Controlling *Lygus* plant bugs (Heteroptera: Miridae) with European *Peristenus relictus* (Hymenoptera: Braconidae) in Canada – risky or not?

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Abstract

The European *Peristenus relictus* Loan (syn. *P. stygicus*) has been considered for biological control of *Lygus* plant bugs native to Canada. Laboratory and field studies were conducted in the area of origin to evaluate the host specificity of *P. relictus*. Laboratory choice and no-choice tests demonstrated that *P. relictus* attacked all non-target species offered (fundamental host range). However, closely related non-target mirids (tribe Mirini) were generally well accepted by *P. relictus*, while hosts from the tribe Stenodemini were less frequently attacked and less suitable for parasitoid development. To validate the laboratory results, a thorough examination of the parasitoid complex of common mirids in Europe was conducted to determine which non-target species may serve as alternative hosts for *P. relictus* in a natural situation (ecological host range). When comparing both approaches, the fundamental host range of *P. relictus* matched its ecological host range. In addition to three *Lygus* species, the ecological host range of *P. relictus* in the area of Schleswig-Holstein, northern Germany, contains at least 16 non-target species, including hosts belonging to the subfamilies Mirinae, Phylinae and Bryocorinae. A broad ecological and fundamental host range suggests that *P. relictus* is a generalist; however, *P. relictus* was not the primary contributor to parasitism of most non-target hosts studied. Although *P. relictus* is assumed to be of minor importance for regulating non-target populations in the area of investigation, the results of the present study indicate that *P. relictus* has the potential to use non-target host populations for reproduction.

Keywords: biological control, host specificity, parasitoids, Miridae, *Lygus*, *Peristenus relictus*, Germany, Canada

Introduction

In Canada, the plant bug genus *Lygus* Hahn (Heteroptera: Miridae) contains 27 native species, of which 14 are recorded as agricultural field pests (Maw *et al.*, 2000). Historical records of *Lygus* spp. damaging agricultural crops in Canada go back to the 1940s (Carlson, 1940; McMahon & Arnason, 1947). Since then, *Lygus* plant bugs have been reported as pests of various crops throughout the country, e.g. vegetable and fruit crops (Broadbent *et al.*, 2002), alfalfa (McMahon & Arnason, 1947; Soroka, 1997), and oilseed rape (Butts & Lamb, 1991; Timlick *et al.*, 1993; Braun *et al.*, 2001). Furthermore, increasing problems with the occurrence of *Lygus* spp. in greenhouses and conifer nurseries have been documented in British Columbia (Shrimpton, 1985;

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Gillespie & Foottit, 1997). Recent outbreaks of *Lygus* spp. in Canada have mainly been controlled by the use of chemical pesticides (May *et al.*, 2003).

The idea of using biological control to suppress *Lygus* populations in North America dates back to the 1960s (Coulson, 1987; Craig & Loan, 1987; Broadbent *et al.*, 2002). Native North American *Lygus* parasitoids in the genus *Peristenus* Förster and *Leiophron* Nees (Hymenoptera: Braconidae) were considered ineffective, as nymphs of the first *Lygus* generation were primarily attacked, and subsequent *Lygus* generations were usually not significantly parasitized (Clancy & Pierce, 1966; Day, 1987; Day *et al.*, 1990; Lachance, 2000; Braun *et al.*, 2001). To increase overall parasitism of native *Lygus* spp., the exotic *Peristenus digoneutis* Loan, a parasitoid of the European tarnished plant bug *Lygus rugulipennis* Poppius, was imported from Europe and released against *Lygus lineolaris* (Palisot de Beauvois) in the 1980s in the north-eastern USA, where it reached high levels of parasitism and significantly decreased *Lygus* densities on alfalfa (Day *et al.*, 1990; Day, 1996). After its release in New Jersey, USA, *P. digoneutis* has naturally dispersed northwards into southern Quebec (Broadbent *et al.*, 1999) and Ontario, Canada. In addition to *P. digoneutis*, a second promising European *Lygus* parasitoid, previously known as *P. stygicus* (Loan & Bilewicz-Pawinska, 1973) and reclassified as *P. relictus* Loan (Varis & van Achterberg, 2001), was released in California against the western tarnished plant bug *Lygus hesperus* Knight. However, the impact of *P. relictus* on North American *Lygus* species is not known as its recovery in California has only recently been reported (Pickett *et al.*, 2005). Despite similar release efforts in the Canadian Prairie Provinces of Alberta and Saskatchewan in the late 1970s, European *Peristenus* species did not successfully establish (Craig & Loan, 1987). Recently, development of resistance of *Lygus* populations to insecticides (Grafton-Cardwell *et al.*, 2000), concerns about the impact of broad-spectrum insecticides on beneficial insects and pollinators, expanding problems with *Lygus* spp. in canola in western Canada, and the success of *P. digoneutis* in the United States have renewed interest in deliberate releases of European *Peristenus* spp. for biological control of *Lygus* pests in Canada (Braun *et al.*, 2001; Broadbent *et al.*, 2002).

Knowledge of the host range of a biological control agent is essential to assess whether non-target native species may be harmed after an agent is introduced to areas outside its native range (Sands & Van Driesche, 2003). The host range of a biological control agent can be defined as the set of potential host species that the agent is able to attack successfully, following a pattern of searching behaviour enabling it to encounter them regularly (Shaw, 1994). Within the last two decades, concerns about past introductions (i.e. more than 20 years ago) of arthropod biological control agents have increased because a lack of careful screening has resulted in the release of exotic generalist predators and parasitoids that have had negative environmental impacts (Obrycki *et al.*, 2000; Henneman & Memmott, 2001; Elkinton & Boettner, 2004). To appease the critics and to address concerns regarding arthropod biological control, new guidelines or methods for host range testing of biological control agents have been developed (van Lenteren *et al.*, 2003; van Driesche & Reardon, 2004; Bigler *et al.*, 2006). Various approaches may be used to assess a species host range more precisely, including reviews of scientific literature (De Nardo & Hopper, 2004; Sands & Van Driesche, 2004), laboratory

host range tests (van Lenteren *et al.*, 2003; van Driesche & Murray, 2004), or field surveys in an agent's native range (Fuester *et al.*, 2001; Kuhlmann & Mason, 2003; Haye & Kenis, 2004). The set of host species used for successful reproduction in laboratory host range tests can be defined as the fundamental (= physiological) host range of a biological control agent (Nechols *et al.*, 1992). However, in the laboratory it is difficult to accurately reproduce the factors that influence host searching and assess behaviour of a parasitoid in its natural environment (Sands, 1993). Thus, it has been suggested that a combination of laboratory observations and field studies of a parasitoid's host range be used to provide the basis for correctly interpreting fundamental host range estimations made via laboratory testing (Onstad & McManus, 1996; Kuhlmann & Mason, 2003). In contrast to the fundamental host range, the set of species that supports the development of a parasitoid in nature is defined as ecological (= realized) host range (Nechols *et al.*, 1992; Onstad & McManus, 1996).

In Europe, hosts other than *Lygus* spp. have rarely been associated with *P. relictus* (Drea *et al.*, 1973; Bilewicz-Pawinska, 1982; Carl & Mason, 1996) and consequently, this species has been regarded as having a narrow effective host range (Day, 1987). Previous laboratory studies, however, indicated a much broader host range for *P. relictus* compared to the somewhat limited field data previously reported (Porter, 1979; Condit & Cate, 1982). Therefore, we assessed host specificity of *P. relictus* by combining information from the literature as well as data from fundamental and ecological host range studies in the area of origin in Europe. This study aimed to contribute to the evaluation of whether the release of *P. relictus* against *Lygus* plant bugs in Canada is likely to be environmentally safe.

Materials and methods

Fundamental host range

Selection of non-target hosts

Selection of non-target hosts for laboratory testing (Kuhlmann & Mason, 2003; Kuhlmann *et al.*, 2005) was based on phylogenetic criteria, availability, spatial and temporal overlap of potential non-target and *Lygus* hosts in their natural habitats in Schleswig-Holstein, northern Germany. The phylogenetic approach involved studying the maximum fit cladogram of *Lygus* and its outgroup taxa (Schwartz & Foottit, 1998) and led to the selection of two Mirini species, *Lygocoris pabulinus* (Linnaeus) and *Liocoris tripustulatus* (Fabricius), as non-target test candidates. Another candidate, the potato bug *Closterotomus norwegicus* (Gmelin), belongs to the same tribe (Mirini) as *Lygus*. This species was selected because it is the most abundant spring mirid in northern Germany (Afscharpour, 1960) and occurs at the same time and habitat as *Lygus* species. To include more distantly related mirid species, four grass bugs (Stenodemini) were selected, including *Leptopterna dolobrata* Linnaeus, *Stenodema calcarata* (Fallén), *Notostira elongata* (Geoffroy), and *Megaloceraea recticornis* (Geoffroy). In this study, *L. rugulipennis* Poppius represented the target host instead of the congeneric North American *Lygus* species. To investigate if variations in acceptance and parasitoid development occur when different *Lygus* hosts are offered, *Lygus maritimus* Wagner which occurs primarily in coastal habitats was included in the testing procedures.

Source and rearing of parasitoids, hosts and potential hosts

To obtain parasitoid adults for laboratory experiments, first and second generation *L. rugulipennis* nymphs were field-collected from *Trifolium pratense* L. and *Matricaria recutita* (L.) habitats in northern Germany. These hosts were reared until parasitoid larvae egressed and spun cocoons. Immediately following emergence from cocoons, adult *Peristenus* were kept in a subterranean insectary (15–18°C) when not used in experiments. Adult wasps were fed a 30% honey-water solution. Before each test, mated parasitoids were acclimated to 25°C, 70% RH for at least 1 h, because parasitoids show a higher oviposition activity at this temperature (B. Broadbent, personal communication, 2005).

In each year, populations used in testing consisted of the progeny of field-collected adults or immatures collected directly from the field. Nymphs of *L. rugulipennis*, *L. maritimus* and *L. tripustulatus* were reared from overwintered adults, and nymphs of *L. pabulinus* were reared from newly emerged spring generation adults. Adult *L. rugulipennis* and *L. maritimus* were collected from *M. recutita* at Rastorf (54°16.55 N, 10°17.21 E) and Dagebuell (54°43.97 N, 8°42.25 E), respectively. Adults of *Liocoris tripustulatus* and *Lygocoris pabulinus* obtained from stinging nettle stands at Rosenfelder See near Kiel (54°16.44 N, 10°15.55 E). Nymphs were reared using a combination of methods described for *Lygus lineolaris* by Stevenson & Roberts (1973) and Snodgrass & McWilliams (1992). Adults were kept at 20°C, 16:8 L:D and provided with lettuce and sprouting potatoes, which served as an oviposition substrate as well as food source for newly emerged nymphs. An *S. calcarata* culture was established by obtaining freshly emerged nymphs from grass ears (into which adults had oviposited) collected at Kiel (54°20.54 N; 10°06.46 E). To obtain nymphs of univoltine non-target mirids that overwinter in the egg stage, such as *C. norwegicus*, *L. dolobrata* and *M. recticornis*, first and second instar nymphs were field-collected at Lindhoeft (54°27.54 N, 9°58.63 E) in early spring. Small instar nymphs of *N. elongata* were collected from Kiel-Molfsee (54°15.26 N, 10°03.15 E) in early July when the second generation started to emerge. In the laboratory, grass bug nymphs were all reared on the host plants they were collected from; nymphs of *C. norwegicus* were fed beans. The risk that field-collected nymphs have already been parasitized during this very early period of nymphal emergence is generally low. However, as an additional control, subsamples of small non-target nymphs were always reared and dissected to assess parasitism.

Nymphs of *Lygus*, *Closterotomus*, *Lygocoris* and *Liocoris* that were attacked during exposure to *P. relictus* females in laboratory tests (described below) were reared individually in small plastic vials (55 mm length, 30 mm diameter) to assess the host acceptability and suitability of parasitoids. Although nymphs of most of the species tested were provided with Romaine lettuce and potato sprouts as a food source, grass bug nymphs were placed on grass ears or leaves instead. A thin layer of moistened vermiculite covered the bottom of the vials and served as a pupation substrate for the emerging parasitoid larvae.

Sequential no-choice test

The test aimed to determine whether *P. relictus* accepts non-target nymphs consistently and whether non-target nymphs are suitable hosts for parasitoid development. Three-day-old, mated, naïve females were first exposed to a single second or early third instar nymph of the target *L. rugulipennis*, the stages usually parasitized (Loan, 1980). The peak oviposition period for *P. relictus* is within the first 5 days following emergence (Haye *et al.*, 2005a), when they are likely to experience the heaviest oviposition pressure. As explained by Withers & Browne (2004), it is preferable to conduct non-target laboratory experiments when the candidate biological control agent is experiencing conditions in which it is most likely to express its maximal host range. Because *P. relictus* has no pre-oviposition period (Haye *et al.*, 2005a), females that did not react to *Lygus* nymphs were presumed unfit and thus excluded from the testing. In the subsequent no-choice tests, females that were presumed ready for oviposition were individually placed into a clear plastic vial (diameter 30 mm, length 55 mm), each containing a second or third instar nymph of a non-target host. Each parasitoid was given a maximum time of 20 min to find and parasitize the nymph. To avoid recording false-negatives in cases where females did not react to the non-target host, a control test was conducted 24 h later. The same experimental procedure was used except that a *L. rugulipennis* nymph was presented to the parasitoid instead of a non-target host. Data recorded included attacks on host, host acceptance, and host suitability. When the parasitoid was observed to insert the ovipositor into the nymph, the host was recorded as attacked ('attacks on hosts'). Host acceptance was noted when evidence of parasitism was discovered (i.e. via dissection of the host or egression of mature larva). A mirid host was classified as suitable when parasitoid larvae successfully completed their development and formed a cocoon outside their hosts ('host suitability').

Behavioural choice test

This test investigated whether the ovipostional behaviour of the female parasitoid on a non-target host changed in the presence of the target host ('host preference'). For this, six to eight-day-old experienced parasitoid females from the no-choice tests and second to third instar nymphs were used. Experienced parasitoids were those individuals that had previously been exposed to hosts in the no-choice tests. For each treatment, a single female wasp was offered three nymphs of the target and three of a non-target species at the same time, in a Petri dish (5 cm diameter). Attacked nymphs were immediately removed with a mouth aspirator and replaced by new, non-parasitized individuals to maintain a constant number of each mirid species in the arena over the 5 min observation period. Each treatment was replicated 20 times. The number of attacked nymphs was recorded.

Statistical analyses

The χ-square test, after McNemar, was used to analyse the data sets obtained from each mirid species tested in the no-choice tests. For comparing the levels of host acceptance, only data obtained from parasitoids which had attacked *Lygus* and non-target nymphs in the no-choice tests were used. Consequently, the number of replicates was automatically reduced. Some of the attacked nymphs died and desiccated during the rearing process and thus could not be dissected for parasitism. In these cases the complete test series was excluded from the analysis and consequently, the number of replicates was further reduced. The ratio of successful parasitoid development in *Lygus* and non-target hosts were compared using the binomial test. Data sets from

small arena behavioural choice tests were analysed using the Wilcoxon paired-sample test. Statistical analyses were performed using the SPSS® 10.0 software (SPSS Inc., 1999).

Ecological host range

Surveys

During the 2001–2003 field seasons, sampling of mirid nymphs was carried out from April to September at more than 60 sites in Schleswig-Holstein, northern Germany. To obtain a broad range of common and rare mirid species, mirid nymphs were collected from various host plants (table 1) in natural or agricultural habitats using a standard sweep net (38 cm diameter). As parasitoid larvae are known to emerge from late nymphal instars and only rarely from teneral adults (Loan, 1980), only fourth and fifth instar mirid nymphs were retained. To investigate whether *P. relictus* are actually specific to the subfamily Mirinae, representatives of other mirid subfamilies, such as Bryocorinae, Orthotylinae and Phylinae were collected.

Rearing of nymphs and their natural enemies

A rearing system consisting of 1.2-litre plastic containers fitted with removable Petri dishes on the bottom was used for samples of up to 50 nymphs. The Petri dishes were filled with moist vermiculite and separated from the rest of the container by gauze (mesh size 1.20×1.38 mm), which allowed larval parasitoids gain access to the Petri dish for pupation (Drea *et al.*, 1973). Larger samples of up to 500 nymphs were kept in plastic buckets with the bottoms removed and replaced with gauze. Plastic funnels, terminating in vermiculite-filled Petri dishes were attached to the bottom of the buckets to collect emerging parasitoid larvae that fell through the gauze. Mirids belonging to the genera *Lygus*, *Adelphocoris*, *Closterotomus* and *Calocoris* were fed with organically grown beans and lettuce. For all other mirid species, the host plants from which they had been collected were added to the rearing cages because they would not accept any other diet. When all nymphs had reached the adult stage or all parasitoid larvae had egressed, Petri dishes containing parasitoid cocoons were removed and stored in an outdoor wooden shelter until adult emergence.

Ecological host range of *P. relictus* and its impact on mirid hosts

For each species studied, overall parasitism (%) was calculated by dividing the number of parasitoid cocoons by the combined number of parasitoid cocoons plus reared mirid adults, multiplied by 100 (see table 1). The proportion of *P. relictus* (and other parasitoids) relative to the total number of emerged parasitoids was also calculated for each mirid species. The proportion of each parasitoid species was then converted into percent parasitism by each species (e.g. if overall parasitism of a given mirid species was 40% and the proportion of *P. relictus* in the nymphal parasitoid community was 25%, then the parasitism rate for *P. relictus* was 10%). For cases when hyperparasitoids emerged from the cocoons (primarily *Mesochorus curvulus* Thomson, C.J. Zwakhals, personal communication, 2005), the actual proportion of the primary parasitoid as well as parasitism by the hyperparasitoid could not be estimated precisely because the primary parasitoid is consumed. This makes it

impossible to determine which primary parasitoid species initially attacked the mirid host and thus, the actual proportion of *P. relictus* in these non-target hosts may have been higher than was estimated from the rearing. However, preliminary molecular data estimating parasitoid species composition in European mirids indicates that the proportion of *P. relictus* in non-target hosts is not significantly influenced by hyperparasitoids (T.D. Gariepy, personal communication, 2005).

Results

Fundamental host range

Sequential no-choice test

Peristenus relictus females attacked the alternative target *Lygus maritimus* and the non-targets, *Liocoris tripustulatus*, *Lygocoris pabulinus* and *C. norwegicus* with nearly the same frequency as the target *Lygus rugulipennis* (table 2). In contrast, the Stenodemini *Leptopterna dolobrata*, *S. calcarata*, *N. elongata*, and *M. recticornis* were significantly less attacked. The acceptance of these Stenodemini and *Lygus* hosts also differed significantly (table 2). The lowest acceptance was for *Leptopterna dolobrata*. Although *P. relictus* attacks on *Lygocoris pabulinus* were frequently observed, in comparison to the *Lygus* control a large proportion of the test nymphs were not accepted. All non-target hosts tested were suitable for parasitoid development (fig. 1). Corresponding with the lower acceptance observed, *P. relictus* completed its development significantly less often in *Leptopterna dolobrata*, *S. calcarata* and *N. elongata* than in *L. rugulipennis*; however, parasitoid development in *M. recticornis* was as successful as that in the target host. *Lygocoris pabulinus* was likely a less suitable host for *P. relictus* but, as data on in-host development were limited due to the poor acceptance of the host ($n = 9$), development in the non-target and *Lygus* host did not differ significantly.

Behavioural choice test

In the choice test, both target and non-target hosts were attacked by *P. relictus* (fig. 2). The parasitoids showed no preference for nymphs of the target *Lygus rugulipennis* when offered simultaneously with those of the non-target species *Liocoris tripustulatus*, *Lygocoris pabulinus*, *S. calcarata*, and *M. recticornis*. However, when nymphs of the potato bug *C. norwegicus* ($Z = -2.73$; $P = 0.006$) or either of the two grass bugs *N. elongata* ($Z = -3.01$; $P = 0.003$) and *Leptopterna dolobrata* ($Z = -3.26$; $P = 0.001$) were present, *P. relictus* attacked the target significantly more often than the non-target. In these cases more than 60% of the attacks observed were on *Lygus* nymphs. Fewest attacks on a non-target species (20%) were observed when *P. relictus* had the choice between *Lygus rugulipennis* and *Leptopterna dolobrata* nymphs.

Ecological host range

In northern Germany, *P. relictus* was reared from 19 mirid species, belonging to the Bryocorinae, Mirinae and Phylinae (table 1). All non-target species accepted by *P. relictus* in laboratory tests were also found to be suitable hosts in the field. The ecological host range of *P. relictus* primarily included hosts belonging to the subfamily Mirinae, of which

Table 1. Mirid species, host plants and details regarding collection and rearing of mirid nymphs collected in Schleswig-Holstein, northern Germany to assess presence and parasitism by *Peristenus relictus* in target and non-target mirids.

Mirid host species	Host plants[a]	No. nymphs taken into rearing	No. mirid adults reared	No. cocoons received	No. parasitoids emerged	No. sites sampled	No. sites with *P. relictus* present	Overall parasitism (%)	% Parasitism		
									Peristenus relictus	*Peristenus* spp.	*Mesochorus* spp.[b]
Bryocorinae											
Dicyphus globulifer (Fallén)	Sv	274	100	69	38	1	1	40.8	1.8	33.7	0
Mirinae: Mirini											
Adelphocoris lineolatus (Goeze)	Ml	568	336	59	35	11	0	14.9	0	14.5	0.4
Apolygus lucorum (Meyer-Dür)	U, Av	1114	466	155	86	13	0	25	0	18.5	6.5
Calocoris affinis (Herrich-Schaeffer)	U	186	120	25	21	8	1	17.2	0.8	15.7	0.7
Calocoris roseomaculatus (De Geer)	A	14	6	2	2	2	1	(25.0)	(12.5)	(12.5)	(0)
Closterotomus norvegicus (Gmelin)	Tp, Mr, G, Pt, U	15149	7639	1828	1357	29	13	19.3	0.8	17.2	1.3
Liocoris tripustulatus (Fabricius)	U	4217	1197	839	409	31	1	41.2	0.4	32.6	8.2
Lygocoris pabulinus (Linnaeus)	U	1955	1127	141	74	27	2	11.1	0.3	6.0	4.8
Lygus maritimus Wagner	Mr	1243	877	80	62	6	3	8.4	7.7	0.6	0.1
Lygus pratensis (Linnaeus)	Mr, Tp	488	363	67	65	13	7	15.6	8.1	5.3	2.2
Lygus rugulipennis Poppius	Mr, Tp	39851	22154	7296	5556	20	17	24.8	9.2	14.4	1.2
Orthops kalmii (Linnaeus)	A	97	64	4	1	4	1	(5.9)	(5.9)	(0)	(0)
Rhabdomiris striatellus (Fabricius)	Q	23	11	8	8	2	0	42.1	0	42.1	0
Stenotus binotatus (Fabricius)	G	911	351	252	200	9	0	41.8	0	35.5	6.3
Mirinae: Stenodemini											
Leptopterna dolobrata (Linnaeus)	G	3285	1218	529	375	23	3	30.3	0.6	20.6	9.1
Leptopterna ferrugata (Fallén)	G	37	25	6	4	1	1	(19.4)	(4.8)	(14.6)	(0)
Megaloceraea recticornis (Geoffroy)	G	2450	1772	75	43	14	4	4.1	2.3	1.4	0.4
Notostira elongata (Geoffroy)	G	7872	4014	757	603	23	4	15.9	0.1	12.9	2.9
Stenodema calcarata (Fallén)	G	1970	1038	458	269	25	10	30.6	2.7	18.7	9.2
Stenodema holsata (Fabricius)	G	486	318	93	80	3	0	22.6	0	21.2	1.4
Stenodema laevigata (Linnaeus)	G	371	224	33	19	13	0	12.8	0	12.8	0
Stenodema trispinosa Reuter	G	102	61	7	3	5	0	10.3	0	3.4	6.9
Trigonotylus caelestialium (Kirkaldy)	G	341	189	68	48	9	5	26.5	11.1	13.8	1.6
Orthotylinae											
Orthotylus moncreaffi (Douglas & Scott)	Al	24	20	1	1	1	0	4.8	0	4.8	0
Orthotylus marginalis Reuter	U	25	13	6	5	1	0	31.6	0	31.6	0
Phylinae											
Europiella artemisiae (Becker)	Av	54	5	18	12	1	0	78.3	0	45.4	32.9
Lopus decolor (Fallén)	U	137	25	3	1	1	1	(10.7)	(10.7)	(0)	(0)
Plagiognathus chrysanthemi (Wolff)	Cv	195	104	64	25	3	1	38.1	15.3	21.3	1.5
Plagiognathus arbustorum (Fabricius)	U	3141	1076	601	375	22	0	35.8	0	29.0	6.8
Amblytylus nasutus (Kirschbaum)	G	1225	820	46	38	8	4	5.3	4.2	0.9	0.2

[a] A, Apiaceae; Al, *Atriplex lacinata* L.; Av, *Artemisia vulgaris* L.; Cv, *Chrysanthemum vulgare* (L.); G, Gramineae; Ml, *Medicago lupulina* L.; Mr, *Matricaria recutita* (L.); Pt, *Phacelia tanacetifolia* Bentham; Q, *Quercus* spp.; Sv, *Silene vulgare* (Moench) Garcke; Tp, *Trifolium pratense* L.; U, *Urtica* spp.

[b] Hyperparasitoids.

Values in parentheses represent data from mirid species from which less than five individual parasitoids emerged (including *P. relictus*), and are likely not representative.

Table 2. Percentage of *Peristenus relictus* females attacking and accepting two *Lygus* and seven non-target hosts in small arena no-choice tests.

Mirid species	% parasitoids that attacked			Acceptance (%)		
	N	Percentage	P-value[1]	N	Percentage	P-value[1]
Lygus rugulipennis[2]	37	95	0.5	25	92	1
Lygus maritimus[2]	14	100	1	13	100	1
Liocoris tripustulatus	29	93	0.5	17	100	0.5
Lygocoris pabulinus	33	82	1	24	33	0.001
Closterotomus norwegicus	34	94	0.5	20	85	1
Leptopterna dolobrata	31	68	0.002	13	54	0.031
Stenodema calcarata	53	68	< 0.001	23	57	0.016
Megaloceraea recticornis	42	67	0.008	17	70	0.008
Notostira elongata	32	75	< 0.001	27	53	0.008

[1]Comparison with the control; [2]*Lygus* control species offered in both experimental runs.
N gives the number of tested parasitoid females for each mirid species investigated. (χ-square test after McNemar.)
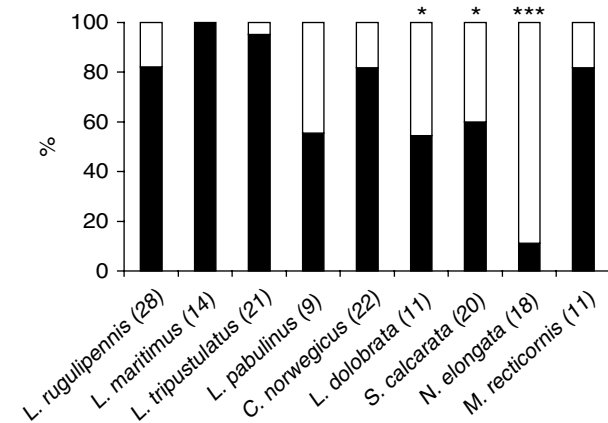
Fig. 1. Host suitability: percentage of *Peristenus relictus* larvae that completed larval development and formed a cocoon outside their host (■) and percentage of larvae that failed to complete development due to host mortality (□). The number of accepted nymphs is given in brackets for each mirid species (binomial test; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$, the frequency for completing development in *Lygus* nymphs (0.82) was used instead of 0.5).

the tribes Mirini and Stenodemini were represented with nine and six hosts, respectively. In the target host *Lygus rugulipennis* as well as in the alternative target hosts *Lygus pratensis* (Linnaeus), and *Lygus maritimus*, the percent of *P. relictus* in the larval parasitoid guild were 37%, 52% and 92%, respectively. *Peristenus relictus* was found at 85%, 54% and 50% of the sampling sites of *L. rugulipennis L. pratensis*, and *L. maritimus*, respectively. The parasitism of the three *Lygus* species by *P. relictus* varied between 7.7% and 9.2%. Sporadically, *P. relictus* developed from the closely related non-target hosts *Liocoris tripustulatus* (<1% of the nymphal parasitoid species composition) and *Lygocoris pabulinus* (3%), but it caused only 0.4% and 0.3% parasitism, respectively. Among non-target hosts from the Mirinae, *P. relictus* was most regularly recorded from *C. norwegicus* (45% of all sites where *C. norwegicus* was collected), *S. calcarata* (40%) and *Trigonotylus caelestialium* (Kirkaldy) (56%). Although frequently recorded, overall parasitism caused by *P. relictus* in *C. norwegicus* and *S. calcarata* was 0.8% and 2.7%, respectively. In contrast, parasitism in *T. caelestialium*

(11.1%) was similar to parasitism in *Lygus* spp.. Parasitism by *P. relictus* in the common grass bugs *M. recticornis*, *L. dolobrata* and *N. elongata* did not exceed 3%. Single specimens of *P. relictus* were also obtained from the Mirini *Calocoris affinis* (Herrich-Schaeffer), *Calocoris roseomaculatus* (De Geer), *Leptopterna ferrugata* (Fallén) and *Orthops kalmi* (Linnaeus).

Among the Phylinae, *P. relictus* developed from *Amblytylus nasutus* (Kirschbaum), *Plagiognathus chrysanthemi* (Wolff), and *Lopus decolor* (Fallén) (single specimen). Parasitized nymphs of *P. chrysanthemi* were only found at one out of three sampled sites; however, at this site the proportion of *P. relictus* in the nymphal parasitoid guild was 40%, resulting in 15.3% parasitism. Furthermore, *P. relictus* occurred at 50% of the sites where *A. nasutus* was collected ($n = 8$), but parasitism by *P. relictus* was only 4.2%. In addition, two specimens of *P. relictus* were obtained from the predator *Dicyphus globulifer* (Fallén) (Bryocorinae).

Discussion

The laboratory data show that non-target species phylogenetically close (i.e. other Mirini) to the target *Lygus* spp. are attacked by *P. relictus* at a similar frequency (table 2) and are as suitable for its development (fig. 1). Non-target species in another tribe (e.g. Stenodemini) were attacked, although significantly less frequently, and were significantly less suitable as hosts for *P. relictus*. The presence of the target host may elicit greater (e.g. *S. calcarata* and *M. recticornis*) or fewer (e.g. *C. norwegicus*) attacks by *P. relictus* (fig. 2) regardless of phylogenetic distance. It was demonstrated previously that in laboratory studies *P. relictus* was able to develop in North American non-target host species (Porter, 1979; Condit & Cate, 1982). Recently, it was suggested that biological control agents should be tested in small and simple structured arenas to provide conditions where the maximal host range is likely to be expressed (Withers & Browne, 2004). In the present study, all non-target species recorded as hosts of *P. relictus* in the laboratory were also found to be suitable hosts in the field and thus, the use of small arenas for laboratory tests was appropriate to identify potential non-target hosts. Furthermore, high non-target parasitism in the laboratory also suggested that the tested non-targets would be frequently parasitized in the field. However, overall parasitism of these species did not exceed 3%. Therefore, laboratory tests may identify potential
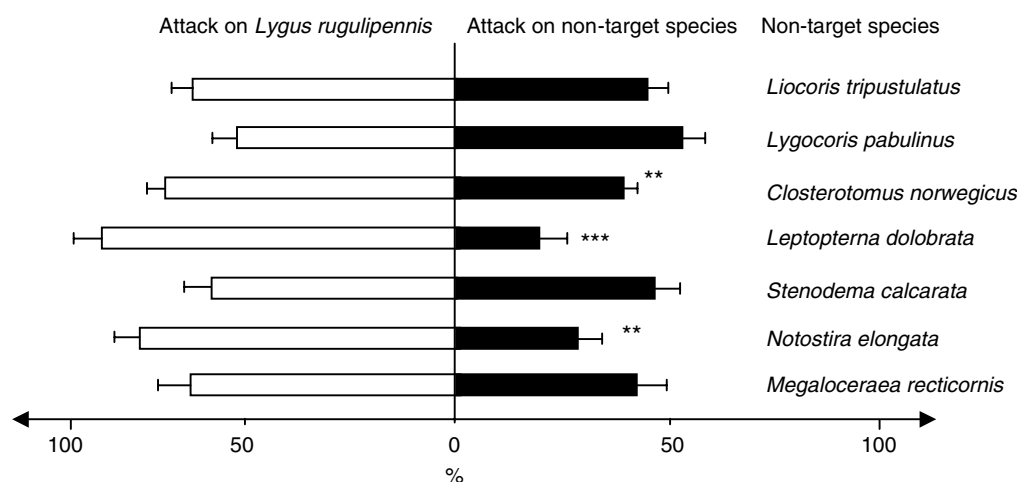
Fig. 2. Mean percent (±SE) of attacks per 5 min on *Lygus* and non-target nymphs when offered to *Peristenus relictus* in small arena behavioural choice tests. For each non-target species 20 females were tested. Bars marked with asterisks indicate a significant difference between attacks on non-target and *Lygus* hosts (Wilcoxon paired-sample test; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$).

non-target hosts, but they cannot predict the impact on a non-target host population in nature (Louda *et al.*, 2003b).

To address concerns regarding the reliability of laboratory tests, these observations should be validated with field studies in the area of origin (Onstad & McManus, 1996; Kuhlmann *et al.*, 2000; Louda *et al.*, 2003a). Day (1987) assumed that the failure of *P. relictus* to establish in the USA on the mirid genus (*Lygus*) and the host plant (alfalfa) it was originally obtained from in Europe, suggests that the ecological host range of *P. relictus* is considerably more restricted than indicated by laboratory tests. Indeed, *P. relictus* has been previously reported from only four mirid hosts in Europe (Drea *et al.*, 1973; Bilewicz-Pawinska, 1977, 1982; Carl & Mason, 1996), but the broad ecological host range of *P. relictus* comprising a total of 21 host records (from the present study and the literature) contradicts the assumption by Day (1987). The previous and the present host range studies all indicate that *P. relictus* has a high potential to attack non-target hosts. This conclusion is also supported by the high lifetime fecundity of *P. relictus* (Haye *et al.*, 2005a), 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). The ecological host range of *P. relictus* is not only restricted to hosts of the subfamily Mirinae, but also contains hosts from the subfamilies Bryocorinae and Phylinae. The development of *P. relictus* in Phylinae hosts, therefore, confirms the finding by Condit & Cate (1982) that *P. relictus* was able to develop successfully from the North American Phylinae *Pseudoatomoscelis seriatus* (Reuter) in the laboratory. The broad ecological host range suggests that in the case of *P. relictus*, the phylogenetic relatedness between *Lygus* and non-target hosts may play a lesser role in predicting the parasitoid's behaviour (Messing, 2001; Hoddle, 2004). For example, Shahjahan (1974) demonstrated that *Erigeron* plants, which are also preferred host plants of *Lygus*, were highly attractive to *Peristenus pseudopallipes* (Loan).

According to Stiling (2004), *P. relictus* should be regarded as a generalist because it has been recorded from a total of

16 genera in Europe. For parasitoid species such as *P. relictus*, a broad host range may suggest that non-target effects are more likely (Stiling, 2004), but it does not necessarily predict severe non-target effects. Examination of the proportion of and the overall parasitism by *P. relictus* in the nymphal parasitoid guild attacking *Lygus* spp. and non-target species may clarify the relationship between the number of hosts attacked by *P. relictus* and its actual impact on mirid communities.

Peristenus relictus was widely distributed in northern Germany, occurring at more than 50% of all sites where *Lygus* spp. were sampled. Surprisingly, overall parasitism by *P. relictus* alone was only 7.7% to 9.2% among *Lygus* spp., and the maximum parasitism level recorded at single collection sites was 46% (data not shown). The relatively low proportion of *P. relictus* in the nymphal parasitoid complex of *L. rugulipennis* (37%) in northern Germany agrees with former observations by Coutinot & Hoelmer (1999) who showed that *P. relictus* is the dominant *L. rugulipennis* parasitoid in Mediterranean areas of southern Europe, but that it is less common in oceanic and suboceanic areas. A critical consideration for releasing *P. relictus* in Canada is that the genus *Lygus* contains 13 native species, which are not recorded as agricultural field pests (Maw *et al.*, 2000). That two native target species, *L. lineolaris* and *L. hesperus* (Condit & Cate, 1982), and three European *Lygus* spp. are suitable hosts for *P. relictus* suggests that all *Lygus* species native to Canada, including those without pest status, could serve as alternate hosts. However, the importance of these *Lygus* species in the ecosystems they belong to is poorly understood.

Although *P. relictus* was regularly obtained from non-target hosts, in particular *S. calcarata* and *C. norwegicus*, parasitism by *P. relictus* in most non-targets did not exceed 5%, except in *T. caelestialium*. These fairly low values are remarkable, because according to the laboratory results, Mirini were expected to be parasitized to a larger extent by *P. relictus*. However, *P. relictus* was not the primary contributor to parasitism of most non-target hosts studied and thus, it may be of minor importance for regulating

non-target populations. Only second generation *T. caelestia-lium* (the first generation was not sampled) was parasitized by *P. relictus* at a similar level as *Lygus*. In Poland, *T. caelestialium* was the only non-target mirid attacked by *P. relictus* (Bilewicz-Pawinska, 1982). However, the data presented were unclear, and despite the fact that the impact appeared to be low, estimation of the actual impact of *P. relictus* on *T. caelestialium* in that study was difficult. As the importance of *P. relictus* for regulating *Trigonotylus* in Europe is currently not clearly understood, the 13 *Trigonotylus* species native to Canada (Maw *et al.*, 2000) may potentially be affected by *P. relictus* and therefore, native *Trigonotylus* species should be considered as test species for host range studies under quarantine conditions in Canada.

A general problem in ecological host range studies is the availability of non-target hosts in the field. As long as non-target hosts are common and parasitoids can be reared in high numbers, the impact of the parasitoid may be easily assessed. However, problems arise when trying to estimate the impact of a parasitoid on rare hosts. For example, the Phylinae species *P. chrysanthemi* was only found at three sites, but at one of these sites, *P. relictus* amounted to 40% of all the parasitoids that emerged (16% parasitism). These results may show the potential of *P. relictus* to parasitize non-target hosts to a larger extent, but general conclusions for risk assessment are impossible as sample size is not representative.

A major concern regarding the introduction of exotic biological control agents is further potential harm to beneficial insects, such as the predatory mirid *Dicyphus hesperus* knight (subfamily Bryocorinae). In the present study, two specimens of *P. relictus* were reared from *D. globulifer*; however, recent laboratory tests conducted in the USA (Day & Fuester, 2003) indicate that *P. relictus* does not represent a threat to beneficial *Dicyphus* species.

Overall, *P. relictus* possesses many desirable qualities for release, e.g. facultative diapause, short developmental time, and high reproductive potential (Broadbent, 1976; Haye *et al.*, 2005a); however, with regard to potential non-target impacts, *P. relictus* is likely less favourable for introduction into Canada than the European *P. digoneutis*, which is rated as being more host specific than *P. relictus* (Bilewicz-Pawinska, 1982; Haye *et al.*, 2005b), which had caused no non-target effects after its release in the USA (Day, 2005), and which has already naturally dispersed into eastern Canada (Broadbent *et al.*, 1999).

Acknowledgements

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