considering their lifetime potential, only a few mature eggs; therefore, both species can be designated as synovigenic. Females were frequently observed to feed on the provided honey during the experiment and consequently, it can be assumed that the consumed nutrients were needed to achieve the full reproductive potential. However, no pre-oviposition period was observed; this is generally a trait associated with pro-ovigenic parasitoids. Mature eggs were found to be small and alecithal (yolk-poor). Therefore, both species can be considered as hydropic (Flanders, 1950), indicating that eggs can be stored for long periods, because embryonic development of the eggs requires insertion into host fluids and absorption of host nutrients (Jervis & Copland, 1996). Once inside a host, hydropic eggs swell within a short period of time (Schlinger & Hall, 1960). This phenomenon has been observed by Carignan et al. (1995), who described the immature stages of *P. digoneutis* in detail and reported that newly laid eggs swelled greatly in width during the first 24 h of incubation.

Peristenus digoneutis and *P. stygicus* oviposited most actively within the first two weeks of their life span, with the maximum average daily oviposition rate after about five days. Similar observations were made by Hormchan (1977) who found that the primary egg laying period of *P. digoneutis* occurred during the first five days (maximum age-specific fecundity after four days). In the present study, it is likely that a constant number of 30 nymphs provided per day resulted in adult reproduction concentrated in the early stage of adult life. It has been demonstrated that parasitoids, when provided with unlimited host access, show a heavily skewed pattern of early adult reproduction, whereas wasps with limited host access distribute their progeny more evenly throughout their adult lives (Harvey et al., 2001; Mehrnejad, 2003). Van Steenwyk & Stern (1976) showed that the total fecundity of *P. stygicus* increased significantly as the host density increased from 25 to 50 nymphs per cage. They also stated the optimal host density for the highest fecundity per day is ca. 100 second instar nymphs per cage. Based on these results, it can be assumed that in the present experiments a higher host density would have led to an even more skewed pattern of early adult reproduction. On the other hand, it seems that the daily oviposition rate of *P. stygicus* was not limited by the number of hosts because a high level of self-superparasitization and a maximum daily oviposition rate of 86 eggs / female was observed during the main oviposition period. Assuming that *P. stygicus* females strongly prefer to oviposit into non-parasitized nymphs, less self-superparasitization, lower daily oviposition rates and a more even pattern of adult reproduction would have been expected. Godfray (1994) stated that deliberate self-superparasitism is probably rare and that most cases likely result from being unable to distinguish between parasitized and non-parasitized hosts. However, a high level of superparasitization does not necessarily mean that a parasitoid is unable to discriminate between parasitized and non-parasitized hosts (van Lenteren, 1981; Godfray, 1994). In the present experiment, high levels of self-superparasitization can be attributed to the fact that in relatively small rearing cages with a limited number of hosts, the number of encounters between parasitoids and hosts is high, and thus the number of non-parasitized hosts decreases with time. In a natural, more complex environment encounters of *P. stygicus* and self-parasitized nymphs are likely rare; this is supported by the observation that field collected *Lygus* nymphs rarely contain more than one *Peristenus* larva. In *P. digoneutis*, self-superparasitization was less abundant than in *P. stygicus*. However, it remains unclear whether this results from a limited number of mature eggs available per day, lower searching activity, or the potential ability to discriminate between parasitized and non-parasitized hosts.

The relatively high potential fecundity of both species may be explained by the host stage attacked. Although *Peristenus* is able to oviposit into all five nymphal stages of *Lygus*, it is known that early instars are more often attacked than the older ones (Brindley, 1939; Lim & Stewart, 1976; van Steenwyk & Stern, 1976). Early instars are probably not preferred, but are much easier to catch. As noted by van Steenwyk & Stern (1976), when older nymphs were attacked, a strong defence reaction by the nymphs was observed. In chapter 2 it was shown that the time of parasitoid adult emergence is highly correlated to the occurrence of small *Lygus* nymphs in the field. Parasitoids attacking young host stages often show a higher fecundity (“balanced mortality hypothesis”, cf. Price, 1974; Godfray, 1994). When parasitoids oviposit into young hosts and delay their development until the host matures, the risk of immature mortality is far greater than for parasitoids that oviposit in pupae or cocoons and kill their host instantly (Godfray, 1994). Many hosts suffer severe mortality in their youngest instars, and therefore parasitoids like *Peristenus*, which attack primarily early instar hosts, would be particularly affected. Another reason for a higher fecundity in parasitoids can be related to the level of predation risk for the host; high levels of predation on a given host also translate into high mortality for its associated parasitoid. Predators feeding on *Lygus* such as *Nabis* sp., *Chrysopa* sp. and crab spiders were observed frequently during field studies in Schleswig-Holstein (Appendix, Figure 1); however, their impact on *Lygus* nymphs is not known.

The results of this study differ greatly from those of Hormchan (1977) and van Steenwyk & Stern (1976). In comparison to the afore-mentioned studies, both *Peristenus* species examined in the present study showed a surprisingly higher potential lifetime fecundity than initially expected. Hormchan (1977) had found an average lifetime fecundity of 32.8 eggs per *P. digoneutis* female, when exposed to 100 L2 nymphs daily and kept at 25 °C, 50-60% RH and 16 h light. Instead of dissecting nymphs for 1st instar parasitoid larvae, nymphs were dissected after 48 hours to record parasitoid eggs. A different method was used by van Steenwyk & Stern (1976), who measured the total fecundity of *P. stygicus* by counting the total adult progeny produced. According to their study, when females were exposed to 100 L1 nymphs per day, and kept at 25 ± 1 °C, 75 ± 5% RH and 14 h light, the highest mean total progeny produced was 179. Although using different methods to measure potential lifetime fecundity, an average lifetime fecundity of 32.8 eggs for *P. digoneutis* and a maximum progeny of 179 for *P. stygicus* seems to be remarkably low when compared with the average lifetime fecundity of *P. digoneutis* (385.4 ± 35.2 eggs) and *P. stygicus* (782.1 ± 65.1 eggs) found in

this study. Different factors in the experimental design could explain the distinct differences observed among studies. When calculating the lifetime fecundity of *Peristenus* parasitoids by the adult progeny produced (van Steenwyk & Stern, 1976), potential superparasitization is neglected because all *Peristenus* species are known to be solitary parasitoids and thus, even when a host is parasitized more than once, only one parasitoid will complete development in a single host. Rearing the parasitized nymphs until parasitoid larvae emerge, also increases the risk of nymphal mortality. Van Steenwyk & Stern (1976) provide no information regarding nymphal mortality during the rearing process; consequently it is unclear whether their fecundity data are based on a total of 100 nymphs that survived the rearing process, or if it is based on a smaller sample size due to mortality. The low lifetime fecundity reported by Hormchan (1977) was likely underestimated based on the fact that nymphs were dissected within 48 hours following parasitization, at a time when the parasitoid eggs are still small and easy to miss (length of a *P. digoneutis* egg after two days: 0.16mm; Carignan et al., 1995). However, the main reason for differences in the studies seems to be the longevity of the parasitoids. In Hormchan's experiments, *P. digoneutis* females were kept continuously at 25 °C, resulting in an average female survival of 13.8 days (min. 7 days, max. 19 days). Van Steenwyk and Stern (1976) also kept *P. stygicus* at 25 ± 1 °C continuously, and observed a maximum average reproductive longevity of 8.1 days. However, maintaining the parasitoids at a constant temperature ignores the fact that in nature temperatures fluctuate daily, with the lowest temperatures occurring at night (Jervis & Copland, 1996). Thus, in the present experiment females were kept at 25 ± 1 °C during the day and at 15 °C during the night in order to simulate natural temperature conditions as they appear in early summer when *Peristenus* parasitoids are active in the field. By keeping the parasitoids at low temperatures over night, adult longevity of *P. digoneutis* and *P. stygicus* was on average 25 and 31 days, respectively. Van Steenwyk & Stern (1976) found an inverse curvilinear relationship between longevity of *P. stygicus* and rearing temperature. Females kept continuously at 15 °C (without the chance to oviposit) survived on average 43.1 days, whereas at a constant temperature of 25 °C females survived 16.2 days. In general, there is an optimum temperature range outside of which the parasitoid either cannot continue ovigenesis and oviposition or is unable to do so for long (Force & Messenger, 1964; Jervis & Copland, 1996). Increasing temperatures may shorten a parasitoid's life span by causing acceleration of development and physiological activities or by exerting a lethal effect (Bilewicz-Pawinska, 1974; Mills, 1981). In consideration of the former statements, keeping the parasitoids at 25 °C continuously, as done

by Hormchan (1977) and van Steenwyk & Stern (1976), likely resulted in early adult mortality and consequently, in a much lower lifetime fecundity.

Under optimal laboratory conditions, single females of both species were able to survive longer than one month. Although both species are closely related, it has been demonstrated that both parasitoids follow a different survivorship curve. *Peristenus digoneutis* follows a type II survivorship curve (Slobodkin, 1962; Southwood & Henderson, 2000), indicating that a constant number of adults die per unit of time and that the risk of mortality is already high during the early adult stage. Remarkably, the first females died immediately after the maximum daily oviposition rates were observed. In contrast, *P. stygicus* follows a type I survivorship curve (Slobodkin, 1962; Southwood & Henderson, 2000) which indicates the risk of mortality is fairly low during the early adult stage. Thus, the mean adult longevity of *P. stygicus* was significantly higher than that of *P. digoneutis*. However, in both species single females were found to survive for more than one month. The results of this study are similar to those in the experiments of Bilewicz-Pawinska (1974), in which more than 76 % of *P. digoneutis* females survived longer than 15 days when kept at temperatures between 20 and 25 °C. However, at temperatures above 25 °C the majority (72.6%) of the females died within the first two weeks. A long post-reproductive survival was rarely observed in either species. The parasitoids generally survived for two to three more days following the cessation of oviposition; only single parasitoids showed a prolonged post-reproductive survival. Harvey et al. (2001) reported a prolonged post-reproductive survival when the parasitoids had unlimited host access, whereas restricting host access to parasitoids resulted in a decrease in the duration of post-reproductive survival.

The relationship between fecundity and longevity and fecundity and oviposition period was almost equal, as post-reproductive survival of *P. digoneutis* was generally short. In both cases a logarithmic relationship was found. During the first two weeks of adult life, fecundity increased greatly with parasitoid age, whereas among older females lifetime fecundity only increased slightly, indicating that the females had reached their maximum reproductive potential. Although differences in longevity among *P. stygicus* females were much lower, lifetime fecundity increased when the age of the females increased. However, this relationship was only significant when individuals with extremely long post-reproductive survival were excluded from the analysis. Thus, a stronger relationship was observed between lifetime fecundity and oviposition period.

Jervis & Copland (1996) suggested that lifetime fecundity in many parasitoids is positively correlated with female body size. It is assumed that larger female parasitoids live longer and have a higher fecundity and progeny production; consequently, body size is often used to estimate the quality of parasitoids in biological control programmes (Reitz & Adler, 1995; Lauzière et al., 2000; Sagarra et al., 2001). In the present study, the relationship between lifetime fecundity and size of female *P. stygicus* and *P. digoneutis* was examined, but only a minor positive correlation was found for *P. stygicus*.

5.4.2 Consequences for biological control

The present results are not in agreement with previous studies on the reproductive potential of *P. stygicus* and *P. digoneutis* by Hormchan (1977) and van Steenwyk & Stern (1976) and thus, the potential of *P. digoneutis* and *P. stygicus* as biological control agents has likely been underestimated.

The relatively high potential lifetime fecundities of both *Peristenus* species suggest that they have a promising potential for biological control. However, it has been shown recently that the hypothesis that the ability to suppress pest populations is positively correlated with parasitoid fecundity cannot be supported in general, but is strongly dependent on host taxon (Lane et al., 1999). Furthermore, potential lifetime fecundity observed under constant optimal laboratory conditions does not necessarily reflect the situation under natural conditions (= realized fecundity). It has been shown that the distribution of realized fecundities in synovigenic parasitoids can be highly skewed, and that a very large proportion of parasitoids never reach their potential fecundity (Weisser et al., 1997; Ellers et al., 1998). When comparing both parasitoid species, it is evident that *P. stygicus* is able to produce on average 50% more eggs over its lifetime than *P. digoneutis* and therefore, this species can be regarded as the potentially more effective biological control agent when only fecundity is taken into consideration. However, high lifetime fecundity of biological control agents is not necessarily an advantage, because parasitoids with high egg loads are more likely to oviposit in nearly every host they encounter, including hosts in which the probability of offspring survival is small (Godfray, 1994). Thus, the risk of non-target effects from these species is assumed to be high. In the case of *P. stygicus*, these concerns are supported by the observations on host specificity described in chapters 3 and 4.

6 Cold-hardiness of *Peristenus digoneutis* and *P. stygicus* (Hymenoptera: Braconidae)

6.1 Introduction

The ability to survive extended periods at temperatures below 0 °C is an essential requirement for most insects inhabiting northern temperate regions (Turnock et al., 1983). Therefore, insects have developed different strategies to adapt to low-temperature extremes (Loomis, 1991). Honey bees for example, can survive the winter because they stay in their hives and maintain a higher temperature by contracting their flight muscles (Southwick & Heldmaier, 1987). Other insects migrate down into the soil or to other protected over-wintering sites. Butterflies, like the Monarch, escape from cold winter temperatures while migrating long distances from North America to Mexico and California (Danks, 1991; Müller, 1992). However, in most insects physiological and biochemical processes lead to cold-hardening. Cold-hardiness is defined as the capacity of an organism to survive exposure to low temperatures (Lee, 1991).

According to a survey of biological control schemes, most of the successes have occurred in warmer, stable climates, where temperatures are favorable for phytophagous pest insects to feed and reproduce throughout the year, providing a continuous supply of hosts for the natural enemy species (DeBach, 1964; Bale, 1991). Moving from environments with warmer climates to regions with temperate and subarctic climates, cold winter periods result in seasonal crop production and require pest species and their natural enemies to seek out hibernation sites, to diapause, and to develop increased cold tolerance. The relative lack of successes in biological control in temperate and colder climates has been assigned to high mortality of natural enemies due to climatic extremes and the need for parasitoids and predators to synchronize their annual life cycle with that of their host. Therefore, in a biological control program it is essential that an introduced natural enemy is as well adapted to the stresses of the winter season as their target pest species (Bale, 1991).

The European *Lygus* parasitoids *Peristenus digoneutis* and *P. stygicus*, both considered for biological control of *Lygus* spp. in Canada, overwinter as adults in cocoons formed by their larvae at a soil depth of 1 to 5 cm. Meteorological records for western Canada indicate that soil temperatures at 5 cm depth reach a minimum of -15 °C throughout 25% of the winters and -20 °C during 5% of the winters (Turnock et al., 1983). Thus, in order to assess the

potential of *Peristenus* spp. to establish in Canada, knowledge of the overwintering biology of the parasitoids is essential.

In this chapter, the question of whether European *Lygus* parasitoids have the ability to survive extended periods of temperature below 0 °C will be addressed. The results are discussed in context with the climatic conditions of potential release areas for *Peristenus* spp. in Canada.

6.2 Material and Methods

6.2.1 Source of parasitoids

Parasitoid cocoons containing overwintering adults of the primary parasitoids *P. digoneutis* and *P. stygicus* were obtained from the second nymphal generation of *L. rugulipennis* collected in Schleswig-Holstein in August 2002 and 2003 (see chapter 2). On rare occasions, the cocoons contained larvae of the hyperparasitoid *Mesochorus curvulus*. In addition, cocoons containing *P. near pallipes* were obtained from nymphs of the univoltine mirid *Closterotomus norwegicus*, which were collected in early June 2002. Members of the *P. pallipes* species complex occasionally occur in *Lygus* spp. in Europe and North America (Loan, 1980; Rämert et al., unpublished), and for that reason, *P. near pallipes* (ex "*Closterotomus*") was partly included in the studies.

Overwintering cocoons were kept in Petri dishes filled with moist vermiculite and stored in a subterranean insectary. In the insectary, which is designed for overwintering insects, the inside temperature decreases when the temperature outside the building is decreasing, too; however, the inside temperature never drops below + 2 °C. Thus, the parasitoid cocoons were not exposed to freezing temperatures before the experiments were started. Storage temperature was monitored with Testostor 175 mini loggers.

6.2.2 Supercooling points

The supercooling point (SCP) of insects is defined as the temperature at which body water spontaneously freezes during the process of continuous cooling (Lee, 1991). Most insects, in particular those that are intolerant of freezing, developed the capacity to supercool as an

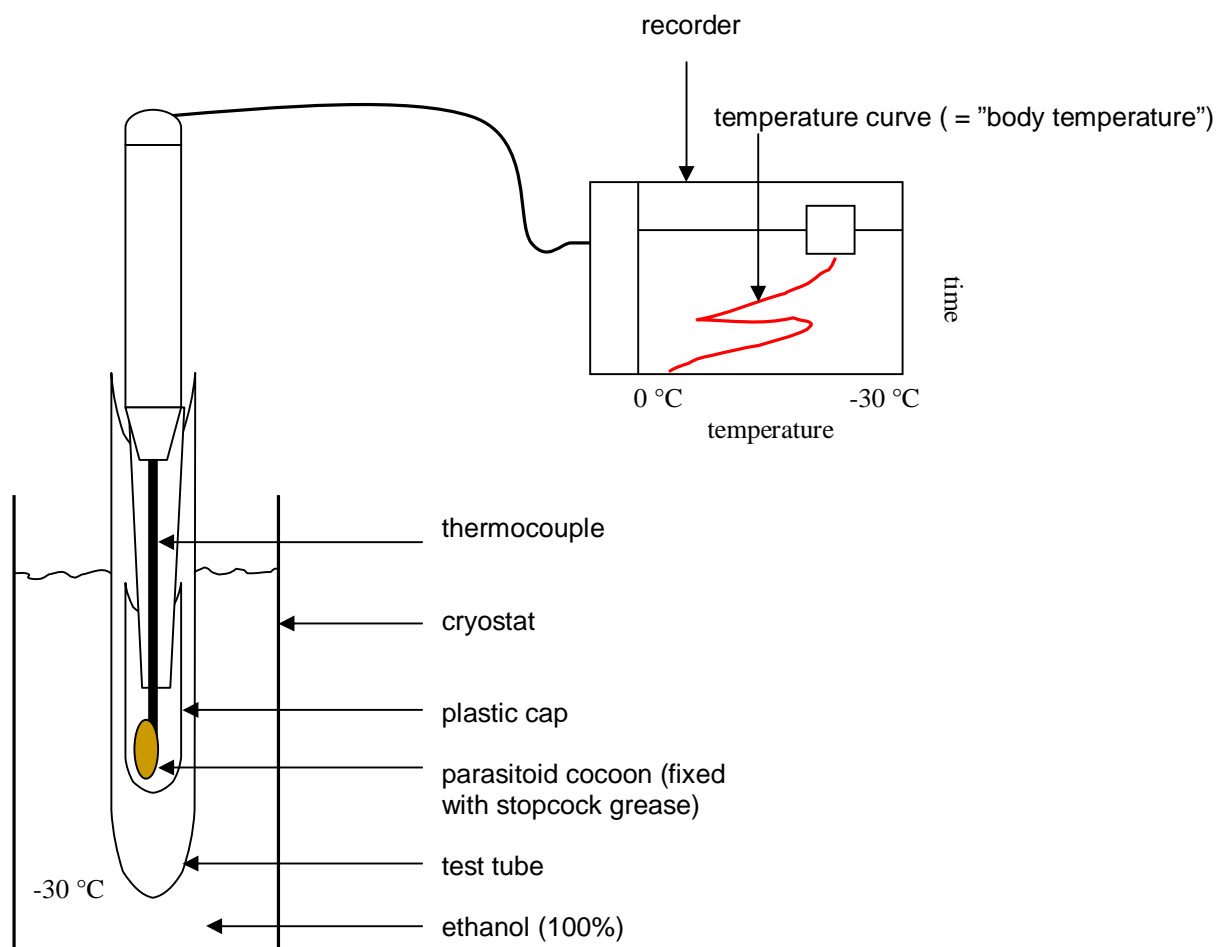


Figure 6.1 Experimental set up for SCP measurement

overwintering strategy. While its reliability has been questioned, the survival potential of a species can be assessed by comparing the measured supercooling point (as an indicator of the lower lethal limit) to minimum winter temperatures (Leather et al., 1993).

The day the experiments were conducted, parasitoid cocoons were transferred directly from the insectary to a 4 °C chamber where they were stored until used in the experiments. This way, the potential influence of room temperature was diminished. Before starting the measurements, small particles of moist vermiculite attached to the parasitoid cocoons were carefully removed with a smooth brush to avoid the disturbing influence of water absorbed in the vermiculite.

The change of the parasitoid's body temperature was measured with a NiCr-NiAl thermocouple (Gulton: Tastoherm D 700) which was connected to a Linseis recorder (Fig. 6.1). The outer surface of the parasitoid cocoon was attached to the temperature-sensitive tip of the thermocouple. To guarantee a close contact between the tip of the thermocouple and the

cocoon, stopcock grease was used as glue. The tip of the thermocouple with the attached cocoon was then covered with a small plastic cap. The measurement was started by transferring the thermocouple with the attached parasitoid cocoon into a thin walled test tube inserted in a cooling bath filled with 100% ethanol (see Block & Young, 1979), so that the cocoons were cooled down at a constant rate of approximately 2 °C / min until they froze spontaneously. Then, the supercooling point was indicated by the sudden increase of temperature, caused by the release of heat of fusion from freezing water (heat of crystallization). The lowest temperature recorded before this temperature increase was taken as the SCP.

Since the cocoons of *Lygus* parasitoids are morphologically indistinguishable, cocoons were dissected for adult parasitoids (or hyperparasitoid larvae) after the experiments were finished. Morphological characteristics of the adults were then used to identify the parasitoid species responsible for the observed SCPs.

6.2.3 The effects of adaptation to high and low temperatures on the SCP

After measuring the SCPs of *Lygus* parasitoids in January 2003, cocoons containing overwintering adult parasitoids that were stored in the insectary at temperatures above 0 °C were subsequently chilled down for four days at -5 °C, followed by four days at -10 °C, and four days at -15 °C. The aim of the experiment was to examine whether the parasitoids are able to adapt their SCP to low temperatures. After the cooling-down period was finished, parasitoid SCPs were measured and compared to the former SCPs that were measured before the experiment had started (= SCPs measured in January).

In March 2003, the SCPs of *Lygus* parasitoids stored in the insectary were measured again and compared to the SCPs measured in January to determine if increasing temperatures in the insectary influenced parasitoid SCPs. To estimate whether parasitoids can also adapt their SCPs to high temperatures, a sample of cocoons was warmed-up for four days at +10 °C followed by four days at +15 °C. The SCPs of the warmed-up parasitoids were compared to the parasitoid SCPs measured in March.

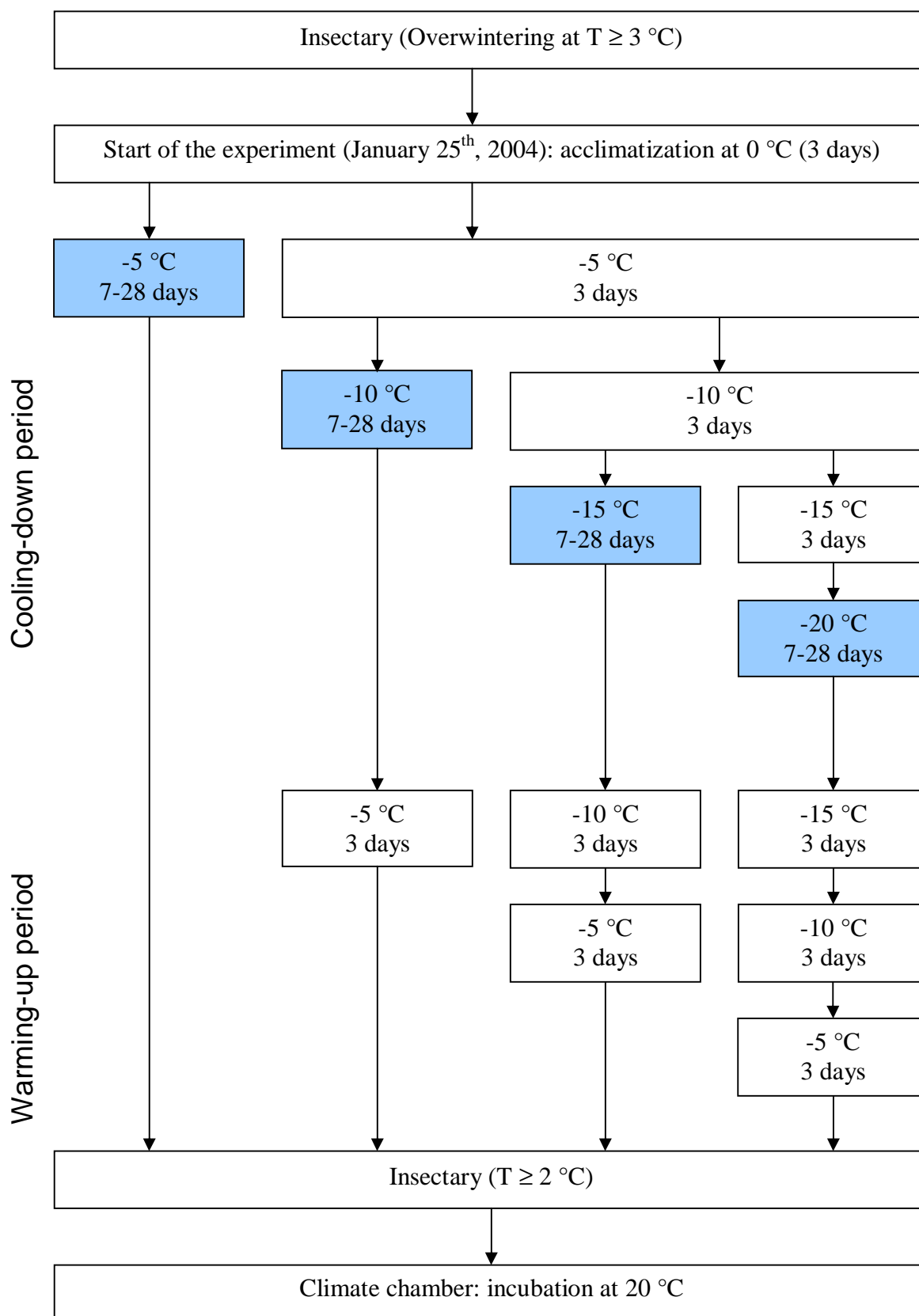


Figure 6.2 Experimental design to determine the effects of subzero temperatures and exposure time on the survival of *Lygus parasitoids*; the blue boxes represent the four experimental groups

6.2.4 Influence of subzero temperatures on parasitoid survival

In January 2004, parasitoid cocoons were transferred from the insectary to a cryostat and acclimatized at 0 °C for three days before exposure to subzero temperatures (Fig. 6.2). All cocoons were kept in Petri dishes filled with vermiculite which buffered the cocoons against rapid temperature changes. The cocoons were acclimatized to -5 °C (cooling rate: 2 °C / h), then they were divided into four experimental groups, each containing five to ten samples of 50 to 150 parasitoid cocoons. Each group was kept at different subzero temperatures (-5 °C, -10 °C, -15 °C, -20 °C) for a period of one to four weeks. Cocoons selected for exposure to temperatures below -5 °C were chilled down in 5 °C steps every three days until the desired storage temperature was achieved. When the period of exposure to subzero temperatures was finished, the cocoons were warmed up in 5 °C steps (lasting three days each) until they reached -5 °C. In this way, the parasitoids were allowed to slowly acclimatize to changes in temperature. Following the experimental period, the cocoons were transferred back to the insectary. After the longest exposure of the cocoons to low temperatures (-20 °C, four weeks), all the cocoons used in the experiments and stored afterwards in the insectary were incubated simultaneously at +20 °C until emergence or death of the parasitoids. To estimate the effect of freezing temperatures on *Lygus* parasitoids, the survival of individuals exposed to subzero temperatures was compared with the survival of individuals in control samples which were kept in the insectary during the entire experimental period and thus, were not exposed to freezing temperatures at any time.

An extra sample of cocoons (n = 136; 102 *P. digoneutis* and 34 *P. stygicus*) was cooled down immediately to -21 ± 1 °C without allowing the parasitoids to acclimatize to falling temperatures; these cocoons were then kept at this temperature for two weeks. The aim of this test was to investigate whether parasitoids would survive a rapid decrease to temperatures just above their SCP.

In order to investigate the parasitoid survival when exposed to natural winter temperatures in Schleswig-Holstein, 312 parasitoid cocoons (a mixture of *P. digoneutis* (57%), *P. stygicus* (42%) and *M. curvulus* (1%)) were kept in Petri dishes filled with vermiculite in an outdoor open wooden shelter until parasitoid emergence in spring. As a control 395 cocoons (*P. digoneutis* (50%), *P. stygicus* (46%), *M. curvulus* (4%)) were stored inside the insectary during winter where the cocoons were never exposed to temperatures below 2 °C.

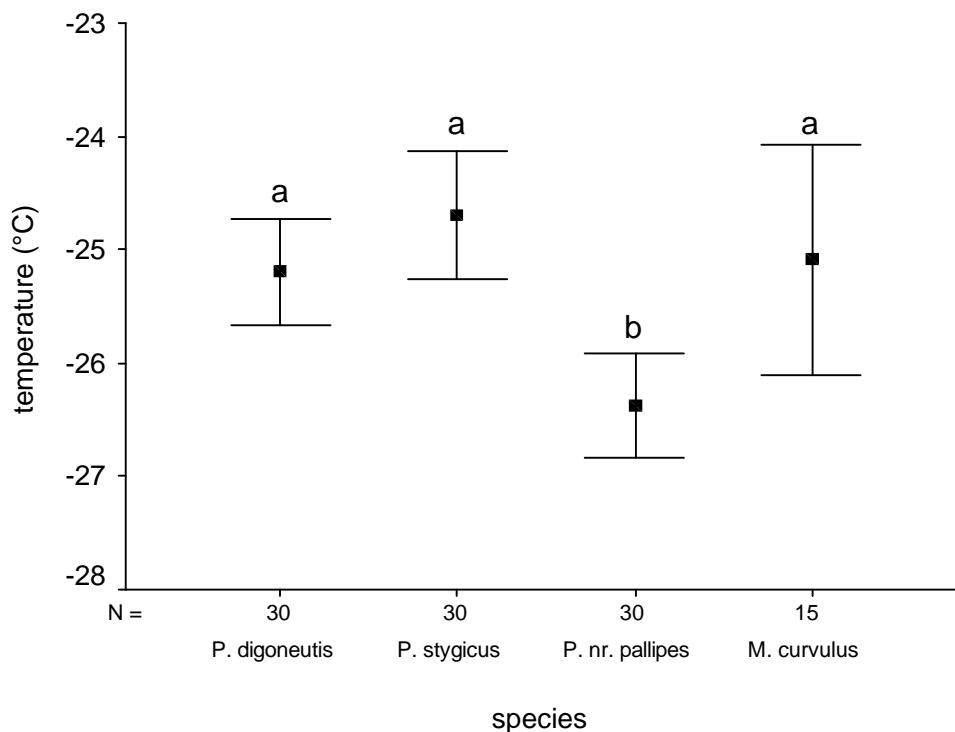


Figure 6.3 Supercooling points of the primary parasitoids *P. digoneutis*, *P. stygicus*, *P. nr. pallipes* and the hyperparasitoid *M. curvulus* in January 2003 after storage at temperatures above 2 °C (N = number of measurements; test statistic: Oneway ANOVA followed by a Bonferroni post hoc test for multiple comparisons, $P > 0.05$, see Table 19 in the Appendix, vertical bars show standard errors and means with the same letter are not different).

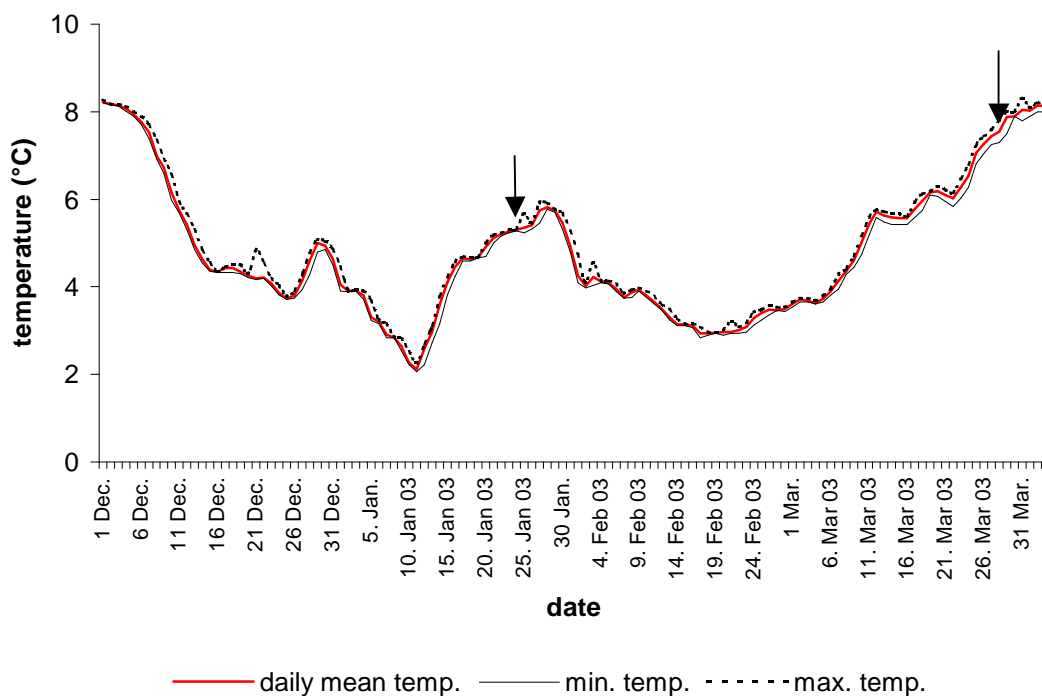


Figure 6.4 Temperatures inside the subterranean insectary between December 1st, 2002 and April 3rd, 2003 (arrows indicate the date when cocoons were removed for SCP measurements)

6.3 Results

6.3.1 Supercooling point and acclimation to high and low ambient temperatures

The mean supercooling points for *P. digoneutis*, *P. stygicus*, and *M. curvulus* measured in January were -25.2 ± 0.2 °C (SE), -24.7 ± 0.2 °C (SE), and -25.1 ± 0.5 °C (SE), respectively (Fig. 6.3). The SCPs of these species, all reared from *L. rugulipennis*, did not differ significantly. However, the SCP of *P. nr. pallipes* reared from *Closterotomus norwegicus* was significantly lower (-26.4 ± 0.2 °C).

A further increase of the SCPs was observed after parasitoid cocoons were stored in the insectary until March and then slowly acclimatized to +15 °C (Figs. 6.5 a, b). In *P. digoneutis* the mean SCP increased significantly from -23.7 ± 0.2 °C (SE) (= SCP in March) to -22.5 ± 0.2 °C (SE). In *P. stygicus*, the mean SCP increased from -23.6 ± 0.2 °C (SE) to -22.9 ± 0.2 °C (SE); however, the sample size was low and SCPs measured before and after the acclimatization did not differ significantly.

After cocoons of *P. digoneutis* and *P. stygicus* were slowly acclimatized to -15 °C, the mean SCPs of both species were significantly lower than those of non-acclimatized individuals in which SCPs were measured before exposure to low temperatures in January (Figs. 6.5 a, b). In *P. digoneutis* the mean SCP decreased from -25.2 ± 0.2 °C (SE) to -26.8 ± 0.2 °C (SE), and in *P. stygicus* it decreased from -24.7 ± 0.2 °C (SE) to -26.2 ± 0.2 °C (SE).

After temperatures inside the insectary had increased in March (Fig. 6.4) – synchronously with increasing temperatures outside (Fig. 6.7) – the mean SCPs of *P. digoneutis* increased significantly from -25.2 ± 0.2 °C (SE) measured in January to -23.7 ± 0.2 °C (SE) measured in March (Fig. 6.5 a); in *P. stygicus*, the mean SCP increased from -24.7 ± 0.2 °C (SE) to -23.6 ± 0.2 °C (SE) (Fig. 6.5 b).

6.3.1 Effect of prolonged exposure to low temperatures on parasitoid survival

Quiescent adults of *P. digoneutis* survived -5 °C exposure with little mortality for as long as three weeks (Fig. 6.6 a). However, when parasitoid adults were exposed to -5 °C for four weeks, survival drastically decreased to 0%. In comparison to survival in untreated

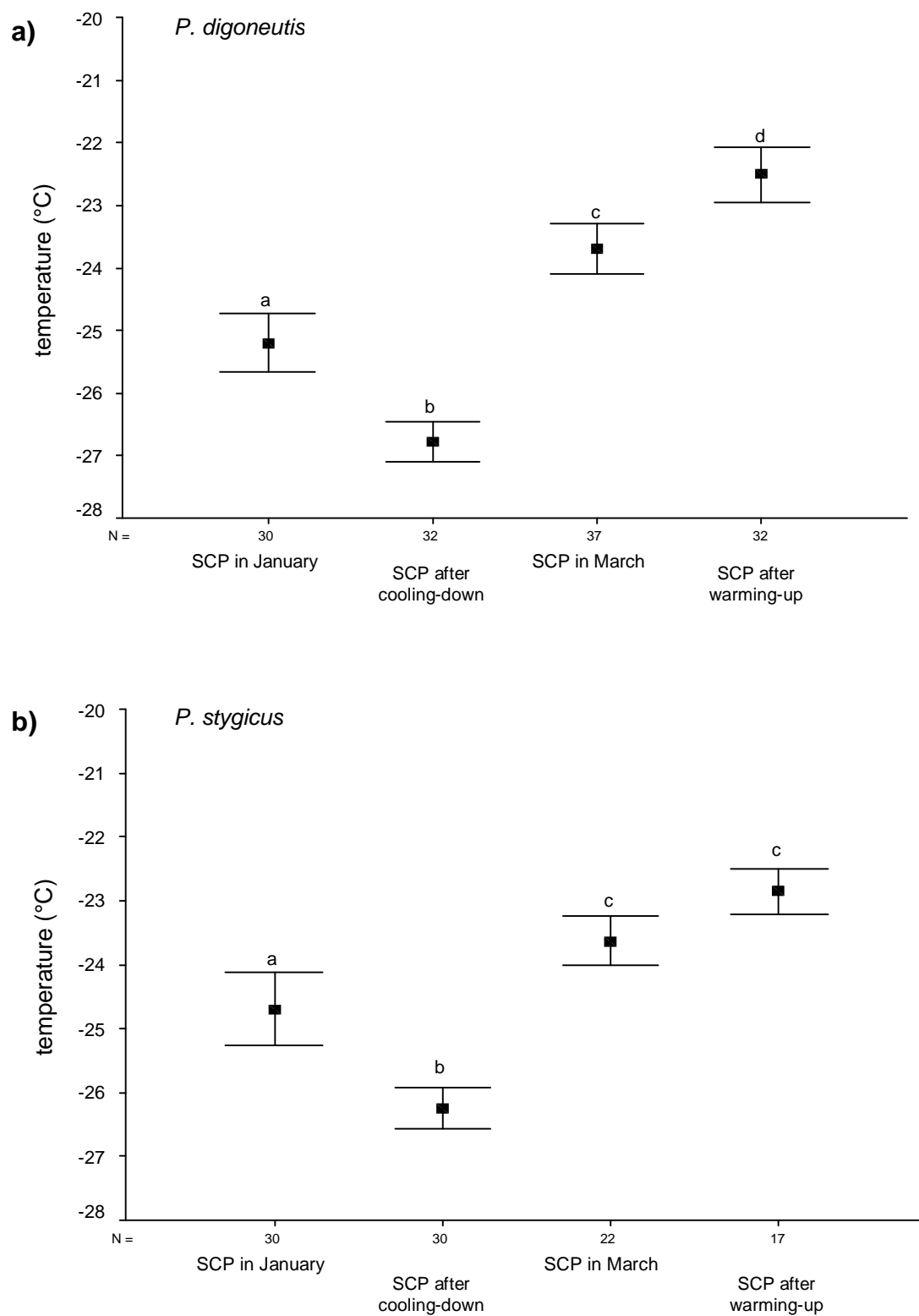


Figure 6.5 Mean supercooling points of (a) *P. digoneutis* and (b) *P. stygicus* in January and March 2003 after storage in the insectary and SCPs after the parasitoids were cooled down to $-15\text{ }^{\circ}\text{C}$ or warmed up to $+15\text{ }^{\circ}\text{C}$ in the laboratory (N = no. of measurements; test statistics: Oneway ANOVA followed by a Bonferroni post hoc test for multiple comparisons, $P > 0.05$, see Appendix, Tables 20, 21; vertical bars show standard errors and means with the same letter are not different).

control samples (99.2%, $n = 13$ samples; 696 individuals), survival of adults exposed to -10 °C significantly decreased after an exposure length of one week (79%) (Binominal test: $P < 0.001$, also see Table 22 in the Appendix). A prolonged exposure of three to four weeks resulted in an even lower survival of 53% and 28%, respectively. However, survival among adults exposed to -10 °C for four weeks was significantly higher than survival among parasitoids that were exposed for four weeks to -5 °C (Appendix, Table 23). *Peristenus digoneutis* exposed to temperatures of -15 °C suffered severe mortality. Only 22% and 25% of the parasitoids survived an exposure of one and two weeks, respectively. After an exposure of three weeks or longer, adult survival significantly decreased to less than 14% ($P < 0.001$). At temperatures of -20 °C, survival (16%) was only observed among adults that were exposed no longer than one week. Group wise comparisons of the different treatments are presented in Tables 22 and 23 in the Appendix.

The proportion of *P. stygicus* in field samples used for the experiments was generally much lower than that of *P. digoneutis* and thus, the results are based on a much lower number of individuals. For that reason, the small numbers of *P. stygicus* from samples that were treated the same way were added (Fig. 6.6 b). Parasitoid adult survival at temperatures between -5 °C and -15 °C was always higher than 50%, even when the length of exposure was four weeks. Thus, in comparison to *P. digoneutis*, survival of *P. stygicus* at low temperatures was remarkably higher. Similar to *P. digoneutis*, only a small proportion of *P. stygicus* adults (17%) survived a one week exposure at -20 °C. No survival was observed when parasitoids were exposed longer than one week at -20 °C. Statistical comparisons of groups were neglected because of the small sample sizes.

From cocoons that were transferred directly to -21 °C and stored at this temperature for two weeks, only a low percentage of adults emerged. In *P. digoneutis*, adults emerged from only 7% (7 out of 102) of the cocoons, whereas in *P. stygicus* 32% emergence was observed (11 out of 34).

6.3.2 Parasitoid survival under natural temperature conditions

In winter 2002/3 the air temperature fluctuated greatly with average daily temperatures frequently dropping below 0 °C. Temperatures falling below 0 °C were measured between mid-October and mid-April (Fig. 6.7). In December and February two extended periods of

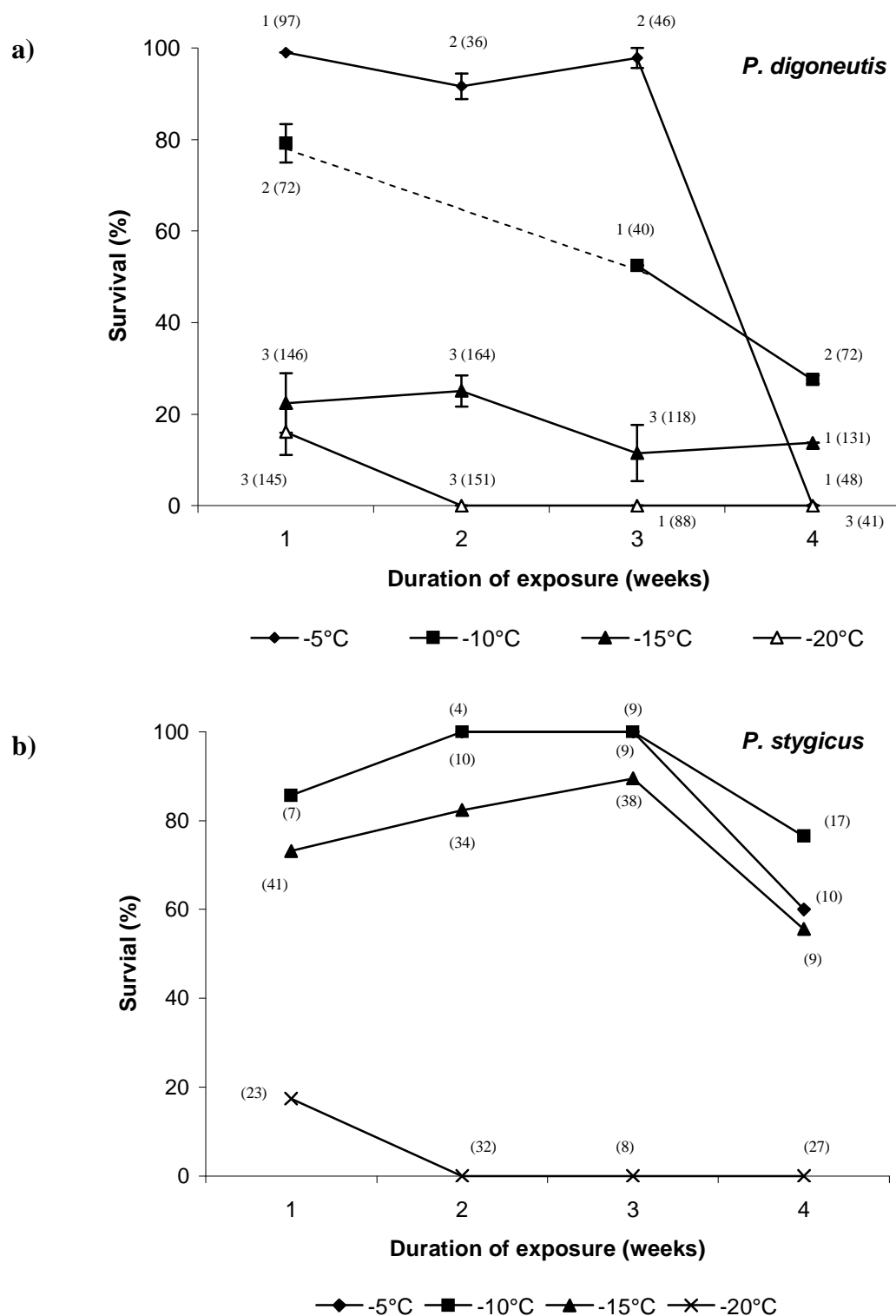


Figure 6.6 Survival following exposure to different low temperatures of variable duration:

- a) *P. digoneutis* (no data for two weeks exposure at -10°C ; vertical bars show standard errors; numbers beside the symbols represent the sample size and numbers in brackets represent the total number of individuals; mean survival in untreated control samples: $99.2\% \pm 0.5$ SE, $n = 13$ samples (696 individuals)).
- b) *P. stygicus* (no data for one week exposure at -5°C ; numbers in brackets beside the symbols represent the total number of individuals; survival in untreated control samples: 94.6% , $n = 99$ individuals; samples were combined because of the low proportion of *P. stygicus* in each sample, see further explanation in the text).

daily mean temperatures between 0 °C and -5 °C were observed. However, the lowest daily mean temperatures were measured in January. For five days the mean temperature was between -5 °C and -10 °C (Table 6.1). The lowest temperature measured was -13.35 °C (January 5th). In total, the parasitoid cocoons were exposed to daily mean temperatures between 0 °C and -5 °C for 43 days and to mean temperatures between -5 °C and -10 °C for five days. The longest period of continuous subzero temperatures was only nine days.

Table 6.1 Number of days per month during which the daily mean temperature (daily minimum temp.) was within 5 °C ranges below 0 °C, October 2002 to April 2003 at Kiel (measured in an open wooden shelter, 30 cm above the soil; lowest temperature measured: -13.35 °C, January 5th).

Month	temperature			
	0 to -5 °C	< -5 to -10 °C	< -10 to -15 °C	< -15 to -20 °C
October 02	2 (8)	0	0	0
November 02	4 (6)	0	0	0
December 02	15 (21)	0 (1)	0	0
January 03	7 (6)	5 (4)	(3)	0
February 03	14 (22)	0 (3)	0	0
March 03	1 (10)	0	0	0
April 03	0 (4)	0	0	0
sum	43 (77)	5 (8)	0 (3)	0

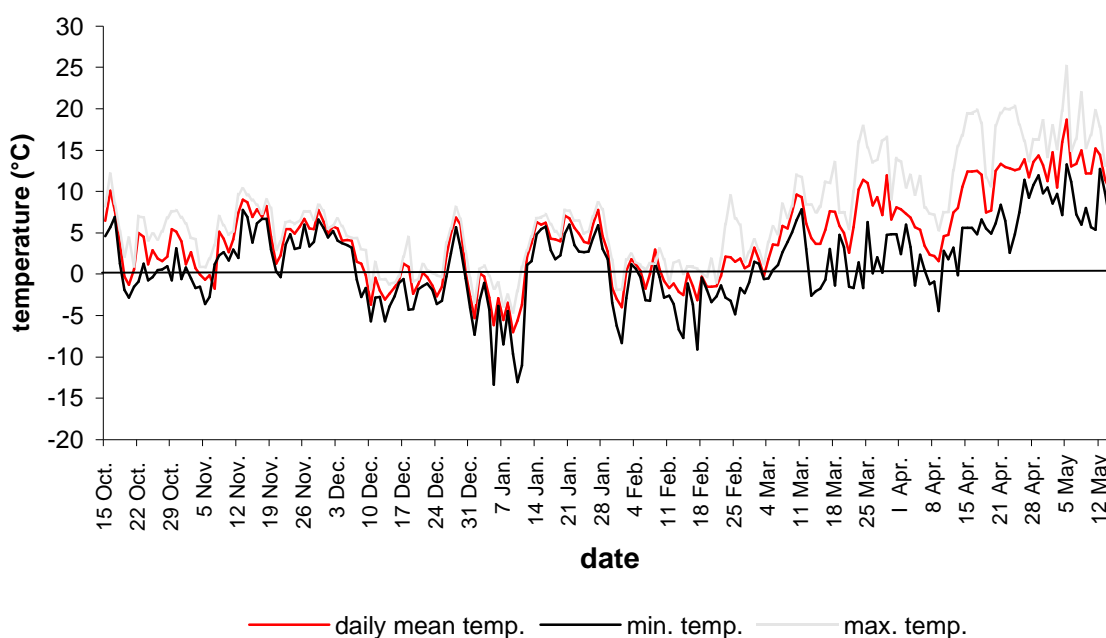


Figure 6.7 Daily mean, minimum and maximum temperatures in October 2002 – May 2003 in Kiel (measured in an open wooden shelter, 30 cm above the soil).

Survival of parasitoid cocoons stored in the insectary at temperatures above 0 °C did not differ significantly from cocoons exposed to outside air temperatures (Kruskal-Wallis test; $P = 0.14$; n.s.). On average, the parasitoid survival was $73.2\% \pm 2.0$ (SE) in samples stored outside ($n = 4$ samples; in total 312 individuals) and $78.2\% \pm 1.84$ (SE) in samples stored in the insectary ($n = 5$ samples; in total 395 individuals).

6.4 Discussion

6.4.1 Cold-hardiness of *P. digoneutis* and *P. stygicus*

In general, two main strategies of cold-hardiness have evolved in insects to withstand temperatures below the freezing point of their body fluids (Baust, 1982). Insects are either freeze-tolerant or freeze-intolerant (syn. freeze-susceptible) depending on their ability to survive the formation of extracellular ice (Salt, 1961).

Freeze-tolerant insects are able to endure extensive ice formation within their body tissues and organs (Leather et al. 1993). This freezing occurs extracellularly to prevent intracellular freezing which would be lethal. Therefore, many freeze-tolerant insects produce haemolymph proteins or lipoproteins (= “Cyroprotectants”) that serve as catalysts for extracellular nucleation of ice at subzero temperatures ranging from -5 to -10 °C (Zachariassen & Hammel, 1976; Lee, 1991). Among these cyroprotectans, glycerol is the most common and abundant substance (Sømme 1964; Zachariassen, 1985).

Freeze-intolerant insects are unable to tolerate the formation of ice in the body tissues and fluids (Leather et al., 1993). Insects belonging to this category avoid freezing by supercooling and by selecting overwintering sites that protect them from extreme temperatures that occur in exposed sites (Turnock et al., 1983). Freeze-intolerant insects can often avoid freezing until their temperature has fallen many degrees below the melting point of their body fluids. This level of supercooling is usually achieved as a two-stage process: first, by the removal or inactivation of any ice nucleating agents from the haemolymph, or gut contents from the digestive system, and secondly by the prewinter accumulation of antifreeze agents, notably polyols, proteins and sugars, e.g. glycerol, sorbitol, trehalose and glucose (Lee, 1991; Leather et al., 1993).

The majority of freeze-tolerant insects have relatively low winter SCPs between -5 and -10 °C, whereas in most freeze-intolerant species the SCP is much lower (Zachariassen 1982, 1985). The low mean SCPs of overwintering *P. stygicus* (-24.7 °C) and *P. digoneutis* (-25.2 °C) which were not cold-acclimated, and at the time of measurement (January) most likely in a period of thermal quiescence (see chapter 2; Braune, 1971), indicate that both parasitoids are probably freeze-intolerant. However, Ring (1982) has described several freeze-tolerant insects with SCPs between -23 °C and -54 °C and thus, a low supercooling point does not necessarily mean that an insect is freeze-intolerant. In the present study, it has also been demonstrated that both parasitoid species are able to acclimatize to changing temperatures with an increase/decrease of their SCPs (= laboratory-induced acclimatization). Although no data are available from individuals kept at natural temperatures, it can be assumed that the SCPs of the parasitoids would change throughout the season (= natural field changes), following the changes in soil temperatures. In nature, the seasonal increase in supercooling from summer through autumn to winter, is a common feature of freeze-intolerant insects (Leather et al., 1983) and thus, the observed acclimatisation of *Peristenus* spp. can be regarded as another indication that the parasitoids are freeze-intolerant. A commonly used method to designate an insect as freeze-tolerant or intolerant is to cool the insect until its SCP is achieved and then warm it up slowly to room temperature and assess survival. However, in the present study this method was not practical, because immediately after measuring the SCPs it was difficult to estimate whether the quiescent parasitoid adults were still alive when dissecting the cocoons.

The SCPs of native *Peristenus* species that are known to occur in the prairie provinces of Canada such as *P. pallipes* and *P. pseudopallipes* (SCP = -22.44 °C ± 1.97; Panneton et al., 1995) are similar to those measured for *P. digoneutis* and *P. stygicus*.

Until recently, freezing was generally regarded as the main mortality factor among overwintering, freeze-intolerant insects (Baust & Rojas, 1985). Consequently, the supercooling point was accepted as a sufficient measure of cold-hardiness. However, in many studies it has been demonstrated that exposure to temperatures above the SCP can cause high mortality in diapausing or quiescent insects (Turnock et al., 1983; Knight et al., 1986; Pullin & Bale, 1988; Bennett & Lee, 1989; Larsen et al., 1992; Turnock & Bilodeau, 1992; Turnock, 1993). Consequently, Bale (1993) suggested that survival at subzero temperatures above the supercooling point should be given more consideration in insect cold-hardiness investigation.

Hou et al. (2001) reported that the mortality of the black carpet beetle larvae *Attagenus unicolor* (Coleoptera: Dermestidae) increased with length of exposure time, even at temperatures above the SCP. Similar trends were observed in the present study: Mortality of *P. digoneutis* increases as temperature decreases and as exposure to low temperatures lengthens. This phenomenon has been first described by Salt (1936). However, the low survival of *P. digoneutis* following an exposure to $-5\text{ }^{\circ}\text{C}$ for four weeks was unexpected and stays in contradiction to the higher survival of *P. digoneutis* when exposed to $-10\text{ }^{\circ}\text{C}$. This contradiction remains unexplained and further investigation is necessary. The response of *P. stygicus* to prolonged exposure to subzero temperatures was less consistent. This inconsistency was probably caused due to the limited number of individuals available for each temperature treatment. However, the present results indicate that at least an exposure to subzero temperatures for four weeks results in an increased mortality. A remarkable difference in cold-tolerance between *P. stygicus* and *P. digoneutis* was observed when parasitoids were kept at $-15\text{ }^{\circ}\text{C}$: Whereas in *P. digoneutis* no more than 25% of the adults survived, survival in *P. stygicus* was minimum 56%. Therefore, *P. stygicus* is likely better adapted to low temperatures than *P. digoneutis*.

Although *P. digoneutis* and *P. stygicus* are able to adapt their SCPs to falling temperatures, the results from laboratory experiments suggest that a SCP lower than $-25\text{ }^{\circ}\text{C}$ is not sufficient enough to protect the parasitoids from dying due to a long-term exposure to low temperatures and that cold injury occurs in *P. digoneutis* at temperatures above the SCP. Furthermore, the SCPs of *P. digoneutis* and *P. stygicus* do not differ significantly, but in the present experiments *P. stygicus* was more resistant to lower temperatures and long-term exposure than *P. digoneutis*. Thus, the SCP of *Peristenus* species is unreliable as a measure of low temperature tolerance. The present study may support other research results which indicate that in freeze-intolerant insects which die at temperatures above their SCP cryoprotectant polyols are likely to play a role in cyroprotection other than depressing SCP of insects (Chen et al., 1987; Pullin et al., 1991). However, polyols in *Peristenus* were not investigated.

The high mortality of *P. digoneutis* and *P. stygicus* exposed to $-20\text{ }^{\circ}\text{C}$ for one week or longer may partly result from the experimental set-up. After it had been shown that the parasitoids are able to adapt their SCP to falling temperatures, parasitoids in the long-term exposure were slowly acclimatized to low temperatures assuming that depressing the SCP would protect the parasitoids from dying due to low temperatures. However, this means that the parasitoids

were already exposed to subzero temperatures for many days before the actual temperature treatment had started. In addition, parasitoids were slowly acclimatized back to warmer temperatures after the treatment was finished. Thus, parasitoids that were for example kept at $-20\text{ }^{\circ}\text{C}$ for one week were actually exposed to subzero temperatures for 25 days (9 days acclimatization to $-20\text{ }^{\circ}\text{C}$ + 7 days exposure + nine days acclimatization to $-5\text{ }^{\circ}\text{C}$; see Fig. 6.2). However, *P. digoneutis* already suffers mortality when exposed to temperatures above $-20\text{ }^{\circ}\text{C}$ and thus, some parasitoids may already have died during the acclimatization process. It is not known how fast *Peristenus* parasitoids can respond to falling temperatures, but it seems possible that a relatively short acclimatization period would result in a higher survival of parasitoids when exposed to $-20\text{ }^{\circ}\text{C}$. No survival was observed in parasitoids which were slowly acclimatized and then kept at $-20\text{ }^{\circ}\text{C}$ for two weeks. In contrast 7% of *P. digoneutis* and 32% of *P. stygicus* adults survived when directly exposed to $-21\text{ }^{\circ}\text{C}$ for two weeks. These results indeed suggest that the prolonged exposure to subzero temperatures due to the experimental acclimatization process caused an increased mortality.

It has been demonstrated that an exposure of *Peristenus* parasitoids to natural winter temperatures (of northern Germany) with a total of 48 days with daily mean temperatures between 0 and $-10\text{ }^{\circ}\text{C}$ does not negatively affect the overwintering parasitoids. Consequently, the parasitoids are well adapted to the winter temperatures of their area of origin. However, outside temperatures rarely dropped below $-5\text{ }^{\circ}\text{C}$ and the longest period of continuous subzero temperatures was only nine days. The successful survival of *Peristenus* under natural winter temperature conditions corresponds greatly with results from laboratory studies which show that *P. digoneutis* and *P. stygicus* hardly suffer mortality due to a $-5\text{ }^{\circ}\text{C}$ exposure for as long as three weeks.

6.4.2 Consequences for biological control

The survival of overwintering *Peristenus*, a crucial factor affecting the successful establishment of the biocontrol agent in Canada, is likely influenced by the climatic conditions in the potential release areas. A key factor may be the winter soil temperature. Since *Lygus* spp. are an increasing problem in canola in Alberta and Saskatchewan (Mason & Soroka, 1998), soil temperatures analysed by Turnock et al. (1983) and Lamb et al. (1985) for the canola growing area in the Canadian prairie provinces can be used to assess the potential for successful overwintering (and establishment) of *Peristenus* in *Lygus* outbreak areas.

In the present study, it has been demonstrated that the majority of overwintering *P. digoneutis* would most likely die due to a prolonged exposure to temperatures of -15°C or lower. Consequently, the most promising regions for a successful establishment of *P. digoneutis* would be the canola producing areas in Alberta, where soil temperatures of -10°C or lower (5 cm depth) only occur on one to three days per year (Fig. 6.8; Lamb et al., 1985). In particular, the outbreak area in southern Alberta (Vulcan area) is likely suitable for releasing *P. digoneutis*. Furthermore, the canola growing areas in eastern Saskatchewan and around Winnipeg seem promising. However, in the area of central Saskatchewan overwintering of *P. digoneutis* may be more critical due to the high number of days with soil temperatures of -10°C or lower. Although, even in an area with 22 days of low soil temperatures (e.g. Saskatoon) parasitoids would not be exposed to low temperatures for more than 22 days continuously. In reality, temperatures generally fluctuate within short periods of time and thus, the periods of low temperatures the parasitoids are exposed to would be much shorter. In the case of *P. stygicus*, the risk of overwintering mortality in potential release areas can be assumed as fairly

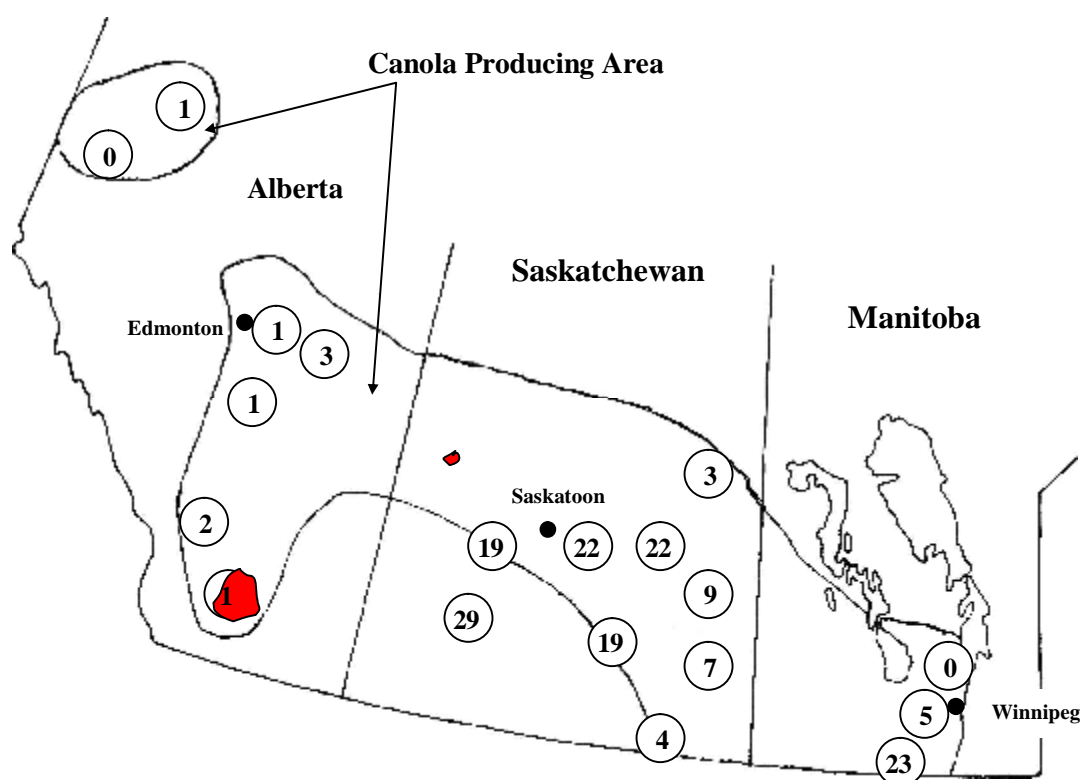


Figure 6.8 The average number of winter days (numbers in circles) with soil temperatures of -10°C or lower at 5 cm soil depth at climatological stations on the agricultural areas of the Canadian prairie provinces from 1969 to 1983 (modified after Lamb et al., 1985). Outbreak regions of *Lygus* spp. (1996-1997) are indicated by red areas within the canola growing area (modified after Mason & Soroka, 1998).

low, because even when exposed to $-15\text{ }^{\circ}\text{C}$ for several weeks more than 50% of the overwintering adults survived.

A snow cover on the soil surface is frequently an important factor in insect survival (Hayhoe & Mukerji, 1987). Turnock et al. (1983) found that soil temperatures in snow-free plots in Manitoba were much lower than in snow covered areas. At 2.5 and 5 cm depth the number of days during which the daily mean soil temperature was between $-15\text{ }^{\circ}\text{C}$ and $-20\text{ }^{\circ}\text{C}$ was 17 and 10 days, respectively (Tab. 6.2; Turnock et al., 1983). Consequently, in areas without snow cover the risk of overwintering mortality in *P. digoneutis* may be considerable. In contrast, the daily mean soil temperature varied little and generally remained above $-5\text{ }^{\circ}\text{C}$ throughout the winter in plots in which snow had accumulated to a depth of 20 to 40 cm and thus, the insulation effect of snow cover (Mackay & Mackay, 1974) would likely protect the overwintering parasitoids from harmful exposure to low temperatures. Even a relatively low snow cover can significantly enhance the survival of insects overwintering in the soil, as has been shown by Lamb et al. (1985) for overwintering pupae of the canola pest, *Mamestra configurata* (Lepidoptera: Noctuidae), in the Canadian prairie provinces. They found that there was a higher survival of pupae in soil (2 to 5 cm depth) covered by deep snow than shallow snow. When snow depth exceeded 20 cm, pupal survival was higher than 90%, and almost zero when there was no snow cover (Turnock et al., 1983). Furthermore, at least in the area of Winnipeg, Manitoba, temperatures fall to $-20\text{ }^{\circ}\text{C}$ at 5 cm depth during only 5% of the winters.

Table 6.2 The relation between soil depth and the number of days during which the daily mean soil temperature was within $5\text{ }^{\circ}\text{C}$ ranges below $-5\text{ }^{\circ}\text{C}$ at Winnipeg, Manitoba at locations where the snow cover was kept at $< 2\text{ cm}$ (November 1st, 1979 – March, 31st, 1980) (after Turnock et al., 1983).

Temperature ($^{\circ}\text{C}$)	soil depth (cm)	
	2.5	5.0
- 5 to -10	50	49
-10 to -15	33	40
-15 to -20	17	10

In Schleswig-Holstein (Germany), *Peristenus* parasitoids are rarely exposed to longer periods of continuous subzero temperatures (maximum nine days in winter 2002/3). In contrast, in the province of Manitoba, soil temperatures between $0\text{ }^{\circ}\text{C}$ and $-5\text{ }^{\circ}\text{C}$ at 5 cm depth can last continuously from the end of November until mid-March in snow covered areas (Turnock et

al., 1983). Thus, overwintering parasitoids would be exposed to freezing temperatures for about 15 weeks. The results of the present study suggest that *P. digoneutis* would suffer severe mortality from a prolonged exposure to -5°C . However, the low survival of *P. digoneutis* when exposed to -5°C for longer than three weeks is not understood, as already mentioned above, and requires further investigation if conclusion are to be drawn on this phenomenon.

In eastern Canada, ongoing field cage experiments conducted at London, Ontario, indicate that *P. digoneutis* is able to overwinter successfully in southwestern Ontario (Bruce Broadbent, personal communication). Furthermore, it has been shown that *P. digoneutis*, after being released in New Jersey (U.S.A.), expanded its range northwards to southern Quebec. The comparison of the future northern expansion of *P. digoneutis* with climatic data from the area of expansion may help to estimate the potential for successful establishment of *P. digoneutis* in other regions of Canada.

Saunders & Hayward (1998) have pointed out that insects with a wide latitudinal distribution, e.g. *P. stygicus* and *P. digoneutis* (see chapter 2, Table 2.1), can show ecophysiological variations in low temperature survival. For this reason, it seems worthwhile to carry out comparative analyses on survival to identify the existence of 'locally adapted' strains (Hart et al., 2002). Sømme (1996) suggested that the presence of a species in areas of severe climatic conditions is actually the best demonstration of its ability to survive prolonged exposure at low temperatures. The results of the present study suggest that *P. stygicus* is obviously more resistant to low temperatures than *P. digoneutis* and thus, the different cold-hardiness of both species may explain why *P. stygicus* is recorded from central Scandinavia whereas *P. digoneutis* is absent in these regions (Rämert et al., unpublished). Since *P. digoneutis* does not occur in central Scandinavia, northern Germany is probably close to the northern limit of *P. digoneutis* distribution and thus, strains from northern Germany are likely better adapted to low temperatures than strains from Southern Europe, which were used for releases in the United States (Day, 1987; Pickett et al., 2002). For release of *P. stygicus*, northern German populations are promising, but populations from coastal Central Sweden may be more suitable for introduction into Canada. Nevertheless, before releasing *Peristenus* into Canada, the overwintering survival of *Peristenus* under natural winter temperatures in the area of potential introduction should be analysed. This is necessary since it is difficult to make direct comparisons between laboratory assessments of cold-hardiness and winter field

survival because of the different conditions experienced in the field, particularly fluctuating temperatures and humidity (McDonald et al., 2000).

7 General discussion and conclusions

7.1 Estimating the potential of European *Lygus* parasitoids for the biological control of *Lygus* spp. in Canada

An essential first step in a biological control program is to survey for natural enemies that could potentially be introduced into the areas where a pest occurs (Cock, 1986). If the target pest is introduced, then the search for natural enemies is usually conducted in the area of origin of the pest (“old associations”). However, native species can reach pest status when native natural enemies are absent or not effective in suppressing the pest population. In this case, surveys would be conducted either in regions where the pest species is present but is not considered a problem, or in regions where closely related species occur, in the hopes of finding natural enemies that may be able to expand their host range to include the target pest (“new associations”; Hokkanen & Pimental, 1984).

In Canada, where native plant bugs of the genus *Lygus* are a serious problem on a wide variety of crops, native natural enemies are considered relatively ineffective (Lachance, 2000; Broadbent et al., 2002). In Europe, *Lygus rugulipennis* holds nearly the same ecological niche as the economically important pest *Lygus lineolaris* in North America (Day, 1987) and thus parasitoids of the European *Lygus* species have been suggested as biological control agents for American *Lygus* populations (Drea et al., 1973; Day, 1987; Coutinot & Hoelmer, 1999). Following this approach of “new associations”, surveys for natural enemies were conducted in several European countries (Coutinot & Hoelmer, 1999). However, natural enemies of *Lygus* spp. have never been investigated in northern Germany, where *Lygus* spp. do not present a problem. To fill this gap, surveys for nymphal parasitoids of *Lygus* spp. were conducted in agricultural and natural habitats in Schleswig-Holstein (chapter 2). The two braconids *Peristenus digoneutis* and *P. stygicus* were the only nymphal parasitoids reared from field-collected samples. Both species are known to be widely distributed in Europe (Coutinot & Hoelmer, 1999). Surprisingly, *P. rubricollis*, a common parasitoid of *Lygus rugulipennis* in Poland (e.g. Bilewicz-Pawinska, 1982), was absent. On the other hand, the abundance of *P. stygicus*, which is said to be more common in southern Europe (Coutinot & Hoelmer, 1999; Broadbent et al., 2002), was unexpected.

If a variety of natural enemies that attack the target pest are found in the surveys, then the question arises which species is likely the best candidate for biological control? When searching for effective biological control agents, certain promising attributes are obvious prior to introduction. Among others, superior searching efficiency, high reproductive potential, high larval survival, balanced sex ratio, phenological synchrony with the pest, host specificity and the ability to adapt to the climatic conditions in the area of potential release have been postulated as desirable attributes of an ideal biological control agent (Doutt & DeBach, 1964; Waage & Hassell, 1982; Cock, 1986; Waage, 1990; Ehler, 1995; Sagarra et al., 2001). Basic information on the association of the target pest and the control agent can be obtained from field studies (e.g. level of parasitization in the host; abundance; habitat preferences; synchronization of host and control agent; occurrence in the field; number of generations per year; see chapter 2). Other characteristics of potential biological control agents (e.g. cold-hardiness and reproductive potential; see chapter 5 and 6) may be easier to investigate in laboratory studies.

Field studies on the *Lygus* parasitoids *P. digoneutis* and *P. stygicus* (chapter 2) demonstrated that both parasitoids, because of their frequency and their levels of parasitization caused, have the potential to become important agents for the biological control of *Lygus* spp. in Canada. Both species are at least partly bivoltine (i.e. some cocoons remain in diapause until the following season) and are well synchronized with the occurrence of early nymphal instars of their host, *L. rugulipennis*, in agricultural (clover fields) and natural habitats (camomile stands). Furthermore, the present study suggests that the highest impact on *Lygus* spp. is obtained in constant environments, where the bivoltine parasitoids can complete their entire annual life cycle (two generations). Interestingly, *P. digoneutis* is clearly dominant in clover fields whereas in camomile stands the proportions of *P. digoneutis* and *P. stygicus* were not significantly different. These findings indicate that the habitat or the host plants may have an influence on the host-searching behavior of the parasitoids. The chemical cues involved in these interactions are unclear, and further investigation is required to elucidate their role in host and habitat location by *Peristenus* spp..

In general, the reproductive rate (a combination of fecundity and life cycle duration) of the control agent should be higher than that of the pest, otherwise the agent will be unable to suppress a pest increase (Cock, 1986). In laboratory tests (chapter 5), it has been demonstrated that (under optimal conditions) *P. stygicus* has the potential to parasitize 50% more *Lygus*

nymphs than *P. digoneutis*. In comparison to the life time fecundity of the North American target species *Lygus lineolaris* (82 to 97 eggs; Bariola, 1969; Khattat & Stewart, 1977; Snodgrass & McWilliams, 1992), *P. digoneutis* (385 eggs) and *P. stygicus* (782 eggs) both have a higher reproductive potential. However, both bivoltine parasitoids are presumably efficient in suppressing *Lygus* populations only in areas where the target consistently has two generations. This is supported by the literature on the distribution of *Lygus* parasitoids in Europe (see chapter 2), which shows that in areas where *Lygus* is univoltine, *P. digoneutis* and *P. stygicus* do not occur. In northern Germany, the bivoltine parasitoids are well synchronized with the occurrence of the two nymphal generations of *L. rugulipennis*. However, the synchronization of European parasitoids and North American *Lygus* species cannot be predicted by field data from Europe, because climatic conditions in the area of release may be different, and the parasitoid-host association has to be regarded as a “new association” (Hokkanen & Pimentel, 1984; Lachance, 2000)

When considering the release of an exotic biological control agent into another country, it is essential that the control agent is as well adapted to the stresses of the winter season as their target pest species (Bale, 1991). For releases of *Peristenus* parasitoids in the United States, strains from Southern Europe were mainly used (Day, 1987; Pickett et al., 2002). However, for releases in more northern areas like Canada, *Peristenus* strains from Northern Europe have been recommended (Coutinot & Hoelmer, 1999), assuming that these would be better adapted to extreme winter conditions. Therefore, the cold-hardiness of *Peristenus* species obtained from field collections in northern Germany was investigated (chapter 6). It has been demonstrated that mortality of *P. digoneutis* (enclosed as adults in the overwintering cocoons) increases as temperature decreases and as exposure to low temperatures lengthens. At temperatures between -10 and -15 °C, the mortality of *P. digoneutis* increases remarkably, whereas *P. stygicus* suffers less mortality. The differences in the cold-hardiness of these two species may explain why *P. stygicus* is recorded from central Scandinavia whereas *P. digoneutis* is absent in this region (see chapter 2). Based on these data only, *P. stygicus* would most likely be more successful in controlling *Lygus* populations in outbreak areas where winter conditions are harsh (e.g. central Saskatchewan).

In the last decade, concerns about potential environmental risks (“non-target effects”) caused by the introduction of exotic arthropod biological control agents have increased and received public attention (Knight, 2001). Host specificity has been pointed out as the key to safety in

biological control (e.g. Nechols et al., 1992; Onstad & McManus, 1996; van Lenteren et al., 2003) and thus, host specificity of European *Lygus* parasitoids was investigated in laboratory and field studies (chapters 3 and 4). The present study may serve as an example to evaluate different approaches to risk assessment studies and to elucidate problems that may arise during the practical work.

A critical issue in laboratory host range studies is the selection of non-target species to test. In general, a procedure similar to the phylogenetic centrifugal method used for the evaluation of weed biological control agents has been suggested (Wapshere, 1974; van Lenteren et al., 2003) because it has been stated that native relatives of target species are most vulnerable to non-target effects (Pemberton, 2000). However, this approach requires that the degree of relatedness between the target and non-target species is well known, but often knowledge is insufficient (Nechols et al., 1992). According to the maximum fit cladogram of *Lygus* and its outgroup taxa (Schwartz & Foottit, 1998), in the present study, two species, *Lygocoris pabulinus* and *Liocoris tripustulatus*, were selected for laboratory host range testing due to their phylogenetic relatedness to the *Lygus* target host. Whereas *L. tripustulatus* was actually a very suitable host for *Lygus* parasitoids, *L. pabulinus* was less suitable for parasitoid development than other non-target species which were assumed to be less closely related to *Lygus*. These findings correspond with other studies which demonstrated that phylogenetic relatedness may not necessarily be a reliable indicator of host suitability (Barratt & Johnstone, 2001; Babendreier et al., 2003). However, this contradiction challenges the centrifugal approach for host selection and may require a broader range of test species.

In general, the aim of laboratory tests on host specificity is to estimate the potential ecological host range of the control agents in the area of release (e.g. Barratt et al., 1997). However, there have been concerns that in laboratory tests, it is difficult to accurately reproduce the factors that influence host searching and assessment behavior of a parasitoid in a natural environment (Nechols et al., 1992; Sands, 1993; Withers & Browne, 1998; Kuhlmann et al., 2000; Benson et al., 2003). To address the concerns regarding the reliability of laboratory tests, it has been suggested that laboratory observations should be used in combination with field studies, as the determination of a parasitoid's host range in the area of origin provides a basis for correctly interpreting physiological host range estimations made via laboratory testing (Onstad & McManus, 1996; Kuhlmann et al., 2000; Louda et al., 2003a). In the present study, laboratory tests were performed on potential non-target hosts that occur in the area of origin of *Lygus* parasitoids (chapter 3). One aim of these tests was to examine how well the ecological host

range (chapter 4) of the control agents can be reproduced in laboratory tests. As recommended in van Lenteren et al. (2003), sequential non-choice tests were followed by simultaneous choice tests.

Surprisingly, all seven potential alternative hosts offered in the laboratory tests were accepted by *P. digoneutis* and *P. stygicus*. However, the non-choice tests indicated the trend that, compared to *P. digoneutis*, *P. stygicus* is more likely to attack non-target hosts. In particular, it was observed that *P. digoneutis* attacks Stenodemini nymphs less frequently than Mirini nymphs. These trends were confirmed by the results of the choice tests, which showed that *P. digoneutis* has a stronger preference for *Lygus* hosts than *P. stygicus*. Therefore, this study is in agreement with similar studies, in which results from non-choice tests corresponded with results from choice tests (Duan & Messing, 2000; Zilahi-Balogh et al., 2002).

One aspect, which can hardly be assessed in laboratory tests, is the actual impact of biological control agents on non-target hosts in the field. The standard laboratory tests used in host specificity testing can only identify potential non-target hosts (“host suitability”), but they cannot predict the response of a non-target host population, variation of host abundance, host phenology, and environmental conditions in nature (Louda et al., 2003 b). Frequent attacks on non-target mirids by *P. stygicus* and *P. digoneutis* in laboratory tests suggest that direct effects on non-target species may be considerable. However, these predictions need to be supported by field-collected data on the actual or realized host range.

Based on the frequent attacks by *P. stygicus* and *P. digoneutis* on non-target mirids in the laboratory, it was expected that both parasitoids would be found in field-collected samples of the mirid species which were used in laboratory tests. Furthermore, it was assumed that *P. stygicus* would be recorded more often from these non-target hosts than *P. digoneutis*. In the case of *P. stygicus*, all non-target species tested in the laboratory were found to be suitable hosts in the field and thus, results from laboratory studies are in agreement with results from field studies. However, the physiological host range of *P. digoneutis* did not correspond with its ecological host range. Three mirid species belonging to the tribe Stenodemini (*Notostira elongata*, *Megaloceraea recticornis*, and *Leptopterna dolabrata*) that were regularly attacked in the laboratory tests, were not recorded as suitable hosts in the field. The laboratory choice and non-choice tests only indicated a trend that *P. digoneutis* would attack Stenodemini less often, but they did not suggest the total absence of the parasitoid in the three Stenodemini species collected in the field. Thus, the evaluation of the physiological host range can lead to

an overestimation of the potential for non-target effects of *P. digoneutis*. However, based on the laboratory tests, *P. digoneutis* appeared to be more selective than *P. stygicus* when choosing hosts for oviposition. This assumption is confirmed by the much smaller number of alternative hosts (7) recorded for *P. digoneutis* from field collections when compared with *P. stygicus*, which was reared from 16 alternative hosts. Field studies clearly indicate that despite observed attacks in the laboratory, the impact of *P. digoneutis* on non-target mirids in nature is negligible.

Furthermore, the ecological host range of biological control agents in their area of origin may be a good predictor for the ecological host range of the agents in the area of release. Day (1999) demonstrated that *P. digoneutis*, after being released in the eastern U.S., behaved similar as observed in Europe. *Peristenus digoneutis* actually attacked three non-target hosts in the area of release, but the impact on these species was negligible. However, these studies were primarily conducted in agricultural habitats and thus, the potential impact on non-target species in natural habitats is unclear.

It has been stressed that conclusions concerning host specificity can rarely be based solely on field data from the area of origin (van Lenteren et al., 2003). This is because some species that were assumed to be monophagous in the area of origin were later found to attack more than one host in the area of release (Barratt et al., 1997). Partly, this contradiction may result from ecological host range studies that were limited to a relatively small area within the distribution of the control agent. The reasons why the ecological host range of a biological control agent can be different when estimated in different areas of the agent's distribution have been discussed in chapter 4 (e.g. overlap of occurrence of the biological control agent and non-targets in the field, number of generations of the non-target). However, the chance of underestimating the potential for non-target effects can be decreased when studies on the ecological host range are expanded to different regions within the area of distribution of the biological control agent. In the case of *Lygus* parasitoids, data are already available from Poland (Bilewicz-Pawinska, 1982), northern Germany and more information will be obtained from collections in Switzerland and southern Germany (see 7.3). Therefore, the ecological host range of *Lygus* parasitoids in the area of origin will likely be a good indicator for host specificity of European *Lygus* parasitoids. These data, however, must be considered together with laboratory tests on mirids native to the area of introduction.

The quality of the host specificity assessment based on the ecological host range is further dependent on the number of potential non-target hosts in the area of origin of the biological control agent. In biological control programs in which the actual number of potential alternative hosts is very limited, it may be easy to assess the complete ecological host range of the biological control agent (as long as the non-target species are common in the field). However, in the case of *Lygus* parasitoids, the number of potential non-target hosts is immense and thus, it is likely impossible to consider the complete ecological host range. In general, the criteria that were proposed for selecting non-targets for laboratory tests can also be applied for collecting potential non-targets in the field. However, the likelihood of neglecting a non-target species that might be harmed by the biological control agent increases with the number of potential non-target hosts occurring in the area of investigation. A general problem in assessing the ecological host range is finding and collecting rare hosts. From a low number of individuals, only a few or no parasitoids will emerge and thus, data are most likely not meaningful. In the present study, nymphs of 39 non-target hosts have been investigated. However, 20 potential non-target species were only found sporadically, or only found in the adult stage and thus, it is not known if these species would be attacked by *Lygus* parasitoids. Nevertheless, the investigation of the ecological host range of *Peristenus* parasitoids clearly indicated that *P. stygicus* has a much higher potential to attack non-target hosts, as indicated by the laboratory tests. Thus, even when the ecological host range of a biological control agent is only partly known, it may still serve as a reliable indicator of host specificity.

A problem that will appear during almost every study on the ecological host range of biological control agents is the lack of adequate rearing protocols for field-collected non-target hosts. In the case of mirids, suitable rearing protocols were only available for *Lygus* hosts, and these were only partly applicable to rearing non-target hosts. Therefore, the lack of information on optimal rearing conditions occasionally resulted in increased rearing mortality. Consequently, the loss of mirid hosts during the rearing process, combined with the overwintering mortality of parasitoid adults, resulted in the requirement for larger collections of nymphs to obtain meaningful numbers of parasitoid adults from each non-target host. Molecular diagnostic tools, which have been developed for European *Peristenus* parasitoids (Gariépy et al., in preparation), may present a good alternative for future investigations on host specificity, because non-target samples can be analyzed without time-consuming rearing and overwintering processes. However, this approach can only indicate if a non-target species is attacked by the biological control agent, but it does not show if it is a suitable host for

successful development. Thus, the rearing approach cannot be replaced completely when assessing the ecological host range via molecular diagnostics.

Although the importance of host specificity of biological control agents has been pointed out frequently (e.g., DeBach 1964; Waage & Greathead, 1988; Nechols et al., 1992), biological control programs following the “new association” approach already imply that the natural enemy is not absolutely specific (= “monophagous”) to the target. Consequently, this leads to the difficult question regarding which degree of host specificity is acceptable in biological control agents? Only recently, van Lenteren et al. (2003) published a first attempt that allows the quantification of the risk of biological control agents (used in *inundative* biological control) by different categories (establishment in non-target habitat; dispersal potential; host range; direct and indirect effects on non-targets). As stated by the authors, some of the principles and approaches also apply for classical biological control. However, in classical biological control, the ability of a biological control agent to self-disperse is a desired feature (for *P. digoneutis*, see Day et al., 1998, 2000), whereas in inundative biological control (= mass releases of control agents for short-term pest control without achieving continuing impact or establishment) it is not. Therefore, the approach by van Lenteren et al. (2003) needs to be adapted to fit in a classical biological control context. The ability of *P. digoneutis* to disperse in the eastern United States has been reported by Day et al., (1998; 2000). For *P. stygicus*, van Steenwyk & Stern (1977) stated that it seems to disperse poorly. The potential of *P. digoneutis* and *P. stygicus* to establish in non-target habitats (natural habitats) has been demonstrated in chapter 2. Both species are frequently found in weedy areas (dominated by camomile) and thus, the parasitoids are likely to invade natural habitats in the area of release, too. As previously discussed, in the area of origin *P. stygicus* has a much broader host range than *P. digoneutis*, suggesting that *P. stygicus* has a higher potential to attack non-target hosts (“direct effects”) in the area of release. The high lifetime fecundity of *P. stygicus* (see chapter 5) supports these concerns, because parasitoids with high egg loads are more likely to oviposit in nearly every host they encounter, including hosts in which the probability of offspring survival is small (Godfray, 1994). Furthermore, preliminary results from laboratory tests conducted in Canada suggest that *P. stygicus* is more likely to attack native mirids in Canada than *P. digoneutis* (Mason et al., unpublished a). However, as previously mentioned, a large number of alternative hosts (as determined in laboratory studies) only indicates an increased potential for non-target effects, but it does not predict the actual effects on non-target populations in the field. The impact of *Lygus* parasitoids on non-target species in the area of

origin is likely negligible (see chapter 4), but it may be different when European *Lygus* parasitoids are confronted with the North American mirid fauna. Furthermore, in the present study it has been shown that all *Lygus* species native to the parasitoid's area of origin, e.g. *L. rugulipennis*, *L. pratensis*, and *L. maritimus*, were frequently attacked. In addition, four North American target species, e.g. *L. lineolaris*, *L. keltoni*, *L. elisius*, and *L. shulli*, are suitable hosts for *P. stygicus* and *P. digoneutis* (Day, 1996; Pickett et al., 2002; Mason et al., unpublished a). Consequently, there is little doubt that all *Lygus* species native to Canada, including those with and without pest status, could serve as alternate hosts for the European parasitoids. The interspecific competition ("indirect effect") between European *Lygus* parasitoids and parasitoids native to Canada was investigated by Lachance et al. (2001). In nearly all tests, *P. digoneutis* and *P. stygicus* were better competitors than the native parasitoids. However, the risk of reducing a native parasitoid species by the introduction of exotic parasitoids has been estimated as low because North American *Lygus* parasitoids (*Peristenus pseudopallipes* and *Leiophron lygivorus*) are primarily found in weedy areas, whereas *P. digoneutis* and *P. stygicus* were assumed to be more adapted to search in alfalfa (Lachance et al., 2001). However, the abundance of *P. stygicus* and *P. digoneutis* in weedy areas (e.g. camomile) in Schleswig-Holstein questions this conclusion.

Overall, it may be stated that from the current point of view, *P. stygicus* possesses many desirable qualities for release (e.g. high reproductive potential, distinct cold-hardiness); however, with regards to potential environmental risks, *P. digoneutis* is more favorable for introduction into Canada. The promising potential of *P. digoneutis* for biological control is further indicated by the recent success of *P. digoneutis* in decreasing *Lygus* densities on alfalfa in the U.S. (Day, 1996).

7.2 Conclusions

The following conclusions can be drawn from the studies on host specificity and biological parameters of the potential biological control agents *P. digoneutis* and *P. stygicus*:

- § Studies on *Lygus rugulipennis* in Schleswig-Holstein indicate that because of their frequency and the levels of parasitization observed, the *Lygus* parasitoids *P. digoneutis* and *P. stygicus* have the potential to become important agents for the biological control of *Lygus* spp. in Canada.

- § Investigation on clover and camomile habitats support the assumption that *P. digoneutis* is more suitable than *P. stygicus* to control *Lygus* spp. in leguminous crops.
- § A review of the literature on the distribution of *P. stygicus* and *P. digoneutis* in Europe indicates that the distribution of *P. digoneutis* is likely limited to southern Scandinavia and Continental Europe, whereas *P. stygicus* occurs further north. *Peristenus rubricollis* is absent in northern Germany.
- § Furthermore, the occurrence of *P. digoneutis* and *P. stygicus* in Europe is dependent on the occurrence of two nymphal *Lygus* generations per year. Therefore, the establishment of European *Lygus* parasitoids in Canada will only be successful in areas where *Lygus* spp. have a constant number of two nymphal generations per year.
- § The present study suggests that the highest impact of *P. stygicus* and *P. digoneutis* is obtained in constant environments, where the bivoltine parasitoids can complete their entire annual life cycle (two generations).
- § *Peristenus stygicus* and *P. digoneutis* occur frequently in agricultural habitats (clover fields) as well as in natural, weedy habitats (camomile stands). Thus, both parasitoid species have the potential to disperse into non-target habitats if introduced into Canada.
- § Laboratory tests on host specificity in combination with investigation on the ecological host ranges of *P. digoneutis* and *P. stygicus* indicate that *P. stygicus* has a higher potential to cause non-target effects than *P. digoneutis*.
- § Low proportions of *P. digoneutis* and *P. stygicus* in the total number of parasitoids emerged from non-target mirids collected in the field indicate that *Lygus* parasitoids have a minor impact on most non-target species in Schleswig-Holstein.
- § Furthermore, laboratory tests on host specificity of *P. digoneutis* show that risk assessment based on laboratory tests, exclusively, can lead to the overestimation of the potential for non-target effects of the biological control agent.
- § The results of the present study suggest that the majority of non-target mirid species have a dominant parasitoid species, with *P. digoneutis* and *P. stygicus* being mainly dominant in *Lygus*. *Peristenus* spp. that develop from more than one host are usually restricted to one mirid genus or to mirid hosts that belong to different genera but occur simultaneously within the same habitat.
- § Studies on the parasitoid complexes of *Lygus* spp. in Schleswig-Holstein suggest that, upon introduction, *P. stygicus* and *P. digoneutis* have the potential to attack all *Lygus* species native to Canada, including those without pest status. This is particularly likely

if there is temporal or spatial overlap between pestiferous and non-pestiferous *Lygus* species.

- § It could be demonstrated that *P. stygicus* has the potential to parasitize 50% more *Lygus* nymphs than *P. digoneutis*. However, in the case of *P. stygicus*, because of its higher life time fecundity, the risk of non-target effects is assumed to be higher than in *P. digoneutis*.
- § The northern distribution of *P. stygicus* and its distinct cold-hardiness indicate that *P. stygicus* is more suitable than *P. digoneutis* to survive in areas of Canada where winter conditions are harsh.

7.3 Outlook

In order to implement non-target risk assessment studies of European *Lygus* parasitoids, a molecular diagnostic tool has been developed to identify *Peristenus* species in non-target hosts from additional areas in Europe (southern Germany, Switzerland). Such an approach is advantageous because it eliminates the need for the time consuming rearing process of field collected mirid nymphs (Garipey et al., unpublished). Furthermore, the molecular tool is considered for post-release monitoring studies to assess the parasitoid establishment, distribution and ecological host range in the area of introduction. In southern Quebec, where *P. digoneutis* has already established after being released in the U.S., the ecological host range of *P. digoneutis* will be investigated to clarify whether the impact on non-target species in these region is as low as in Europe (Mason et al., unpublished, b). Non-target risk assessment in the area of origin, and studies assessing the displacement of native *Lygus* parasitoids in the area of introduction, would be facilitated by molecular techniques.

Studies on *Lygus* parasitoids in high elevation and sub-alpine habitats will be conducted in Switzerland to address possible elevation limits of the parasitoids and to determine if non-target mirids of concern in sensitive habitats could serve as hosts for the European parasitoids in British Columbia (Haye et al., unpublished). Furthermore, in areas where the release of European *Peristenus* species is considered, the ecological importance of *Lygus* species without pest status should be investigated.

In addition to host specificity investigations in Europe, physiological host range studies are

currently in progress in Canada to determine if species of native Miridae are likely to serve as non-target hosts for *P. stygicus* and *P. digoneutis* (Mason et al., unpublished a).

Studies on the cold-hardiness of *Peristenus* parasitoids suggest that it would be helpful to develop a model (e.g. CLIMEXTM model, see Dossall et al., 2002; Mason et al., 2003; Olfert et al., 2004) which predicts the potential distribution of European *Peristenus* parasitoids after release in Canada. The model should be based on the knowledge regarding the distribution of the parasitoids in Europe. Furthermore, data on the current distribution of *P. digoneutis* following its release in the eastern United States can be integrated. A model for the potential distribution of *Lygus* parasitoids in combination with survival experiments under natural winter conditions in Canada would be a good tool to predict whether the European *Lygus* parasitoids have the potential to establish in *Lygus* outbreak areas.

8 Summary

In Canada, native plant bugs of the genus *Lygus* (Heteroptera: Miridae) are serious pests of a wide variety of agricultural crops. *Lygus* spp. outbreaks in western Canada have renewed interest in introducing European *Lygus* parasitoids. The intention of this thesis was to provide information on the host specificity of *Lygus* parasitoids belonging to the genus *Peristenus* (Hymenoptera: Braconidae), in an effort to assess potential risks for introducing these European parasitoids as biological control agents of native *Lygus* species in Canada. In a first step, investigations were made on the distribution, seasonal abundance and synchronization of *Lygus* spp. and their associated nymphal parasitoids, *P. stygicus* and *P. digoneutis*, in camomile and clover habitats in Schleswig-Holstein. In addition, the physiological host range of the parasitoids was defined using laboratory tests. The results were compared with data on the ecological host range of the parasitoids, obtained from extensive field studies in the area of investigation (Schleswig-Holstein, Germany). In addition, lifetime fecundity and cold-hardiness of the parasitoids were studied to guide the choice of parasitoid species for possible introduction, and to improve the chances of successful parasitoid establishment.

In Schleswig-Holstein, *Lygus* spp. are parasitized by the bivoltine nymphal parasitoids *P. digoneutis* and *P. stygicus*. Occasionally, larvae of *Peristenus* are hyperparasitized by the ichneumonid *Mesochorus curvulus*. The occurrence of the parasitoid adults is well synchronized with the occurrence of early instar *Lygus* nymphs in the field. The common host, *L. rugulipennis*, has two nymphal generations per year, the first of which is generally characterized by a lower parasitization level (4-27%). On average, the level of parasitization in the second nymphal generation is above 32%, occasionally reaching more than 60%. *Peristenus digoneutis* dominates in clover habitats whereas in camomile stands, *P. stygicus* and *P. digoneutis* appear with the same frequency.

Physiological host range studies demonstrated that *P. stygicus* and *P. digoneutis* attack non-target mirids consistently under laboratory conditions and that both parasitoids are able to develop in hosts other than *Lygus*. *Peristenus stygicus* and *P. digoneutis* were capable of attacking the seven selected non-target species they were presented with in laboratory non-choice and simultaneous choice tests. Closely related non-target mirids belonging to the tribe Mirini were generally well accepted by the parasitoids, while mirids of the tribe Stenodemini were less frequently attacked and less suitable for parasitoid development. When non-target

nymphs were offered simultaneously with *Lygus* nymphs, *P. digoneutis* showed a stronger preference for the target nymphs than *P. stygicus*.

Ecological host range studies on *Peristenus* spp. in Schleswig-Holstein indicate that in addition to attacking three *Lygus* species, *P. stygicus* also attacks at least 16 non-target species, whereas *P. digoneutis* attacks only seven non-target species. *Peristenus stygicus* and *P. digoneutis* were reared from field-collected non-target mirids; however, their proportion in comparison to the total number of parasitoids that emerged from non-target hosts is relatively low. The comparison of the ecological and physiological host range of *P. stygicus* and *P. digoneutis* suggests that *P. stygicus* is a generalist whereas *P. digoneutis* is assumed to be an oligophagous parasitoid. Among each genus of non-target mirids, little overlap of parasitoid species occurs. Almost each mirid genus has a dominant parasitoid species, with *P. digoneutis* and *P. stygicus* being mainly dominant in *Lygus*. *Peristenus* spp. that develop from more than one host species are usually restricted to one mirid genus or to mirid hosts that belong to different genera but occur simultaneously within the same habitat. In total, 19 species of *Peristenus*, two species of *Leiophron* and one species of *Mesochorus* (hyperparasitoid of *Peristenus* spp.) were reared from nymphs of 27 non-target mirid species (20 genera). Furthermore, several new mirid-parasitoid associations have been identified.

The laboratory experiments demonstrated that the synovigenic parasitoids, *P. digoneutis* and *P. stygicus*, oviposit most actively within the first two weeks of their life span, with a maximum average daily oviposition rate after approximately five days. On average, *P. digoneutis* and *P. stygicus* survived 25 and 32 days, respectively. *Peristenus stygicus* females have an average total lifetime fecundity of 782 eggs, whereas the lifetime fecundity of *P. digoneutis* (385 eggs) is approximately 50% lower. Furthermore, the lifetime fecundity of the parasitoids is strongly dependent on the length of their oviposition period.

Peristenus stygicus and *P. digoneutis*, enclosed as adults in the overwintering cocoons, have supercooling points below -20 °C and are able to acclimatize to changing temperatures with an increase/decrease of their supercooling points. However, mortality occurs at temperatures above the supercooling point. The mortality of *P. digoneutis* increases as temperature decreases and as exposure to low temperatures lengthens. Furthermore, *P. stygicus* is most likely better adapted to low temperatures than *P. digoneutis*.

Host specificity, fecundity, and cold hardiness of *P. stygicus* and *P. digoneutis* parasitoids are discussed in the context of biological control of *Lygus* populations native to Canada.

9 Zusammenfassung

Alljährlich verursachen Weichwanzen der Gattung *Lygus* (Heteroptera: Miridae) große Schäden an einer Vielzahl landwirtschaftlicher Nutzpflanzen in Kanada. Massenaufreten von *Lygus* spp. im Westen Kanadas haben das Interesse an der Einfuhr europäischer *Lygus*-Parasitoiden geweckt. In der vorliegenden Arbeit wurde die Wirtsspezifität von *Lygus*-Parasitoiden der Gattung *Peristenus* (Hymenoptera: Braconidae) untersucht, um mögliche Risiken bei der Einfuhr europäischer Parasitoiden zur biologischen Schädlingsbekämpfung der in Kanada heimischen *Lygus*-Populationen abschätzen zu können. Zunächst galt es, die Verbreitung, das saisonale Auftreten sowie die Synchronisation von *Lygus* spp. und der mit ihnen assoziierten Larvalparasitoiden, *P. stygicus* und *P. digoneutis*, an verschiedenen Kamille- und Kleestandorten in Schleswig-Holstein zu untersuchen. Anschließend wurde das physiologische Wirtsspektrum dieser Parasitoiden in Laborversuchen bestimmt und mit dem ökologischen Wirtsspektrum beider Arten verglichen, das durch umfangreiche Stichprobenentnahmen von Nymphen im Freiland ermittelt wurde. Um die Auswahl eines besonders geeigneten Parasitoiden für die potenzielle Einfuhr nach Kanada zu erleichtern und die Aussichten einer erfolgreichen Ansiedlung zu erhöhen, wurde die Fekundität und die Kälteresistenz der Parasitoiden untersucht.

Die in Schleswig-Holstein vorkommenden *Lygus* Arten werden vorwiegend von den bivoltinen Larvalparasitoiden *P. digoneutis* und *P. stygicus* parasitiert, deren Larven gelegentlich durch die Ichneumonide *Mesochorus curvulus* hyperparasitiert sind. Das Erscheinen der Parasitoiden-Imagines im Freiland ist exakt an das jahreszeitliche Auftreten der frühen Larvalstadien ihrer Wirte angepasst. Der mit Abstand häufigste Wirt, *Lygus rugulipennis*, bringt zwei Nymphengenerationen pro Jahr hervor, von denen die erste generell weniger stark parasitiert ist (4-27%). In der zweiten Nymphengeneration beträgt der Grad der Parasitierung im Durchschnitt mehr als 32%, kann aber gelegentlich mehr als 60% erreichen. *Peristenus digoneutis* dominiert vor allem in Kleefeldern, wohingegen an Kamillestandorten beide Arten in der Regel gleichermaßen häufig anzutreffen sind.

Untersuchungen zum physiologischen Wirtsspektrum von *P. stygicus* und *P. digoneutis* ergaben, dass beide Parasitoiden unter Laborbedingungen auch Nicht-Ziel-Arten (Miriden, die nicht der Gattung *Lygus* angehören) angreifen und fähig sind, sich in den Nymphen dieser Wirte erfolgreich zu entwickeln. Im Labor parasitierten *P. stygicus* und *P. digoneutis* alle

sieben der ihnen in Wahl- und Nicht-Wahl-Versuchen angebotenen Nicht-Ziel-Arten. Dabei wurden nah verwandte Nicht-Ziel-Arten, die dem Tribus Mirini angehören, im hohen Masse akzeptiert, wohingegen Miriden vom Tribus Stenodemini seltener angegriffen wurden und sich zudem als weniger geeignet für die Entwicklung der Parasitoiden erwiesen. Wenn den Parasitoiden *Lygus*-Nymphen gleichzeitig mit Nymphen von Nicht-Ziel-Arten angeboten wurden, zeigte *P. digoneutis* eine deutlich stärkere Präferenz für *Lygus*-Nymphen als *P. stygicus*.

Untersuchungen zum ökologischen Wirtsspektrum von *Peristenus* spp. in Schleswig-Holstein ergaben, dass neben den drei heimischen *Lygus*-Arten noch mindestens 15 weitere Nicht-Ziel-Arten von *P. stygicus* parasitiert werden, während für *P. digoneutis* nur sieben Alternativwirte nachgewiesen werden konnten. Obwohl *P. stygicus* und *P. digoneutis* aus zahlreichen im Freiland gesammelten Nicht-Ziel-Arten gezüchtet wurden, war ihr Anteil an der Gesamtzahl aller aus ihnen geschlüpften Parasitoiden relativ gering. Auf Grund des Vergleichs der physiologischen und ökologischen Wirtsspektren beider Parasitoiden-Arten, kann *P. stygicus* eher als Generalist und *P. digoneutis* als oligophager Parasitoid eingestuft werden. Zwischen den einzelnen Miriden-Gattungen konnte jeweils nur eine geringere Überschneidung der Parasitoidenkomplexe festgestellt werden. Nahezu jede Miriden-Art besitzt einen für sie typischen Parasitoiden, wobei *P. digoneutis* und *P. stygicus* charakteristisch für *Lygus* spp. sind. Insgesamt wurden 19 *Peristenus*, zwei *Leiophron* und eine *Mesochorus*-Art (= Hyperparasitoid von *Peristenus* spp.) aus 27 Nicht-Ziel-Arten (20 Gattungen) gezogen. Weiterhin konnten zahlreiche bisher unbekannte Beziehungen zwischen Miriden und ihren Parasitoiden festgestellt werden.

In Laborversuchen ließ sich zeigen, dass die Weibchen der synovigenen Parasitoiden *P. stygicus* und *P. digoneutis* den größten Anteil ihrer Eier in den ersten zwei Lebenswochen ablegen. Die durchschnittliche, tägliche Eiablage rate war fünf Tage nach dem Schlupf der Parasitoiden am höchsten. Im Durchschnitt betrug die Lebensspanne von *P. digoneutis* 25 Tage und die von *P. stygicus* 32 Tage. Ein einzelnes *P. stygicus* Weibchen legt durchschnittlich 782 Eier, während die *P. digoneutis* Weibchen mit durchschnittlich 385 Eiern eine um 50% geringere Fekundität aufweisen. Die Höhe der Fekundität beider Arten hängt im starkem Maße von der Länge der Eiablageperiode ab.

Peristenus stygicus und *P. digoneutis* überdauern die Wintermonate als Adulte in einem von ihren Larven angefertigten Kokon. Die Parasitoiden zeichnen sich dadurch aus, dass ihr

Unterkühlungspunkt unterhalb von -20 °C liegt und sie in der Lage sind, sich durch die Absenkung bzw. Anhebung ihrer Unterkühlungspunkte an wechselnde Temperaturen anzupassen. Trotz dieser Anpassungsfähigkeit stirbt die Mehrheit der Parasitoiden schon bei Temperaturen, die noch oberhalb des Unterkühlungspunktes liegen. Die Mortalität von *P. digoneutis* steigt um so mehr an, je stärker sich die Temperaturen dem Unterkühlungspunkt nähern und je länger sie den tiefen Temperaturen ausgesetzt sind. *Peristenus stygicus* scheint wesentlich besser an tiefe Temperaturen angepasst zu sein als *P. digoneutis*.

Aspekte der Wirtsspezifität, Fekundität und Kälteresistenz von *P. stygicus* und *P. digoneutis* werden im Zusammenhang mit dem möglichen Einsatz beider Parasitoiden zur biologischen Schädlingsbekämpfung in Kanada heimischer *Lygus*-Populationen diskutiert.

10 References

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Appendix

Table 1 Level of parasitization and parasitoid species composition in mass collections from camomile sites 2002–2003 (1st nymphal generation of *L. rugulipennis*); n = no. of parasitoids emerged; dominating plants: m = *Matricaria recutita*, t = *Tripleurospermum perforatum*; the proportion of a species is equivalent with the dominance index d (e.g. if the proportion of species a is 55%, then the dominance index for species a is 0.55).

date	site	# nymphs collected	% parasitization (dissect.)	# cocoons obtained	Species composition			
					n	% <i>P. stygicus</i>	% <i>P. digoneutis</i>	% <i>M. curvulus</i>
20 Jun. 02	Wulfshagen I (m)	660	16	48	40	55	43	2
5 Jul. 03	Heikendorf (m)	550	6	23	23	61	35	4
25 Jun. 03	Gettorf (m)	269	14	35	22	82	18	0
30 Jun. 03	Laboe (m)	700	4	11	9	33	56	11
30 Jun. 03	Lutterbek (m)	205	(0)	34	29	30	35	35
18 + 25 Jun. 03	Wulfshagen II (m)	1320	27	267	249	26	66	8
9 + 18 Jun. 02	Rastorf (m,t)	2929	11	300	274	88	8	4
14 + 21 Jun. 02	Sophienhof (m,t)	2341	6	131	104	53	44	3

Table 2 Level of parasitization and parasitoid species composition in mass collections from 14 clover fields 2001–2003 (2nd nymphal generation of *L. rugulipennis*); n = no. of parasitoids emerged; w = white clover, r = red clover, p = Persian clover; the proportion of a species is equivalent with the dominance index d (e.g. if the proportion of species a is 13%, then the dominance index for species a is 0.13).

date	site	# nymphs collected	% parasitization (dissect.)	# cocoons obtained	Species composition *			
					n	% <i>P. stygicus</i>	% <i>P. digoneutis</i>	% <i>M. curvulus</i>
22 Aug. 01	Rastorfer Passau (w)	1042	10	71	64	13	87	0
24 Aug. 01	Lindhöft I (w)	1074	6	53	42	40	60	0
24 Aug. 01	Lindhöft II (r)	938	42	116	73	23	75	2
14 Aug. 02	Lütjenbrode II (r)	5185	22	609	453	11	88	1
21 Aug. 02	Lindhöft III (r)	1000	16	82	69	0	94	6
3 Aug. 03	Busdorf (r)	600	56	270	50	40	56	4
6 Aug. 03	Krück (p)	180	59	71	50	16	84	0
12 Aug. 03	Schirnau (r)	400	62	43	43	28	67	5
12 Aug. 03	Osdorf (r)	600	58	270	50	0	100	0
17 Aug. 03	Satrup (r)	100	13	19	19	0	100	0
17 Aug. 03	Idstedt (r)	250	34	60	50	16	84	0
19 Aug. 03	Ausackerholz (r)	1000	28	167	50	24	76	0
19 Aug. 03	Estrup (p)	2000	38	281	50	18	82	0
20 Aug. 03	Burg a. F. (r)	375	12	23	23	48	52	0

Table 3 Level of parasitization and parasitoid species composition in mass collections from 12 camomile sites 2001–2003 (2nd nymphal generation of *L. rugulipennis*); n = no. of parasitoids emerged; dominating plants: m = *Matricaria recutita*, t = *Tripleurospermum perforatum*; the proportion of a species is equivalent with the dominance index d (e.g. if the proportion of species a is 70%, then the dominance index for species a is 0.70).

date	site	# nymphs collected	% parasitization (dissect.)	# cocoons obtained	Species composition			
					n	% <i>P. stygicus</i>	% <i>P. digoneutis</i>	% <i>M. curvulus</i>
14 Aug. 01	Rastorf I (m,t)	328	30	68	66	70	26	4
20 Aug. 01	Birkensee (t)	1117	14	167	147	67	22	11
30 Aug. 01	Silberstedt (t,m)	552	14	54	34	97	0	3
13 Aug. 02	Lindhöft IV (m)	653	27	140	49	57	43	0
17 Aug. 02	Molfsee (m)	586	17	83	64	28	64	8
9 Aug. 02	Sophienhof (t,m)	1187	63	504	297	56	31	13
10 Aug. 03	Schilksee (m)	1100	62	428	50	34	66	0
13 Aug. 03	Probsteierhagen (m)	6900	44	1955	50	18	74	8
13 Aug. 03	Heikendorf (m)	250	38	55	50	38	54	8
15 Aug. 03	Lindhöft V (m)	460	38	103	50	36	54	10
15 Aug. 03	Gettorf (m)	2830	48	793	50	8	88	4
17 Aug. 03	Stolk (t)	170	38	33	33	6	88	6

Table 4 Level of parasitization and parasitoid species composition in mass collections (2nd nymphal generation of *L. rugulipennis*) from Lütjenbrode (* for species composition only subsamples were incubated); n = no. of parasitoids emerged

date	# nymphs collected	% parasitization (dissect.)	# cocoons obtained	Species composition			
				n	% <i>P. digoneutis</i>	% <i>P. stygicus</i>	% <i>M. curvulus</i>
11 Aug. 01	4985	24	1090*	77	26	58	16
19 Aug. 01	20597	19	2071*	92	37	60	3
29 Aug. 01	7035	9	130	84	15	81	4
14 Aug. 02	5185	22	609	453	88	11	1

Table 5 Trellis diagrams: Comparison of camomile sites (mass collections, 1st nymphal generation of *L. rugulipennis*): a) RENKONEN's coefficient (above the diagonal line), JACCARD's coefficient (underneath the diagonal line), and b) K_W after Wainstein (mean RC = 0.69 ± 0.03 (SE); mean JC = 92 ± 3 (SE); mean K_W = 64 ± 4 (SE); dominating plants: m = *Matricaria recutita*, t = *Tripleurospermum perforatum*).

a)

	1	2	3	4	5	6	7	8
1		0.92	0.73	0.78	0.67	0.71	0.65	0.98
2	100		0.79	0.72	0.69	0.65	0.73	0.91
3	67	67		0.51	0.48	0.44	0.90	0.71
4	100	100	67		0.76	0.90	0.45	0.80
5	100	100	67	100		0.69	0.42	0.68
6	100	100	67	100	100		0.38	0.73
7	100	100	67	100	100	100		0.64
8	100	100	67	100	100	100	100	

b)

	1	2	3	4	5	6	7	8
1								
2	92							
3	49	53						
4	78	72	34					
5	67	69	32	76				
6	71	65	30	90	69			
7	65	73	60	45	42	38		
8	98	91	48	80	68	73	64	

1 = Wulfshagen I (m)
 2 = Heikendorf (m)
 3 = Gettorf (m)
 4 = Laboe (m)

5 = Lutterbek (m)
 6 = Wulfshagen II (m)
 7 = Rastorf (m,t)
 8 = Sophienhof (m,t)

Table 6 Trellis diagrams: Comparison of parasitoid communities at clover sites (mass collections, 2nd *Lygus* generation): a) RENKONEN's coefficient (above the diagonal line), JACCARD's coefficient (underneath the diagonal line), and b) K_W after Wainstein (Mean RC = 0.81 ± 0.01 (SE); mean JC = 69 ± 3 (SE); mean $K_W = 57 \pm 2$ (SE); w = white clover, r = red clover, p = Persian clover).

a)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1		0.73	0.88	0.98	0.87	0.69	0.97	0.80	0.87	0.87	0.97	0.89	0.95	0.65
2	100		0.83	0.71	0.60	0.96	0.76	0.88	0.60	0.60	0.76	0.84	0.78	0.92
3	67	67		0.87	0.77	0.81	0.91	0.92	0.75	0.75	0.91	0.98	0.93	0.75
4	67	67	100		0.89	0.68	0.95	0.79	0.88	0.88	0.95	0.87	0.93	0.63
5	33	33	67	67		0.60	0.84	0.72	0.94	0.94	0.84	0.76	0.82	0.52
6	67	67	100	100	67		0.72	0.84	0.56	0.56	0.72	0.80	0.74	0.92
7	100	100	67	67	33	67		0.83	0.84	0.84	1	0.92	0.98	0.68
8	67	67	100	100	67	100	67		0.67	0.67	0.83	0.91	0.85	0.80
9	50	50	33	33	50	33	50	33		1	0.84	0.76	0.82	0.52
10	50	50	33	33	50	33	50	33	100		0.84	0.76	0.82	0.52
11	100	100	67	67	33	67	100	67	50	50		0.92	0.98	0.68
12	100	100	67	67	33	67	100	67	50	50	100		0.94	0.76
13	100	100	67	67	33	67	100	67	50	50	100	100		0.70
14	100	100	67	67	33	67	100	67	50	50	100	100	100	

b)

	1	2	3	4	5	6	7	8	9	10	11	12	13	14
1														
2	73													
3	59	56												
4	66	48	87											
5	29	20	52	60										
6	46	64	81	68	40									
7	97	76	61	64	28	48								
8	54	59	92	79	48	84	56							
9	44	30	25	29	47	19	42	22						
10	49	30	25	29	47	19	42	22	100					
11	97	76	61	64	28	48	100	56	42	42				
12	89	84	66	58	25	54	92	61	38	38	92			
13	95	78	62	62	27	50	98	57	41	41	98	94		
14	65	92	50	42	17	62	68	54	26	26	68	76	70	

1 = Rastorfer Passau (w)
 2 = Lindhöft I (w)
 3 = Lindhöft II (r)
 4 = Lütjenbrode II (r)
 5 = Lindhöft III (r)
 6 = Busdorf (r)
 7 = Krück (p)

8 = Schirnau (r)
 9 = Osdorf (r)
 10 = Satrup (r)
 11 = Idstedt (r)
 12 = Ausackerholz (r)
 13 = Estrup (p)
 14 = Burg a. F. (r)

Table 7 Trellis diagrams: Comparison of chamomile sites (mass collections, 2nd *Lygus* generation): a) RENKONEN's coefficient (above the diagonal line), JACCARD's coefficient (underneath the diagonal line), and b) K_W after Wainstein (Mean RC = 0.65 ± 0.03 (SE); mean JC = 85 ± 2 (SE); mean K_W = 56 ± 3 (SE); dominating plants: m = *Matricaria recutita*, t = *Tripleurospermum perforatum*).

a)

	1	2	3	4	5	6	7	8	9	10	11	12
1		0.93	0.73	0.83	0.58	0.86	0.60	0.48	0.68	0.66	0.38	0.36
2	100		0.70	0.79	0.58	0.89	0.56	0.48	0.68	0.68	0.34	0.34
3	67	67		0.57	0.31	0.59	0.34	0.21	0.41	0.39	0.11	0.09
4	67	67	33		0.71	0.87	0.77	0.61	0.81	0.79	0.51	0.49
5	100	100	67	67		0.67	0.92	0.90	0.90	0.90	0.60	0.76
6	100	100	67	67	100		0.65	0.57	0.77	0.77	0.43	0.30
7	67	67	33	100	67	67		0.84	0.88	0.88	0.74	0.72
8	100	100	67	67	100	100	67		0.80	0.80	0.86	0.86
9	100	100	67	67	100	100	67	100		0.98	0.66	0.66
10	100	100	67	67	100	100	67	100	100		0.66	0.66
11	100	100	67	67	100	100	67	100	100	100		0.98
12	100	100	67	67	100	100	67	100	100	100	100	

b)

	1	2	3	4	5	6	7	8	9	10	11	12
1												
2	93											
3	49	47										
4	56	53	19									
5	58	58	21	48								
6	86	89	40	58	67							
7	40	38	11	77	62	44						
8	48	48	14	41	90	57	56					
9	68	68	28	54	90	77	59	80				
10	66	68	26	53	90	77	59	80	98			
11	38	34	7	34	76	43	50	86	66	66		
12	36	34	6	31	76	43	48	86	66	66	98	

1 = Rastorf I (m,t)
 2 = Birkensee (t)
 3 = Silberstedt (t,m)
 4 = Lindhöft IV (m)
 5 = Molfsee (m)
 6 = Sophienhof (t,m)

7 = Schilksee (m)
 8 = Probsteierhagen (m)
 9 = Heikendorf (m)
 10 = Lindhöft V (m)
 11 = Gettorf (m)
 12 = Stolk (t)

Table 8 No. of parasitoid adults emerged without diapause or entered diapause, when reared from the 1st nymphal generation of *L. rugulipennis* in 2002

date	site	No diapause - emerged in summer 2002			In diapause - emerged in 2003		
		<i>P. stygicus</i>	<i>P. digoneutis</i>	<i>M. curvulus</i>	<i>P. stygicus</i>	<i>P. digoneutis</i>	<i>M. curvulus</i>
9 Jun.02	Rastorf	151	13	0	60	3	0
14 Jun.02	Sophienhof	15	17	0	27	7	1
18 Jun.02	Rastorf	21	6	6	10	1	3
20 Jun.02	Wulfshagen	20	17	1	2	0	0
21 Jun.02	Sophienhof	13	22	0	0	0	2
	Total #	220	75	7	99	11	6

Table 9 Rearing data from sequential non-choice tests

Parasitoid species	Non-target species tested	1 st <i>Lygus</i> control						Non-target testing						2 nd <i>Lygus</i> control						
		# attacks observed	# cocoons reared	# adults reared	# parasitized nymphs (dissected)	# nonparasitized nymphs (dissected)	# dried out nymphs	# attacks observed	# cocoons reared	# adults reared	# parasitized nymphs (dissected)	# nonparasitized nymphs (dissected)	# dried out nymphs	# parasitoids emerged	# attacks observed	# cocoons reared	# adults reared	# parasitized nymphs (dissected)	# nonparasitized nymphs (dissected)	# dried out nymphs
<i>P. stygicus</i>	Lygus control	37	25	4	5	2	1	35	23	3	5	2	2	20	37	24	1	3	5	4
	<i>L. maritimus</i>	14	9	1	2	1	1	14	14	0	0	0	0	13	14	12	0	2	0	0
	<i>C. norwegicus</i>	34	26	2	2	0	4	32	18	0	4	4	6	15	34	25	1	2	3	3
	<i>L. pabulinus</i>	33	23	0	4	3	3	33	5	10	4	11	3	3	32	19	1	4	4	4
	<i>L. tripustulatus</i>	29	19	3	1	3	3	27	20	0	1	0	6	16	29	22	0	1	2	4
	<i>L. dolabrata</i>	31	22	0	3	0	6	21	6	2	5	8	0	3	31	19	0	4	1	7
	<i>S. calcarata</i>	53	36	8	4	2	3	36	12	2	8	9	5	8	53	32	3	4	4	10
	<i>N. elongata</i>	42	28	0	12	0	2	28	2	0	16	10	0	1	42	19	0	17	2	4
<i>M. relicticornis</i>	32	19	0	6	0	7	24	9	9	2	4	0	7	32	20	0	10	0	2	
<i>P. digoneutis</i>	Lygus control	22	17	3	1	0	1	21	13	1	5	1	1	11	22	15	1	3	0	3
	<i>L. maritimus</i>	29	26	0	3	0	0	27	23	0	2	1	1	20	27	21	1	2	2	1
	<i>C. norwegicus</i>	31	25	3	0	1	2	23	15	1	4	1	2	13	30	23	4	1	1	1
	<i>L. pabulinus</i>	31	28	0	3	0	0	29	3	14	1	1	10	2	31	20	0	10	1	0
	<i>L. tripustulatus</i>	32	24	4	2	1	1	22	12	4	1	0	5	10	32	19	3	5	1	4
	<i>L. dolabrata</i>	26	19	1	2	3	1	14	5	1	4	4	0	3	26	21	1	2	1	1
	<i>S. calcarata</i>	30	19	0	9	1	1	13	4	6	1	1	1	3	30	13	0	17	0	0
	<i>N. elongata</i>	46	35	0	4	5	2	16	4	3	7	0	2	4	45	31	1	10	0	3
<i>M. relicticornis</i>	35	29	4	1	0	1	10	0	6	1	3	0	0	35	19	2	9	0	5	

Table 10 Host acceptance of *P. stygicus* and *P. digoneutis* in sequential non-choice tests (n = number of females tested).

Test parasitoid species	Non-target species tested	n	No. nymphs attacked			Cochran's Q test	Mc Nemar tests (group wise)		
			1 st <i>Lygus</i> control	Non-target	2 nd <i>Lygus</i> control		<i>Lygus</i> 1- <i>Lygus</i> 2	<i>Lygus</i> 1-non-target	<i>Lygus</i> 2-nontarget
<i>P. stygicus</i>	Lygus control	37	37	35	37	ns	ns	ns	ns
	<i>Lygus maritimus</i>	14	14	14	14	ns	ns	ns	ns
	<i>Closterotomus norwegicus</i>	34	34	32	34	ns	ns	ns	ns
	<i>Lygocoris pabulinus</i>	33	33	33	32	ns	ns	ns	ns
	<i>Liocoris tripustulatus</i>	29	29	27	29	ns	ns	ns	ns
	<i>Leptopterna dolobrata</i>	31	31	21	31	P < 0.001	ns	P < 0.01	P < 0.01
	<i>Stenodema calcarata</i>	53	53	36	53	P < 0.001	ns	P < 0.001	P < 0.001
	<i>Notostira elongata</i>	42	42	28	42	P < 0.001	ns	P < 0.001	P < 0.001
	<i>Megaloceraea relicticornis</i>	32	32	24	32	P < 0.001	ns	P < 0.01	P < 0.01
<i>P. digoneutis</i>	Lygus control	22	22	21	22	ns	ns	ns	ns
	<i>Lygus maritimus</i>	29	29	27	27	ns	ns	ns	ns
	<i>Closterotomus norwegicus</i>	31	31	23	30	P = 0.001	ns	P < 0.01	P < 0.05
	<i>Lygocoris pabulinus</i>	31	31	29	31	ns	ns	ns	ns
	<i>Liocoris tripustulatus</i>	32	32	22	32	P < 0.001	ns	P < 0.01	P < 0.01
	<i>Leptopterna dolobrata</i>	26	26	14	26	P < 0.001	ns	P < 0.001	P < 0.001
	<i>Stenodema calcarata</i>	30	30	13	30	P < 0.001	ns	P < 0.001	P < 0.001
	<i>Notostira elongata</i>	46	46	16	45	P < 0.001	ns	P < 0.001	P < 0.001
	<i>Megaloceraea relicticornis</i>	35	35	10	35	P < 0.001	ns	P < 0.001	P < 0.001

Table 11 Numbers of attacks on non-target and *Lygus* hosts by *P. stygicus* and *P. digoneutis* that resulted in successful parasitoid development in sequential non-choice tests (n = number of females that attacked nymphs in all three runs).

Test parasitoid species	Non-target species tested	n	# of successful attacks			Cochran's Q test	Mc Nemar tests		
			1 st <i>Lygus</i> control	Non-target	2 nd <i>Lygus</i> control		<i>Lygus</i> 1- <i>Lygus</i> 2	<i>Lygus</i> 1-non-target	<i>Lygus</i> 2-nontarget
<i>P. stygicus</i>	Lygus control	25	23	23	22	ns	ns	ns	ns
	<i>Lygus maritimus</i>	13	11	13	13	ns	ns	ns	ns
	<i>Closterotomus norwegicus</i>	20	20	17	17	ns	ns	ns	ns
	<i>Lygocoris pabulinus</i>	24	22	8	19	P < 0.001	ns	P < 0.001	P = 0.001
	<i>Liocoris tripustulatus</i>	17	14	17	15	ns	ns	ns	ns
	<i>Leptopterna dolobrata</i>	13	13	7	13	P < 0.01	ns	P < 0.05	P < 0.05
	<i>Stenodema calcarata</i>	23	19	13	20	P < 0.01	ns	P < 0.05	P < 0.05
	<i>Notostira elongata</i>	27	27	19	27	P < 0.001	ns	P < 0.01	P < 0.01
	<i>Megaloceraea relicticornis</i>	17	17	9	17	P < 0.001	ns	P < 0.01	P < 0.01
<i>P. digoneutis</i>	Lygus control	21	20	19	20	ns	ns	ns	ns
	<i>Lygus maritimus</i>	23	23	22	20	ns	ns	ns	ns
	<i>Closterotomus norwegicus</i>	14	14	14	13	ns	ns	ns	ns
	<i>Lygocoris pabulinus</i>	19	19	4	19	P < 0.001	ns	P < 0.001	P < 0.001
	<i>Liocoris tripustulatus</i>	15	12	11	11	ns	ns	ns	ns
	<i>Leptopterna dolobrata</i>	13	10	8	11	ns	ns	ns	ns
	<i>Stenodema calcarata</i>	12	11	5	12	P < 0.01	ns	P < 0.05	P < 0.05
	<i>Notostira elongata</i>	12	10	10	11	ns	ns	ns	ns
	<i>Megaloceraea relicticornis</i>	9	8	1	9	P = 0.001	ns	P < 0.05	P < 0.01

Table 12 *P. stygicus*: Length of time until the first sting and duration of oviposition in non-choice tests (¹ = P-value for Kruskal-Wallis test; ² = P-values for group wise U-tests after Mann-Whitney; significant values in bold). (see next page)

P. stygicus		Length of time until the first sting (sec)			Duration of oviposition (sec)		
mirid species	parameter	Lygus (1)	Non-target (2)	Lygus (3)	Lygus (1)	Non-target (2)	Lygus (3)
<i>L. rugulipennis</i>	Mean ± SE	258 ± 55	116 ± 30	101 ± 24	9 ± 2	8 ± 1	9 ± 1
	Median (sec)	81	46	39	7	7	7
	Range (sec)	2-1157	15-885	1-616	2-67	3-17	2-40
	n	37	35	37	37	35	37
	P value ¹	0.616			0.313		
<i>L. maritimus</i>	Mean ± SE	130 ± 45	48 ± 10	82 ± 35	6 ± 1	6 ± 1	6 ± 1
	Median (sec)	48	27	56	5	5,5	6
	Range (sec)	12-513	10-100	6-524	2-14	3-11	3-13
	n	14	14	14	14	14	14
	P value ¹	0.147			0.639		
<i>L. pabulinus</i>	Mean ± SE	126 ± 23	70 ± 13	67 ± 19	8 ± 1	8 ± 2	12 ± 5
	Median (sec)	71	46	29	6	5	6
	Range	12-513	8-303	4-524	2-41	3-62	3-165
	n	33	33	32	33	33	32
	P value	0.048			0.512		
	M.-W. ²	1-2 0.048	1-3 0.006	2-3 0.222			
<i>L. tripustulatus</i>	Mean ± SE	237 ± 53	124 ± 40	121 ± 28	11 ± 1	6 ± 1	9 ± 1
	Median (sec)	145	33	42	8	5	6
	Range (sec)	18-1170	5-903	2-550	4-30	4-14	4-24
	n	29	27	29	29	27	29
	P value ¹	0,013			0,001		
	M.-W. ²	1-2 0.007	1-3 0.019	2-3 0.646	1-2 0.001	1-3 0.053	2-3 0.03
<i>C. norwegicus</i>	Mean ± SE	318 ± 45	221 ± 46	219 ± 49	17 ± 6	11 ± 3	7 ± 1
	Median (sec)	212	221	113	7	7	6
	Range (sec)	13-826	3-1160	13-1072	2-175	3-80	2-18
	n	34	32	34	34	32	34
	P value ¹	0.054			0.846		
<i>L. dolobrata</i>	Mean ± SE	329 ± 50	305 ± 49	218 ± 47	8 ± 1	13 ± 2	12 ± 1
	Median (sec)	255	115	89	7	11	10
	Range (sec)	33-1165	14-1140	7-696	2-24	4-30	5-40
	n	31	21	31	31	21	31
	P value ¹	0.064			0.01		
	M.-W. ²				1-2 0.008	1-3 0.015	2-3 0.47
<i>S. calcarata</i>	Mean ± SE	215 ± 36	126 ± 25	159 ± 28	15 ± 4	13 ± 2	15 ± 4
	Median (sec)	121	72	62	9	10	9
	Range (sec)	5-1138	3-696	1-943	3-162	4-47	2-200
	n	53	36	53	53	36	53
	P value ¹	0.267			0.186		
<i>N. elongata</i>	Mean ± SE	189 ± 24	123 ± 21	76 ± 14	7 ± 1	13 ± 2	7 ± 1
	Median (sec)	150	91	53	6	8	5
	Range (sec)	1-613	9-364	1-560	3-19	2-50	2-24
	n	42	28	42	42	28	42
	P value ¹	< 0.001			0.019		
	M.-W. ²	1-2 0.081	1-3 0.000	2-3 0.083	1-2 0.021	1-3 0.521	2-3 0.009
<i>M. recticornis</i>	Mean ± SE	212 ± 46	190 ± 48	92 ± 18	10 ± 2	11 ± 2	9 ± 1
	Median (sec)	112	71	52	7	8	8
	Range (sec)	7-899	2-777	5-409	3-67	3-38	3-45
	n	32	24	32	32	24	32
	P value ¹	0.038			0.52		
	M.-W. ²	1-2 0.32	1-3 0.009	2-3 0.24			

Table 13 *P. digoneutis*: Length of time until the first sting and duration of oviposition in non-choice tests (¹ = *P*-value for Kruskal-Wallice test; ² = *P*-values for group wise U-tests after Mann-Whitney; significant values in bolt).

<i>P. digoneutis</i>		Length of time until the first sting (sec)			Duration of oviposition (sec)		
mirid species	parameter	Lygus (1)	Nontarget (2)	Lygus (3)	Lygus (1)	Nontarget (2)	Lygus (3)
<i>L. rugulipennis</i>	Mean ± SE	256 ± 48	330 ± 80	327 ± 62	38 ± 12.6	5 ± 1.3	10 ± 3.1
	Median (sec)	228	128	190	9	3	4
	Range (sec)	37-848	12-1010	16-1106	2-202	2-25	2-55
	n	22	21	22	22	21	22
	P value ¹	0.858			0.006		
	M.-W. ²				1-2 0.003	1-3 0.032	2-3 0.189
<i>L. maritimus</i>	Mean ± SE	262 ± 57	186 ± 39	129 ± 37	3 ± 0.3	3 ± 0.4	3 ± 0.4
	Median (sec)	135	92	69	3	3	3
	Range (sec)	4-1179	5-834	9-915	2-6	2-14	2-13
	n	29	27	27	29	27	27
	P value ¹	0.243			0.589		
<i>L. pabulinus</i>	Mean ± SE	204 ± 36	190 ± 48	158 ± 35	3 ± 0.3	4 ± 0.4	4 ± 0.4
	Median (sec)	120	111	84	3	3	3
	Range (sec)	13-898	11-1191	13-872	1-7	1-12	2-9
	n	31	29	31	31	29	31
	P value ¹	0.401			0.640		
<i>L. tripustulatus</i>	Mean ± SE	301 ± 48	334 ± 61	187 ± 35	37 ± 10.7	17 ± 7.5	10 ± 4.0
	Median (sec)	237	237	149	8	4	3
	Range (sec)	24-1093	21-1007	9-902	2-273	2-123	2-110
	n	32	22	32	32	22	32
	P value ¹	0.052			0.007		
	M.-W. ²				1-2 0.06	1-3 0.002	2-3 0.3
<i>C. norwegicus</i>	Mean ± SE	325 ± 48	230 ± 59	312 ± 69	20 ± 6.2	12 ± 2.6	6 ± 0.9
	Median (sec)	249	100	113	7	5	5
	Range (sec)	13-922	3-1160	5-1138	2-175	2-80	2-18
	n	31	23	30	31	23	30
	P value ¹	0.197			0.228		
<i>L. dolobrata</i>	Mean ± SE	298 ± 62	312 ± 55	276 ± 63	14 ± 5.6	13 ± 7.1	4 ± 0.4
	Median (sec)	178	355	139	4	5	3
	Range (sec)	12-1144	9-638	2-1191	2-145	2-104	2-10
	n	26	14	26	26	14	26
	P value ¹	0.467			0.125		
<i>S. calcarata</i>	Mean ± SE	169 ± 36	191 ± 36	268 ± 47	6 ± 1.4	4 ± 0.7	3 ± 0.3
	Median (sec)	101	134	171	3	4	3
	Range (sec)	16-872	24-613	38-1063	2-36	1-9	1-8
	n	30	13	30	30	13	30
	P value ¹	0.07			0.028		
	M.-W. ²				1-2 0.824	1-3 0.006	2-3 0.167
<i>N. elongata</i>	Mean ± SE	311 ± 43	309 ± 75	203 ± 37	9 ± 3.7	4 ± 0.9	4 ± 0.3
	Median (sec)	210	232	125	3	3	3
	Range (sec)	13-1131	47-1109	16-1192	1-150	2-14	2-10
	n	46	16	45	46	16	45
	P value ¹	0.084			0.5		
<i>M. recticornis</i>	Mean ± SE	337 ± 47	276 ± 113	295 ± 50	31 ± 8.9	4 ± 0.5	17 ± 7.0
	Median (sec)	259	124	165	5	4	4
	Range (sec)	37-1069	23-1096	16-1086	2-202	2-7	2-199
	n	35	10	35	35	10	35
	P value ¹	0.353			0.087		

Table 14 Simultaneous choice tests: % attacks on *Lygus* and non-target nymphs; test statistic: Wilcoxon paired-sample test (n = 20/ non-target species).

Parasitoid species	non-target species	% attacks on <i>Lygus</i>	% attacks on non-target	P	Z	Sign.
<i>P. stygicus</i>	<i>L. pabulinus</i>	48.8	51.2	0.621	-0.494	ns
	<i>L. tripustulatus</i>	58.4	41.6	0.117	-1.568	ns
	<i>C. norwegicus</i>	63.4	36.6	0.006	-2.73	**
	<i>L. dolobrata</i>	80	20	0.001	-3.262	***
	<i>S. calcarata</i>	55.3	44.7	0.311	-1.013	ns
	<i>N. elongata</i>	72.3	27.7	0.003	-3.012	**
	<i>M. relicticornis</i>	59.4	40.6	0.175	-1.357	ns
<i>P. digoneutis</i>	<i>L. pabulinus</i>	56.6	43.4	0.305	-1.026	ns
	<i>L. tripustulatus</i>	65	35	0.029	-2.186	*
	<i>C. norwegicus</i>	70	30	0.035	-2.103	*
	<i>L. dolobrata</i>	84.6	15.4	0.001	-3.428	***
	<i>S. calcarata</i>	82.6	17.4	0.001	-3.272	***
	<i>N. elongata</i>	75.7	24.3	0.003	-2.933	**
	<i>M. relicticornis</i>	80	20	0.006	-2.731	**

Table 15 Unspecific mortality among non-attacked mirid nymphs and nymphs after being attacked by *P. digoneutis* or *P. stygicus* (¹unspecific mortality in rearing control = percentage of nymphs that died before reaching the adult stage; ²unspecific mortality of attacked nymphs = nymphs died before reaching the adult stage or before a parasitoid larva had emerged; test-statistic: binominal test)

Mirid species	Rearing control (non-attacked nymphs)		Nymphs attacked by <i>P. stygicus</i>			Nymphs attacked by <i>P. digoneutis</i>		
	n (# of nymphs)	unspecific nymphal mortality ¹ (%)	n (# nymphs attacked)	unspecific nymphal mortality after attack ² (%)	Signif. (P)	n (# nymphs attacked)	unspecific nymphal mortality after attack ² (%)	Signif. (P)
<i>L. rugulipennis</i>	25	20	35	26	ns	21	33	0.001
<i>L. maritimus</i>	15	7	14	0	0.005	27	15	0.002
<i>L. tripustulatus</i>	25	20	27	26	ns	22	27	ns
<i>L. pabulinus</i>	14	14	33	55	P<0.000	29	41	P<0.000
<i>C. norwegicus</i>	25	32	32	44	0.007	23	30	ns
<i>L. dolobrata</i>	20	40	21	62	P<0.000	14	57	P<0.000
<i>N. elongata</i>	15	60	28	93	P<0.000	16	56	ns
<i>S. calcarata</i>	25	24	36	61	P<0.000	13	23	ns
<i>M. relicticornis</i>	24	29	24	25	ns	10	40	0.01

Table 16 Non-target mirid species recorded in Schleswig-Holstein during the seasons 2001-2003; further information is given about the occurrence of the mirids in Canada as well as their pest status (i = invasive; n = native; **ip** = invasive species with pest status; modified after Maw et al. 2000).

Mirid subfamily	Species (nymphs collected)	Species (only adults collected)
Bryocorinae	<i>Dicyphus globulifer</i>	<i>Dicyphus epilobii</i>
		<i>Dicyphus pallidus</i>
		<i>Macrolophus pygmaeus</i>
Deraeocorinae	<i>Deraeocoris ruber</i>	
Mirinae	<i>Acetropis carinata</i>	<i>Adelphocoris ticinensis</i>
	<i>Adelphocoris lineolatus</i> (ip)	<i>Apolygus limbatus</i>
	<i>Adelphocoris quadripunctatus</i>	<i>Closterotomus fulvomaculatus</i> (n)
	<i>Adelphocoris seticornis</i>	<i>Lygocoris viridis</i>
	<i>Apolygus lucorum</i> (i)	<i>Phytocoris dimidiatus</i> (i)
	<i>Calocoris affinis</i>	<i>Phytocoris tiliae</i> (i)
	<i>Calocoris roseomaculatus</i>	<i>Phytocoris ulmi</i> (i)
	<i>Capsus ater</i> (ip)	<i>Phytocoris varipes</i>
	<i>Closterotomus norwegicus</i> (ip)	<i>Pithanus mearkeli</i> (i)
	<i>Creontiades pallidus</i>	<i>Trigonotylus cf. ruficornis</i>
	<i>Grypocoris sexguttatus</i>	<i>Trigonotylus psammaecolor</i>
	<i>Leptopterna dolabrata</i> (ip)	
	<i>Leptopterna ferrugata</i> (n)	
	<i>Liocoris tripustulatus</i>	
	<i>Lygocoris pabulinus</i> (n)	
	<i>Megaloceraea recticornis</i> (i)	
	<i>Notostira elongata</i>	
	<i>Orthops kalmi</i>	
	<i>Polymerus nigrinus</i>	
	<i>Rhabdomiris striatellus</i>	
	<i>Stenodema calcarata</i>	
	<i>Stenodema holsata</i>	
	<i>Stenodema laevigata</i>	
	<i>Stenodema trispinosa</i> (n)	
	<i>Stenotus binotatus</i> (ip)	
	<i>Trigonotylus caelestialium</i> (n)	
	Orthotylinae	<i>Cyllecoris histrionicus</i>
<i>Heterotoma planicornis</i> (i)		<i>Orthocephalus coriaceus</i> (i)
<i>Orthotylus marginalis</i>		<i>Orthotylus tenellus</i>
<i>Orthotylus moncreaffi</i>		
Phylinae	<i>Amblytulus nasutus</i> (i)	<i>Harpocera thoracica</i>
	<i>Europiella arthemisiae</i> (n)	<i>Phylus melanocephalus</i>
	<i>Lopus decolor</i> (i)	<i>Psallus</i> sp.
	<i>Macrotylus solitarius</i>	
	<i>Oncotylus punctipes</i>	
	<i>Plagiognathus chrysanthemi</i> (ip)	
<i>Plagiognathus arbustrorum</i> (i)		

Table 17 Non-target mirid collections 2001-2003 (* level of parasitization estimated by rearing instead of dissections).

Mirid species	Host plant ¹	No. sites sampled	No. collections	No. nymphs collected	No. sites with parasitization	No. sites with <i>P. digoneutis</i>	No. sites with <i>P. stygicus</i>	Parasitization		
								No. of samples	Range	Median
<i>Dicyphus globulifer</i>	BC	1	3	274	1	0	1	3	0-49*	29
<i>Deraeocoris rubber</i>	S	2	2	3	0	0	0			
<i>Adelphocoris lineolatus</i>	BM	11	20	635	5	0	0	12	0-46*	16
<i>Adelphocoris seticornis</i>	BM	1	1	1	0	0	0			
<i>Adelphocoris quadripunctatus</i>	G	1	1	6	0	0	0			
<i>Acetropis carinata</i>	G	1	1	2	0	0	0			
<i>Apolygus lucorum</i>	S, Mu	13	29	1217	12	1	0	4	11-50	40
<i>Calocoris affinis</i>	S	8	13	186	7	1	1	6	3-30*	18
<i>Calocoris roseomaculatus</i>	A	2	3	14	1	0	1			
<i>Closterotomus norwegicus</i>	C, Ca,G,P, S,W,	29	65	16388	27	9	13	29	0-78	19
<i>Liocoris tripustulatus</i>	S	31	65	4776	28	4	1	16	0-82	17
<i>Lygocoris pabulinus</i>	S	27	45	2217	19	2	2	7	21-50	33
<i>Orthops kalmi</i>	A	4	6	97	3	0	1			
<i>Rhodomiris striatellus</i>	O	2	3	23	2	0	0			
<i>Stenotus binotatus</i>	G	9	9	974	9	0	0	8	30-71*	50
<i>Polymerus nigrinus</i>	S	3	4	70	1	0	0			
<i>Creontiades pallidus</i>	G	2	2	3	0	0	0			
<i>Grypocoris sexguttatus</i>	P	1	1	5	0	0	0			
<i>Capsus ater</i>	G	1	1	3	0	0	0			
<i>Leptopterna dolobrata</i>	G	23	33	3800	22	0	3	14	3-56	30
<i>Leptopterna ferrugata</i>	G	1	2	37	1	0	1			
<i>Megaloceraea relicticornis</i>	G	14	20	2761	11	0	4	10	0-43	4
<i>Notostira elongata</i>	G	23	30	8473	21	0	4	14	0-38	7
<i>Stenodema calcarata</i>	G	25	39	2105	21	1	9			
<i>Stenodema holsata</i>	G	3	3	516	3	1	0			
<i>Stenodema laevigata</i>	G	13	16	371	9	0	0	13	0-38*	11
<i>Stenodema trispinosa</i>	G	5	5	123	2	0	0			
<i>Trigonotylus caelestialium</i>	G	9	12	341	7	0	5	8	0-44*	14
<i>Orthotylus moncreaffi</i>	AL	1	1	24	1	0	0			
<i>Orthotylus marginalis</i>	S	1	1	25	1	0	0			
<i>Heterotoma planicornis</i>	S	3	3	13	0	0	0			
<i>Cyllecoris histrionicus</i>	O	1	2	8	0	0	0			
<i>Europiella arthemisiae</i>	Mu	1	1	54	1	0	0			
<i>Lopus decolor</i>	G	1	2	137	1	0	1			
<i>Plagiognathus chrysanthemi</i>	T	3	5	195	3	0	1	5	0-50*	39
<i>Plagiognathus arbustrorum</i>	S	22	37	3170	20	0	0	8	28-46	36
<i>Amblytulus nasutus</i>	G	8	9	1289	6	0	4	3	5-21*	12
<i>Macrotylus solitarius</i>	HN	1	1	92	0	0	0	0	0	0
<i>Oncotylus punctipes</i>	T	3	3	175	0	0	0	0	0	0
sum				50603						

¹ A = Apiaceae
 AT = *Atriplex lacinata*
 BC = Bladder campion (*Silene vulgaris*)
 BM = Black medick (*Medicago lupulina*)
 C = Clover (*Trifolium pratense*)
 Ca = Camomile (*Matricaria recutita* and
Tripleurospermum perforatum)

G = Gramineae
 HN = Hedge nettle (*Stachys silvatica*)
 Mu = Mugwort (*Artemisia vulgaris*)
 O = Oak (*Quercus* spp.)

P = Tansy Phacelia (*Phacelia tanacetifolia*)
 S = Stinging nettles (*Urtica* spp.)
 T = Tansy (*Chrysanthemum vulgare*)
 W = Winter oilseed rape (*Brassica* spp.)

Table 18 Non-target mirid samples, from which *P. stygicus* or *P. digoneutis* emerged (only samples are presented from which a total of more than ten parasitoids emerged; * parasitization estimated by rearing instead of dissections).

Mirid host	Collection site	Collection date	# nymphs coll.	% parasitization*	# parasitoids emerged	species composition			parasitization caused by <i>P. stygicus</i>	parasitization caused by <i>P. digoneutis</i>
						# <i>P. stygicus</i>	# <i>P. digoneutis</i>	# other parasit.		
<i>L. dolobrata</i>	Kiel-Wellsee	12 Jun. 03	200	43*	58	6 (10.34%)	0	52	4.50%	0.00%
<i>L. dolobrata</i>	Osdorf	13 Jun. 03	470	48	72	2 (2.78%)	0	70	1.30%	0.00%
<i>L. pabulinus</i>	Kiel	4 Aug. 03	88	44*	13	0	1(8%)	12	0.00%	3.38%
<i>S. calcarata</i>	Rolfshörn	24 Jun. 03	74	25*	12	8 (66.7%)	1(8.3%)	3	16.67%	2.10%
<i>S. calcarata</i>	Molfsee II	26 Jun. 03	181	23	38	1 (2.6%)	0	37	0.61%	0.00%
<i>M. recticornis</i>	Wellsee	12 Jun. 03	550	6*	21	20 (95.2%)	0	1	5.71%	0.00%
<i>N. elongata</i>	Molfsee II	17 Aug. 02	1170	10	69	1 (1.5%)	0	68	0.15%	0.00%
<i>N. elongata</i>	Bohnert	22 Jun. 03	118	10*	12	1 (8.3%)	0	11	0.83%	0.00%
<i>C. norwegicus</i>	Achterwehr	19 Jun. 01	250	27*	52	2 (3.9%)	0	50	1.04%	0.00%
<i>C. norwegicus</i>	Achterwehr	25 Jun. 01	1030	56	184	22 (12%)	0	162	6.70%	0.00%
<i>C. norwegicus</i>	Achterwehr	30 Jun. 01	110	40	15	1 (6.7%)	0	14	2.67%	0.00%
<i>C. norwegicus</i>	Lindhöft*	13 Jun. 01	915	5*	12	1 (8.3%)	1 (8.3%)	10	0.42%	0.42%
<i>C. norwegicus</i>	Lindhöft*	26 Jun. 01	705	9*	26	1 (3.9%)	2 (7.7%)	23	0.35%	0.69%
<i>C. norwegicus</i>	Kochendorf	6 Jun. 02	1350	66	218	6 (2.8%)	2 (0.92%)	210	1.82%	0.61%
<i>C. norwegicus</i>	Schwedeneck	7 Jun. 02	412	29	56	0	5 (8.9%)	51	0.00%	2.59%
<i>C. norwegicus</i>	Lindhöft	7 Jun. 02	899	5*	45	4 (8.9%)	4 (8.9%)	37	0.44%	0.44%
<i>C. norwegicus</i>	Rastorf	9 Jun. 02	1081	9	42	1 (2.4%)	3 (7.1%)	38	0.21%	0.64%
<i>C. norwegicus</i>	Sophienhof	14 Jun. 02	410	8	29	2 (6.9%)	1(3.5%)	26	0.55%	0.28%
<i>C. norwegicus</i>	Lindhöft	15 Jun. 02	117	22*	19	2 (10.5%)	5 (26.3%)	12	2.31%	5.79%
<i>C. norwegicus</i>	Brux	19 Jun. 02	653	13	75	0	5 (6.7%)	70	0.00%	0.87%
<i>C. norwegicus</i>	Scharnhagen	9 Jun. 03	936	28	19	1 (5.3%)	2 (10.5%)	16	1.47%	2.95%
<i>C. norwegicus</i>	Rastorf	12 Jun. 03	450	30	62	4 (6.5%)	2 (3.2%)	56	1.94%	0.97%
<i>C. norwegicus</i>	Bohnert	22 Jun. 03	150	40	31	1 (3.2%)	0	30	1.29%	0.00%
<i>C. norwegicus</i>	Schnaap	23 Jun. 03	150	16	25	1 (4%)	0	24	0.64%	0.00%
<i>C. norwegicus</i>	Kiel-Holtenuau	25 Jun. 03	320	78	98	0	1(1.02%)	97	0.00%	0.80%
<i>C. norwegicus</i>	Kragholm	27 Jun. 03	202	34	39	1 (2.6%)	0	38	0.87%	0.00%
<i>A. nasutus</i>	Wellsee	12 Jun. 03	119	21	22	21 (95.5%)	0	1	20.05%	0.00%
<i>P. chrysanthemii</i>	Achterwehr	30 Jun. 01	130	41*	15	6 (40%)	0	0	16.40%	0.00%
<i>D. globulifer</i>	Achterwehr	4 Jul. 02	182	49*	32	2 (6.25%)	0	30	3.06%	0.00%
<i>S. holsata</i>	Wulfshagen	14 Jul. 03	260	21*	54	0	1(1.9%)	53	0.00%	0.39%
<i>T. caelestialium</i>	Rastorfer Passau	3 Aug. 02	70	44*	16	3 (18.8%)	0	13	8.25%	0.00%
<i>T. caelestialium</i>	Rastorfer Passau	13 Aug. 02	70	40*	12	1 (8.3%)	0	11	3.33%	0.00%

Table 19 Descriptive statistics, ANOVA table, and Bonferroni tests for comparison of SCPs of *Peristenus* spp. and *M. curvulus* measured in January (N = no. of replicates; SD = Standard Deviation; SE = Standard Error).

Descriptive statistics

Species	N	Mean SCP	SD	SE	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
<i>P. digoneutis</i>	30	-25.1967	1.2394	0.2263	-25.6595	-24.7339	-27.60	-22.60
<i>P. stygicus</i>	30	-24.6967	1.5103	0.2757	-25.2606	-24.1327	-26.80	-21.30
<i>P. nr. pallipes</i>	30	-26.3733	1.2199	0.2227	-26.8289	-25.9178	-28.20	-21.90
<i>M. curvulus</i>	15	-25.0867	1.8314	0.4729	-26.1009	-24.0725	-28.20	-20.70
Total	105	-25.3743	1.5402	0.1503	-25.6724	-25.0762	-28.20	-20.70

ANOVA

	Sum of Squares	df	Mean Square	F	Sig. (P)
Between Groups	45.905	3	15.302	7.696	<0.001
Within Groups	200.815	101	1.988		
Total	246.721	104			

Multiple Comparisons (Bonferroni)

Species compared		Mean Difference (I-J)	SE	Sig. (P)	95% Confidence Interval	
Species (I)	Species (J)				Lower Bound	Upper Bound
<i>P. digoneutis</i>	<i>P. stygicus</i>	-0.5000	0.364	1.000	-1.4798	0.4798
	<i>P. nr. pallipes</i>	1.1767*	0.364	0.010	0.1969	2.1565
	<i>M. curvulus</i>	-0.1100	0.446	1.000	-1.3100	1.0900
<i>P. stygicus</i>	<i>P. digoneutis</i>	0.5000	0.364	1.000	-0.4798	1.4798
	<i>P. nr. pallipes</i>	1.6767*	0.364	<0.001	0.6969	2.6565
	<i>M. curvulus</i>	0.3900	0.446	1.000	-0.8100	1.5900
<i>P. nr. pallipes</i>	<i>P. digoneutis</i>	-1.1767*	0.364	0.010	-2.1565	-0.1969
	<i>P. stygicus</i>	-1.6767*	0.364	<0.001	-2.6565	-0.6969
	<i>M. curvulus</i>	-1.2867*	0.446	0.029	-2.4867	-0.087
<i>M. curvulus</i>	<i>P. digoneutis</i>	0.1100	0.446	1.000	-1.0900	1.3100
	<i>P. stygicus</i>	-0.3900	0.446	1.000	-1.5900	0.8100
	<i>P. nr. pallipes</i>	1.2867*	0.446	0.029	8.665E-02	2.4867

* The mean difference is significant at the 0.05 level.

Table 20 *P. digoneutis*: Descriptive statistics, ANOVA table, and Bonferroni tests for comparison of SCPs measured in January and March and after cooling-down and warming-up periods in the laboratory (N = no. of replicates; SD = Standard Deviation; SE = Standard Error).

Descriptive statistics

SCP	N	Mean	SD	SE	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
SCP in January	30	-25.1967	1.2394	0.2263	-25.6595	-24.7339	-27.60	-22.60
SCP after cooling-down	32	-26.7844	0.8792	0.1554	-27.1014	-26.4674	-29.00	-25.10
SCP in March	37	-23.6865	1.1973	0.1968	-24.0857	-23.2873	-26.10	-19.60
SCP after warming-up	32	-22.5094	1.2161	0.2150	-22.9478	-22.0709	-24.60	-18.90
Total	131	-24.5015	1.9588	0.1711	-24.8401	-24.1629	-29.00	-18.90

ANOVA

	Sum of Squares	df	Mean Square	F	Sig. (P)
Between Groups	332.837	3	110.946	84.900	<0.001
Within Groups	165.962	127	1.307		
Total	498.800	130			

Multiple Comparisons (Bonferroni)

SCP (I)	SCP (J)	Mean Difference (I-J)	SE	Sig. (P)	95% Confidence Interval	
					Lower Bound	Upper Bound
SCP in January	SCP after cooling-down	1.5877*	0.291	<0.001	0.8091	2.3663
	SCP in March	-1.5102*	0.281	<0.001	-2.2629	-0.7574
	SCP after warming-up	-2.6873*	0.291	<0.001	-3.4659	-1.9087
SCP after cooling-down	SCP in January	-1.5877*	0.291	<0.001	-2.3663	-0.8091
	SCP in March	-3.0979*	0.276	<0.001	-3.8375	-2.3582
	SCP after warming-up	-4.2750*	0.286	<0.001	-5.0410	-3.5090
SCP in March	SCP in January	1.5102*	0.281	<0.001	0.7574	2.2629
	SCP after cooling-down	3.0979*	0.276	<0.001	2.3582	3.8375
	SCP after warming-up	-1.1771*	0.276	<0.001	-1.9168	-0.4375
SCP after warming-up	SCP in January	2.6873*	0.291	<0.001	1.9087	3.4659
	SCP after cooling-down	4.2750*	0.286	<0.001	3.5090	5.0410
	SCP in March	1.1771*	0.276	<0.001	0.4375	1.9168

* The mean difference is significant at the 0.05 level.

Table 21 *P. stygicus*: Descriptive statistics, ANOVA table, and Bonferroni tests for comparison of SCPs measured in January and March and after cooling-down and warming-up periods in the laboratory

Descriptive statistics

SCP	N	Mean	SD	SE	95% Confidence Interval for Mean		Min.	Max.
					Lower Bound	Upper Bound		
SCP in January	30	-24.6967	1.103	0.2757	-25.2606	-24.1327	-26.80	-21.30
SCP after cooling-down	30	-26.2367	0.48	0.1561	-26.5559	-25.9175	-27.70	-24.50
SCP in March	22	-23.6318	0.49	0.1844	-24.0153	-23.2484	-25.10	-22.00
SCP after warming-up	17	-22.8529	0.11	0.1676	-23.2083	-22.4976	-24.10	-20.80
Total	99	-24.101	1.408	0.1649	-24.9373	-24.2829	-27.70	-20.80

ANOVA

	Sum of Squares	df	Mean Square	F	Sig. (P)
Between Groups	153.140	3	51.047	43.811	<0.001
Within Groups	110.689	95	1.165		
Total	263.830	98			

Multiple Comparisons (Bonferroni)

SCP (I)	SCP (J)	Mean Difference (I-J)	SE	Sig. (P)	95% Confidence Interval	
					Lower Bound	Upper Bound
SCP in January	SCP after cooling-down	1.5400*	0.279	<0.001	0.7890	2.2910
	SCP in March	-1.0648*	0.303	0.004	-1.8813	-0.2484
	SCP after warming-up	-1.8437*	0.328	<0.001	-2.7267	-0.9607
SCP after cooling-down	SCP in January	-1.5400*	0.279	<0.001	-2.2910	-0.7890
	SCP in March	-2.6048*	0.303	<0.001	-3.4213	-1.7884
	SCP after warming-up	-3.3837*	0.328	<0.001	-4.2667	-2.5007
SCP in March	SCP in January	1.0648*	0.303	0.004	0.2484	1.8813
	SCP after cooling-down	2.6048*	0.303	<0.001	1.7884	3.4213
	SCP after warming-up	-0.7789	0.349	0.167	-1.7181	0.1604
SCP after warming-up	SCP in January	1.8437*	0.328	<0.001	0.9607	2.7267
	SCP after cooling-down	3.3837*	0.328	<0.001	2.5007	4.2667
	SCP in March	0.7789	0.349	0.167	-0.604	1.7181

* The mean difference is significant at the 0.05 level.

Table 22 Binominal tests for comparisons of *P. digoneutis* survival at different lengths of exposure (comparisons within groups of different subzero temperatures); as test proportion, the proportion of survived *P. digoneutis* in the previous temperature treatment was used, respectively, instead of the usual test proportion of 0.5 (e.g. 1 week at -5°C vs. 2 weeks at -5°C : test proportion 0.99; tests were not possible when No. survived = 0); control samples were kept at temperatures above 0°C .

-5°C							
exposure length	No. survived	No. dead	prop. survived	test groups	<i>P</i>	test vs. control	<i>P</i>
1 week	96	1	0.99			1-control	0.68
2 weeks	33	3	0.92	1-2	<0.001	2-control	<0.001
3 weeks	45	1	0.98	2-3	0.12	3-control	<0.48
4 weeks	0	48	0	3-4	-	4-control	-
-10°C							
exposure length	No. survived	No. dead	prop. survived	test groups	<i>P</i>	test vs. control	<i>P</i>
1 week	56	16	0.78			1-control	<0.001
2 weeks							
3 weeks	21	19	0.53	1-3	<0.001	3-control	<0.001
4 weeks	20	52	0.28	3-4	<0.001	4-control	<0.001
-15°C							
exposure length	No. survived	No. dead	prop. survived	test groups	<i>P</i>	test vs. control	<i>P</i>
1 week	36	110	0.25			1-control	<0.001
2 weeks	47	117	0.29	1-2	0.16	2-control	<0.001
3 weeks	21	97	0.18	2-3	0.005	3-control	<0.001
4 weeks	18	113	0.14	3-4	1.24	4-control	<0.001
-20°C							
exposure length	No. survived	No. dead	prop. survived	test groups	<i>P</i>	test vs. control	<i>P</i>
1 week	24	121	0.17			1-control	<0.001
2 weeks	0	151	0	1-2	-	2-control	-
3 weeks	0	88	0	2-3	-	3-control	-
4 weeks	0	141	0	3-4	-	4-control	-
control	688	8	0.99				

Table 23 Binominal tests for comparisons of *P. digoneutis* survival at different subzero temperatures (comparisons within groups of different exposure length).

exposure length	test groups	<i>P</i>
1 week	-5 $^{\circ}\text{C}$ vs. -10 $^{\circ}\text{C}$	<0.001
	-10 $^{\circ}\text{C}$ vs. -15 $^{\circ}\text{C}$	<0.001
	-15 $^{\circ}\text{C}$ vs. -20 $^{\circ}\text{C}$	0.12
2 weeks	-5 $^{\circ}\text{C}$ vs. -15 $^{\circ}\text{C}$	<0.001
	-15 $^{\circ}\text{C}$ vs. -20 $^{\circ}\text{C}$	-
3 weeks	-5 $^{\circ}\text{C}$ vs. -10 $^{\circ}\text{C}$	<0.001
	-10 $^{\circ}\text{C}$ vs. -15 $^{\circ}\text{C}$	<0.001
	-15 $^{\circ}\text{C}$ vs. -20 $^{\circ}\text{C}$	-
4 weeks	-5 $^{\circ}\text{C}$ vs. -10 $^{\circ}\text{C}$	-
	-10 $^{\circ}\text{C}$ vs. -15 $^{\circ}\text{C}$	<0.001
	-15 $^{\circ}\text{C}$ vs. -20 $^{\circ}\text{C}$	-

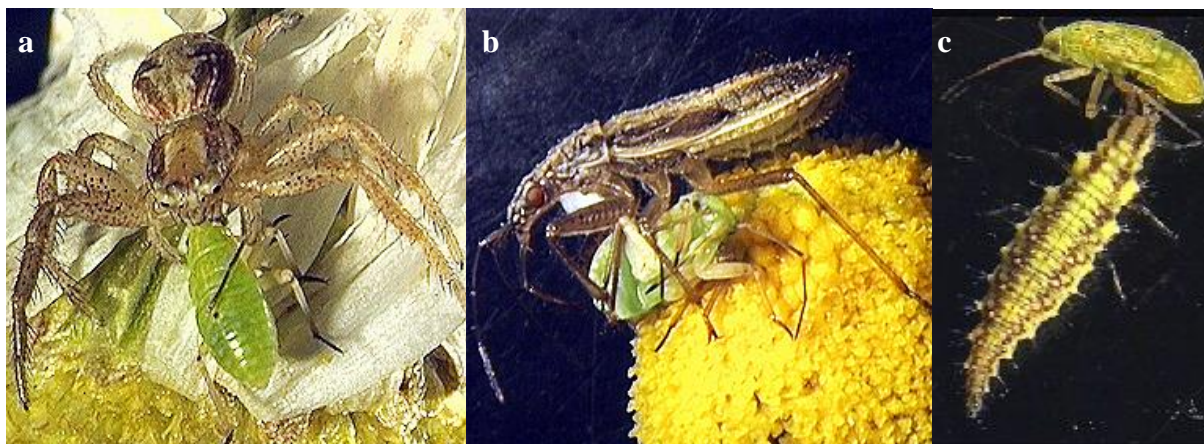


Figure 1 *Lygus* predators: a) crab spider (Araneida: Thomisidae), b) L5 nymph of *Nabis* sp. (Heteroptera: Nabidae), and c) larva of the lace wing *Chrysopa* sp. (Planipennia: Chrysopidae) (Photos: T. Haye).



Figure 2 *Leiofron reclinator*: a) adult female; b) female locating the host *Liocoris tripustulatus*; c) and d) female attacking *Liocoris tripustulatus* adult (Photos: U. Wyss & T. Haye).

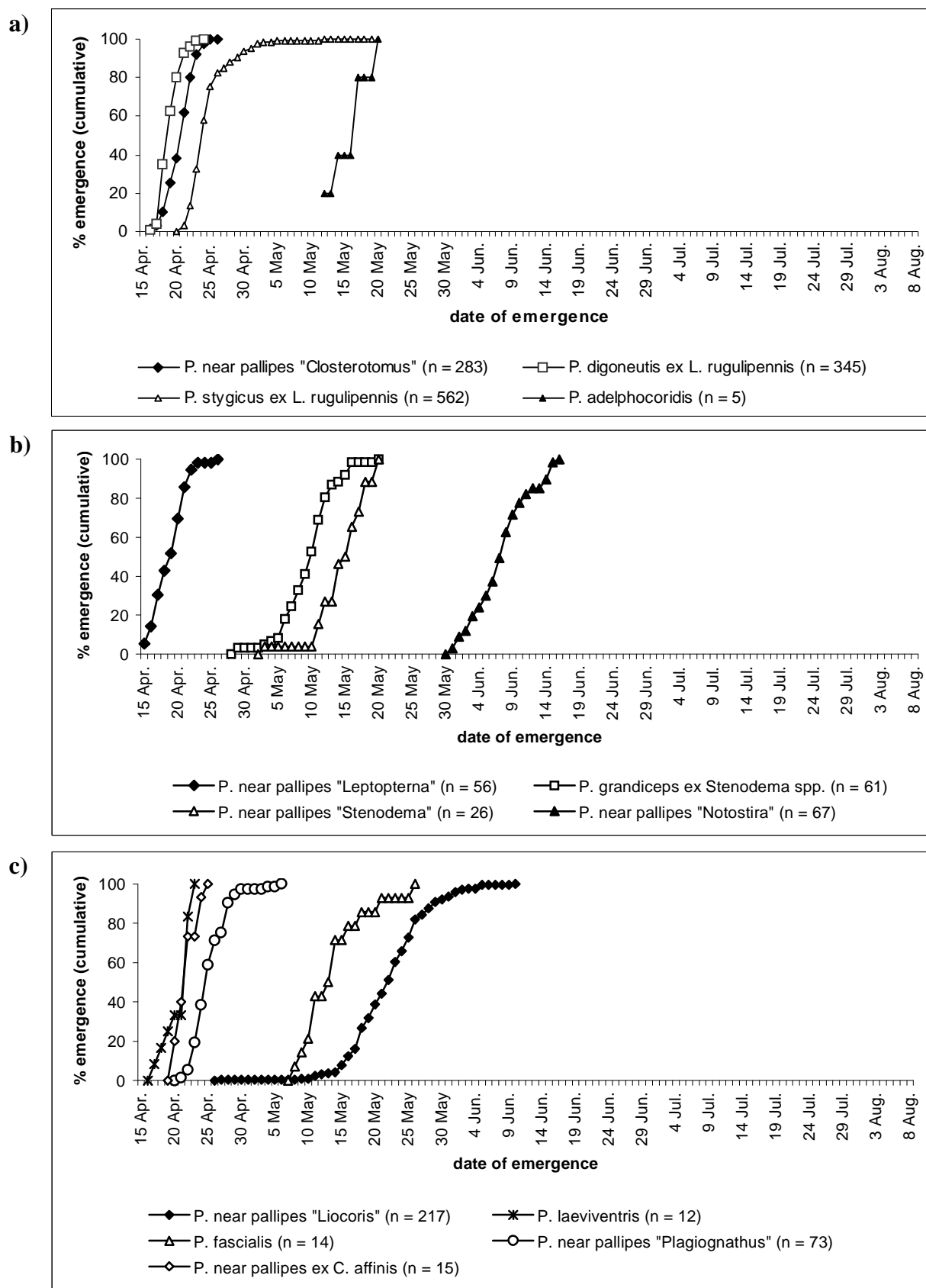


Figure 3 Cumulative emergence of overwintered parasitoids in the laboratory at 21 °C in 2002; parasitoids were reared from mirid hosts collected in a) chamomile or clover stands, b) grasslands, and c) stinging nettle stands (n = number of specimens emerged).

Acknowledgements

I want to thank Dr. Ulrich Kuhlmann for establishing the first contact to the CABI-Bioscience Centre, Switzerland back in 1998, for his outstanding support and for integrating me into the “*Lygus* working group”. Thanks for all the motivation and optimism and for all the technical support for the “CABI-Bioscience-Substation, Kiel” throughout the last years, and in spite of the geographical distance, for placing confidence in my work.

I would like to thank my supervisor Prof. H.J. Braune for his steady support and help during my study and for his valuable comments and suggestions, which improved my work considerably.

I would like to express special thanks to Dr. Peter Mason for inviting me to work with his team in Ottawa and giving me the great experience of living and working in Canada. Further I am grateful for the countless motivating discussions, many suggestions on my work and all his support over the years.

I also want to thank Dr. Henri Goulet for the identification of parasitoids and explaining the secrets of the *Peristenus* systematics to me, and to Caroline Boudreault for preparing all my parasitoid specimens. Further I thank everyone who supported me during my stay at E.C.O.R.C, Ottawa, in particular Ana Maria Farmakis, Mike Sarazin, Dr. John Huber, and Dr. Bob Footitt.

Further I would like to express special thanks to Dr. Bruce Broadbent, Jay Whistlecraft, and Lola Gualteri for their support during my stay in London, Ontario. It was a great pleasure to work with you!

I would like to thank Dr. Dave Gillespie for his support and his help when collecting *Lygus* in northern Germany.

I also want to thank Heidrun Liesegang, Heidrun Mard-Azad, Ina Berndt, Gabi König, Hannes Freitag, Dr. Fritz Sick and Dr. Marko Rohlf for always being helpful and encouraging during my studies in Kiel.

In particular, I would like to thank Prof. Oliver Betz for his support and patience in discussing statistical questions with me. I also want to thank Prof. Urs Wyss for his enthusiasm in taking wonderful pictures of my insects.

I would like to acknowledge the assistance of Dr. Birte von Patay, Eva-Maria Meyer, Antje Petersen, Christine Gülden-zoph, Kristin Moreth, and Marc Schierding with the field and laboratory work. I enjoyed their dedication and team spirit, without which it would not have been possible to undertake this study.

I am grateful to the following taxonomists for identification and information: Dr. Albert Melber and Dr. Mike Schwartz (mirids), Dr. C. van Achterberg (adult parasitoids), and Dr. K. van Zwakhals and Dr. Reijo Jussila (hyperparasitoids). Also, thanks to Sven Helqist who kindly supplied information on *Lygus* parasitoids in Sweden.

I want to thank all farmers who allowed me to collect in their fields and properties, in particular Wulf Kruse (Eichtal, Lütjenbrode), Michaela Schlathölter (P.H. Petersen Saatzucht Lundsgaard, Grundhof), and Sabine Mues (Versuchsgut Lindhof).

Special thanks to Tara, the Canadian part of our “Lygus Team Germany”, for all the help and fun during field and laboratory work, for making the completion of this thesis possible by patiently improving my written and my spoken English, and for being supportive all the time.

Special thanks to Jan, Torge, Jesko, and Julia for being my best friends and for letting me have a life outside the laboratory.

Finally, I am eternally grateful to my parents for all their support during my studies. To my sister for helping me through difficult times during the last three years, for always listening to me, and in short, for being my sister.

This study was financed by the Christian-Albrechts-University at Kiel, CABI-Bioscience, Switzerland, and Agriculture and Agri-Food Canada.

Curriculum vitae

Personal data

Name: Tim Haye
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Aug. 1979 – June 1983 Primary school in Heiligenhafen

Aug. 1983 – May 1992 Secondary school in Oldenburg in Holstein, qualification: Allgemeine Hochschulreife (Abitur)

July 1992 – Sept. 1993 Community service (in place of military service) at the psychiatric hospital, Heiligenhafen

Oct. 1993 – Sept. 1994 Studies in mineralogy at the Christian-Albrechts-University (2 semesters)

Oct. 1994 – April 2000 Studies in biology at the Christian-Albrechts-University at Kiel (12 semesters)

March 1997 – Nov. 1997 Research assistant at the Institute of Micropaleontology (C.A.U. Kiel)

May 1998 – July 1998 Summer student at CABI Bioscience Center Switzerland, Delémont. Research Assistant on the biology and ecology of parasitoids considered for biological control of Canadian forest insect pests

May 1999 – April 2000 Diploma student at the Christian-Albrechts-University, Kiel, Germany and CABI Bioscience Center Switzerland, Delémont (for practical work); Title of thesis: "Ecological studies on the parasitoid complex of *Lilioceris lili* (Scop.) (Coleoptera: Chrysomelidae) in Central Europe." („Ökologische Studien zum Parasitoidenkomplex von *Lilioceris lili* (Scop.) (Coleoptera: Chrysomelidae) an ausgewählten mitteleuropäischen Standorten").

May 2000 – Aug. 2000 Project entomologist at CABI Bioscience Center Switzerland, Delémont. Responsible for the biological control project against the lily leaf beetle *Lilioceris lili* (in collaboration with the University of Rhode Island, U.S.A.)

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Erklärung

Hiermit versichere ich,

- § dass ich diese Arbeit selbstständig angefertigt habe,
- § dass ich wörtlich oder inhaltlich aus anderen Quellen entnommene Stellen als solche kenntlich gemacht habe,
- § dass ich die Inanspruchnahme persönlicher Hilfe namentlich aufgeführt habe,
- § dass die Arbeit bisher weder ganz noch zum Teil im Rahmen eines Prüfungsverfahrens vorgelegen hat.

Für die mündliche Prüfung wähle ich die Form der Disputation.

Einer Zulassung von Zuhörerinnen und Zuhörern bei der mündlichen Prüfung widerspreche ich nicht.