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ARTICLE

A comparison of molecular screening *versus* dissection and rearing methods to estimate parasitism of *Lygus* nymphs by *Peristenus* wasps: size matters

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Abstract
Several broadleaf crops on the Canadian prairies suffer economic damage by *Lygus* bugs (Hemiptera: Miridae), populations of which are suppressed by parasitoids in the genus *Peristenus* (Hymenoptera: Braconidae). We used three different methods to assess levels of this parasitism for different instars of *Lygus* collected in southern Alberta, Canada. Screening *Lygus* for *Peristenus* DNA using a molecular polymerase chain reaction (PCR) method identified levels of parasitism ranging from 35 (second and third instars) to 47 (fifth instars) per cent. With dissection, parasitoids were recovered from 13 (second instars) to 44 (fifth instars) per cent of *Lygus*. For *Lygus* collected in the field and reared in the lab, *Peristenus* emerged from about 22 per cent of individuals. Our results show that use of PCR or dissection for fifth-instar larvae provides comparable estimates of parasitism. For earlier instars, PCR identifies levels of parasitism undetected using dissection – that is, 2.7-fold more for second instars in the present study. For the purposes of pest management and conservation biological control, dissection can provide adequate estimates of parasitism to inform a decision to reduce insecticides to protect *Peristenus* parasitoids.

Introduction

Feeding by *Lygus* bugs (Hemiptera: Miridae) causes economic damage to several broadleaf crops in North America. Affected crops include seed alfalfa, *Medicago sativa* (Fabaceae), faba bean, *Vicia fava* (Fabaceae), and canola, *Brassica rapa* and *Brassica napus* (Brassicaceae) (Otani and Cárcamo 2011). *Lygus* adults overwinter in leaf litter, emerge in spring to feed on the meristematic tissue of noncrop plants, and then move onto crops when the plants become suitable for feeding and oviposition. *Lygus* populations are bivoltine in southern regions of Canada and univoltine further north. Economically important *Lygus* species include *L. lineolaris* (Palisot de Beauvois), *L. borealis* (Kelton), *L. elisus* (Van Duzee), and *L. keltoni* (Schwartz), which vary in relative abundance with region, crop type, and stage (Cárcamo *et al.* 2002).

Lygus damage varies with crops. In canola, *Lygus* causes economic losses by reducing seed weight (ca. 200 kg/ha) when numbers reach more than three bugs per sweep at the early pod stage (Cárcamo *et al.* 2024). In faba beans, *Lygus* may not impact seed weight (Cárcamo, unpublished data), but even low abundance of one or fewer per sweep (Cárcamo, unpublished data) can downgrade seed quality by causing necrotic spots on seeds (Smith *et al.* 2019). In seed alfalfa, *Lygus* bugs are part of the pest complex that includes alfalfa weevil (*Hypera postica*) and alfalfa

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plant bug (*Adelphocoris lineolatus*), and together, they can drastically reduce yield (Soroka and Murrell 1993). Insecticides are often the only option for control, but those products lose efficacy when insecticide resistance develops (Menke 1954; Snodgrass 1996; Snodgrass *et al.* 2009; Dorman *et al.* 2020). For this reason, there has been sustained interest in the use of parasitoids as biocontrol agents to target the *Lygus* pest complex (Craig and Loan 1984; Broadbent *et al.* 2002, 2013; Seymour *et al.* 2005; Haye *et al.* 2006; Day 2007).

Lygus are parasitised by a complex of species in the genus *Peristenus* (Hymenoptera: Braconidae). Adult wasps oviposit in early-instar *Lygus* nymphs in the spring or summer, with the third-instar parasitoid larva emerging from older juvenile hosts to form a cocoon, pupate in the soil, overwinter, and then emerge as an adult (Brindley 1939; Loan 1965). Research on the use of *Peristenus* species as biological control agents has been hampered by a lack of taxonomic tools and knowledge on species diversity within the genus (Goulet and Mason 2006). Currently, membership in this species complex for the Canadian Prairies is recognised as containing *P. dayi* Goulet, *P. mellipes* (Cresson), and *P. howardi* Shaw (Zhang *et al.* 2017). In southern Alberta, *P. dayi* has a spring activity period on an unknown host, but it attacks mainly *Adelphocoris lineolatus* (Hemiptera: Miridae) and, to a lesser extent, first-generation nymphs of *Lygus*. *Peristenus mellipes* has a broader phenology from late spring to mid-summer and occurs mostly on the first *Lygus* generation (Loan 1965; Goulet and Mason 2006). The third species, *P. howardi*, emerges later in the season to attack *Lygus* nymphs of the second generation (Fernández *et al.* 2018). In the Pacific Northwest of the United States of America, *P. howardii* is bivoltine, or it is represented by two species (Seymour *et al.* 2005). In eastern Canada, *P. mellipes* has distinct temporally separated populations on the first and second generations of *Lygus lineolaris* (Carignan *et al.* 2007; Zhang *et al.* 2017), which were previously recognised as distinct species (*P. mellipes* and *P. pseudopallipes* (Loan); Goulet and Mason 2006).

Levels of parasitism by *Peristenus* can be assessed by dissecting *Lygus* nymphs to recover immature parasitoids, rearing *Lygus* for parasitoid emergence, or by using molecular polymerase chain reaction (PCR) methods to detect parasitoid DNA in the host. Dissection is the most convenient method (Day 2007) but is best done using fresh nymphs and is more prone to overlooking parasitoids at earlier stages of development. Host rearing requires holding nymphs for extended periods on suitable plant material until parasitoid emergence, and some nymphal mortality occurs during the process, affecting results. Use of PCR is time-consuming and requires specialised equipment and training; however, nymphs can be frozen or preserved in ethanol for processing, and PCR can identify higher levels of parasitism than either dissection or host rearing does. For *Lygus* collected in alfalfa fields near Saskatoon, Saskatchewan, Canada, *Peristenus* parasitism percentage was estimated by each method to be 71 per cent *via* PCR, 59 per cent *via* dissection, and 25 per cent *via* host rearing (Ashfaq *et al.* 2004). However, molecular screening does not necessarily provide the best estimates of parasitism. Dissection and PCR methods provided similar estimates of parasitism for *Lygus* in New York state, United States of America (Tilmon *et al.* 2000) and for mirid bugs in Europe (Gariepy *et al.* 2008). In their European study, Gariepy *et al.* (2008) suggested that parasitoids could be detected using PCR within the host at earlier stages of development, but they did not present data to support this assertion. Verifying whether the assertion is correct will aid understanding of parasitism in Western Canadian *Lygus* populations.

In the present study, we compared estimates of parasitism by *Peristenus* for different *Lygus* instars obtained using dissection *versus* PCR. To our knowledge, this type of comparison has not been done previously. We also assessed parasitism for some of the same collection sites using host rearing.

Materials and methods

Field collections

Samples of *Lygus* nymphs (mainly *L. keltoni*, with minor representation by *L. elisus* and *L. borealis*) were collected by sweep net from different sites in southern Alberta from 16 June

Table 1. Cultivated and uncultivated plants where *Lygus* nymphs were sampled to estimate parasitism by *Peristenus* wasps using dissection, rearing, and PCR screening in southern Alberta, Canada

Family	Species	Common name (*Lygus* sample)	Sample date
Amaranthaceae	*Chenopodium album* Linnaeus	Lamb's quarters (210)	17 July 2023
Brassicaceae	*Brassica napus* Linnaeus	Canola (428)	25, 27 July 2023
	Capsella bursa-pastoris Linnaeus	Shepherd's purse (58)	21 June 2022
	Descurainia sophia Linnaeus	Flixweed (82)	21 June 2022
	Neslia paniculate Linnaeus	Ball mustard (58)	8 July 2022
	Sinapis alba Linnaeus	Yellow mustard (71)	26 July 2022
	Sinapis arvensis Linnaeus	Wild mustard (63)	2 August 2023
Cannabaceae	*Cannabis sativa* Linnaeus	Hemp (578)	24 July, 2 August 2023
Fabaceae	*Medicago sativa* Linnaeus	Alfalfa (683)	16, 21, 28 June 2022; 19, 29 June, 27 July 2023
	Onobrychis viciifolia Scop.	Sainfoin (361)	17, 29 June 2022; 15, 19 June 2023
	Melilotus spp. + *M. sativa* Linnaeus	Clover–alfalfa (44)	13 July 2023
Linaceae	*Linum usitatissimum* Linnaeus	Flax (68)	18 July 1023

through 26 July 2022 and from 15 June through 17 August 2023. Previous studies by Fernández *et al.* (2018) identified these periods as the ideal times to sample the primary *Peristenus* parasitoids occurring on the first and second generations of *Lygus* bugs when older instars are sufficiently abundant. Host plants included a mix of field and forage crop and weedy species (Table 1), documented during a more extensive ongoing survey, based on dissections, to be reported elsewhere (Pawluk, Ibarra-Galvis, Roy, and Cárcamo, unpublished data). The range of dates and host plants, shown in Table 1, allowed for recovery of different instars and an expected range of low to high levels of parasitism.

Dissection method

Field-collected nymphs were stored in a 0.5-L plastic container with romaine lettuce at 12 °C for 24–48 hours before dissection. Ethanol diluted to 20–50% was used to anesthetise the nymphs in Petri dishes immediately before dissection with insect pins under a stereoscope at 10–20× magnification. The number of nymphs dissected for a sample ranged from 8 to 74. The number of parasitoid larvae were recorded and stored in 95% ethanol.

PCR method

In preliminary work, we compared the methods described by Tilmon *et al.* (2000) and Erlandson *et al.* (2003) to detect *Peristenus* developing in *Lygus* hosts. Both methods use primer pairs specific to *Peristenus* DNA. However, the Tilmon method uses a primer pair that amplifies a region of the mitochondrial gene cytochrome *c* oxidase 1 (*CO*1), whereas the Erlandson method uses a primer pair that amplifies the internal transcribed spacer region between the 5.8*S* and 18*S* nuclear rRNA genes (*ITS*2). When testing the two methods using the same sample template DNA, we found the Tilmon method to be more sensitive for detecting *Peristenus* DNA, an observation that Erlandson *et al.* (2003) also made. For this reason, we used the Tilmon method in the present study.

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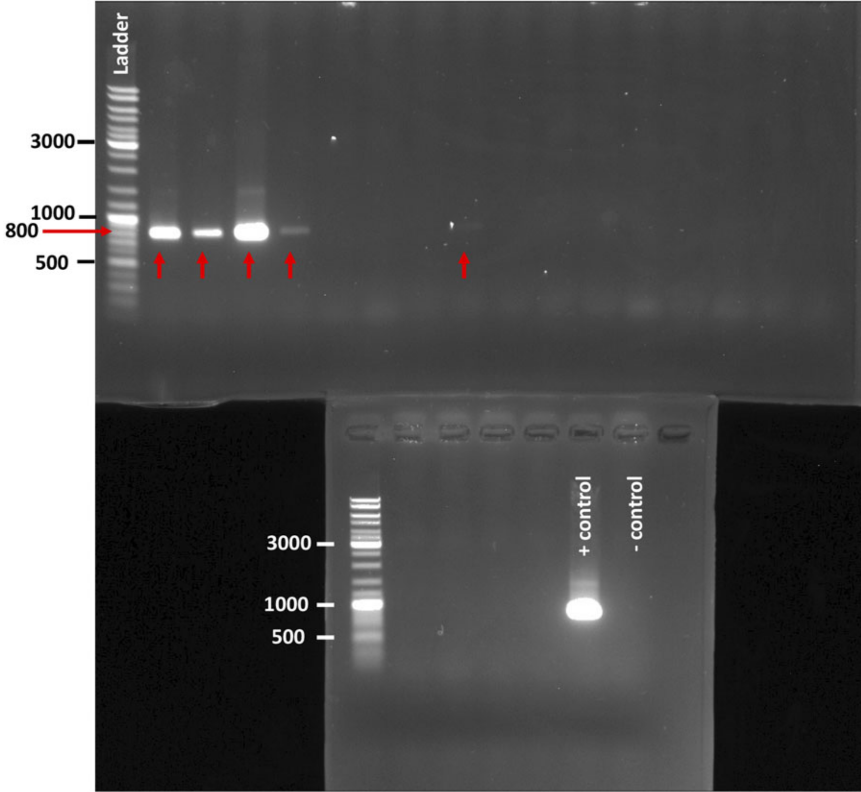

Figure 1. Polymerase chain reaction results for 20 samples of second-instar *Lygus* screened for the presence of *Peristenus* parasitoids using the primers Peristenus Ia and TL2-N-3014. When present, *Peristenus* DNA amplifies to form a band of 820 bp. Samples 1–4 and 8 (very faint) produced bands of the correct size and were considered positive for *Peristenus* (red arrows). All other samples were considered negative. No sample was run on the last lane (to the right of the negative control).

Field-collected nymphs were preserved in 95% EtOH shortly after they were collected. Before DNA extraction, nymphs were surface-sterilised by three washes with distilled water. The DNA was then extracted from individual nymphs using DNeasyVR Blood and Tissue kits (Qiagen Group, Hilden, Germany) as per manufacturer protocols. Amplification of *Peristenus* DNA was performed using the *Peristenus* Ia primer in conjunction with the primer, TL2-N-3014 (Fig. 1) and methods as described by Tilmon *et al.* (2000).

Host rearing method

Field-collected nymphs of mixed instars (second to fifth) were reared in 0.75-L (≤ 50 nymphs) or 5-L (> 50 nymphs) containers equipped with meshed screens at the bottom to contain the larger *Lygus* juveniles and allow *Peristenus* larvae to fall onto a container with fine, moist vermiculite in which they formed cocoons (Whistlecraft *et al.* 2010). Nymphs were fed romaine lettuce twice weekly until they died or reached the adult stage. Rearing took place at room temperature (22 °C), under a photoperiod of 16:8 hours light:darkness and relative humidity of approximately 40%. Parasitoid larvae were allowed to mature in the rearing containers for the remainder of the summer. In September and October, the vermiculite in each Petri dish was searched, and the number of cocoons were counted using a stereoscope at 10× magnification.

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Statistical analysis

For comparison between dissection and PCR methods, we paired samples of *Lygus* for instar, host plant, and date and location of collection. The mean ± number of nymphs per sample was as follows: dissection – 12.6 ± 1.0 (range: 8–74); PCR – 9.9 ± 0.05 (range: 7–10). The number of parasitised individuals per sample was converted into percentage parasitism. We then tested for an effect of method on percentage parasitism using Wilcoxon signed-rank tests (critical P-value = 0.05). We repeated this analysis for second, third, fourth, and fifth instars and for all samples combined.

For comparison between dissection and host-rearing methods, we used a different set of *Lygus* samples. These samples were paired for instar (several instars/sample), host plant, and date and location of collection. The mean ± number of nymphs per sample was as follows: dissection – 27.4 ± 2.0 (range: 10–50); rearing – 47.7 ± 10.3 (range: 10–162). The number of parasitised individuals per sample was converted into percentage parasitism. We then tested for an effect of method on percentage parasitism using Wilcoxon signed-rank tests (critical P-value = 0.05).

With each Wilcoxon signed-rank test, we also performed a Pearson correlation to assess how well estimates of percentage obtained by using dissection predicted results obtained by using either PCR or host rearing.

All analyses were performed using Systat, version 13 (Systat Software, Inc, San Jose, California, United States of America; https://systatsoftware.com/).

Results and discussion

Of the three methods examined, PCR detected overall higher levels of parasitism than did dissection, which detected higher levels of parasitism than did host rearing. When PCR was compared with dissection, the difference was significant ($P \leq 0.028$) for second, third, and fourth instars and for all instars combined, but no significant differences were found for fifth instars (Fig. 2; Table 2). This pattern is explained easily: parasitoids in early-instar nymphs are more often present as eggs or first instars and are more likely to be overlooked when hosts are dissected. When dissection was compared with host rearing, mean (± standard error) percentage parasitism was 31.1 (± 4.6) *versus* 19.8 (± 4.7), respectively (Wilcoxon signed-rank test: Z = –1.834, $P = 0.067$). This same order of sensitivity was reported for a survey of *Lygus* parasitism in Saskatchewan that did not assess parasitism of different host instars (Ashfaq *et al.* 2004).

Pearson's correlation values provide further insight into the values of the three methods used in the present study. For estimates of parasitism obtained using dissection *versus* PCR of samples within instars and combined across instars, values ranged from 0.633 to 0.804 ($P \leq 0.002$; Fig. 2). Thus, even though dissection underestimated parasitism, the two methods similarly ranked samples by percentage parasitism. In contrast, the value for estimates of parasitism obtained using dissection *versus* rearing was 0.316 ($P = 0.116$): percentage parasitism estimated from reared hosts did not reflect parasitism in paired samples of dissected hosts, and rearing therefore should be used only when adult *Peristenus* specimens are required.

Use of PCR *versus* dissection also clarifies when the host is parasitised (Table 1). With PCR, estimates of parasitism were unaffected by instar stage (Kruskal–Wallis test statistic = 1.838, $df = 3$, $P = 0.607$). This finding is consistent with reports that parasitism of hosts normally occurs in second and third instars (Loan 1965). With dissection, estimates of parasitism increased with instar (Kruskal–Wallis test statistic = 9.353, $df = 3$, $P = 0.025$), which could incorrectly be interpreted as evidence that cohorts of *Lygus* become more parasitised with maturity. In the present study, we collected at multiple time periods for the first and second generations of *Lygus* bugs and pooled all the results shown in Table 2. Because of this, we expected no significant differences among instars, as confirmed by the statistical test of samples from the PCR screening. With limited dates of sampling for a given generation of *Lygus* nymphs, one would expect major

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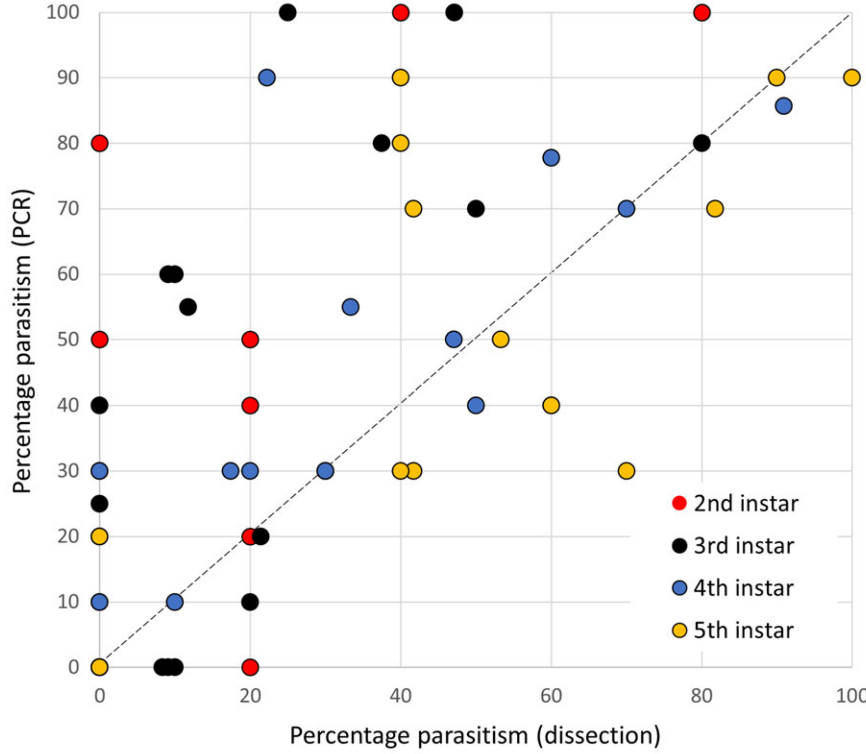

Figure 2. Parasitism by *Peristenus* for different instars of *Lygus*. Each data point is based on a sample of *Lygus* for which a subset of individuals (typically 10) was assessed for parasitism either by host dissection or using polymerase chain reaction.

differences of parasitism among instars depending on when the parasitoid adults were active and the age distribution of nymphs, as shown in Goulet and Mason (2006). In our case, both methods suggested that we sampled at the early spectrum of the oviposition period because the highest incidence of parasitism occurred in the older fourth- and fifth-instar nymphs. A peak of parasitism in the younger instars would indicate sampling of nymphs too late in the season for a given site. This type of data is further complicated by the tendency of *Lygus* to be attracted to plants during their reproductive stage when flowering is more advanced, so that temperature alone may not accurately predict their phenology (Cárcamo, unpublished data). Detailed phenological studies among adjacent crops planted at different dates would elucidate these interactions.

Differences in parasitism levels among *Lygus* instars can be further confounded by host–parasitoid interactions. In Day's (2007) study with *L. lineolaris* and *P. digoneutis* in New Jersey and with *L. hesperus* and *P. howardi* in Idaho, United States of America, parasitism was significantly higher in fourth instars than in fifth instars and also in fourth instars than in earlier first and second instars. The low levels of parasitism in the youngest instars was attributed to limited exposure time relative to older instars and was similar to the pattern that we observed in southern Alberta for *P. howardi* and our *Lygus* species complex dominated by *L. keltoni*. According to Day (2007), possible encapsulation and killing of *Peristenus* larvae by fifth instars of the two *Lygus* species may explain the pattern of lower parasitism in fifth instars relative to fourth instars. In the present study, PCR screening and dissections estimated higher parasitism in fifth instars than in younger nymphal stages. It therefore appears that encapsulation of our local *P. mellipes* and *P. howardi* does not occur to a major extent by the local assemblage of *Lygus* pests in southern Alberta.

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Table 2. Percentage parasitism (mean ± standard error) by *Peristenus* wasps for different instars of *Lygus* bugs. Comparisons are made between paired samples for which the host was either dissected or screened for *Peristenus* DNA using a polymerase chain reaction (PCR) method (Wilcoxon signed-rank test)

Instar	n*	Dissection	PCR	*Z*-value	*P*-value
2	17	12.9 ± 5.1	35.3 ± 8.9	2.809	0.005
3	25	18.4 ± 4.4	34.4 ± 6.6	2.681	0.007
4	16	28.2 ± 7.1	38.0 ± 7.7	2.197	0.028
5	15	43.9 ± 8.6	47.3 ± 8.3	0.511	0.609
Total	73	24.5 ± 3.2	38.1 ± 3.9	4.254	< 0.001

*Number of pairwise comparisons for samples typically containing 10 individuals (dissection: range of 8–74; PCR: range of 7–10).

Even with its greater ability to detect parasitoids, the PCR method used in the present study may still underestimate parasitism in *Lygus* nymphs, depending on the geographic region and the age of the parasitoid egg. The primers used were designed to detect *Peristenus* DNA and were validated during primer development using *P. rubricollis* (Thomson) (as *P. conradi* Marsh), *P. pallipes* (as either *P. dayi*, *P. mellipes*, or *P. howardi*), and *P. digoneutis* Loan (Tilmon *et al.* 2000). In eastern North America, several species of *Leiophron* (Hymenoptera: Braconidae) also parasitise *Lygus* nymphs (Goulet and Mason 2006), and studies conducted in that region using the primers we used in the present study would not detect those species. In Alberta, parasitoids of *Lygus* may include, albeit at low levels, tachinid flies (Clancy and Pierce 1966) that also would go undetected. Furthermore, parasitoid eggs less than three days old may not be detected consistently by PCR methods, as was shown in Gariepy *et al.*'s (2008) study with European *Peristenus*. We note that none of the three methods examined in the present study would have detected parasitism of *Lygus* eggs (Cárcamo *et al.* 2008) or adults (Day 1995). Eggs may be parasitised by *Anaphes*, *Telenomus*, and *Polynema* wasps at levels below 5% (Cárcamo *et al.* 2008), and adults can be parasitised, although very rarely, by other wasps and tachinid flies (Clancy and Pierce 1966, Day 1995; Broadbent *et al.* 2002).

Dissection and rearing, despite their lower accuracy, may still be required in addition to PCR screening to gain a more complete ecological understanding of the trophic interactions of parasitoids and their prey. For taxonomic studies of species that lack prior genomic inventories and primers, rearing parasitoids to the adult stage is the only option to identify them using morphological keys (Goulet and Mason 2006). For species that suffer high mortality during the rearing of the hosts, such as *Lygus* nymphs, and during the overwintering stage of the parasitoid (Gariepy *et al.* 2005), the best option is to rear large numbers of individuals under conditions that avoid crowding and to ensure proper moisture during and after overwintering. For native *Peristenus* of southern Alberta, we observed around 50% mortality during the cocoon stage, even before overwintering (Daniels, Cárcamo, and Ibarra-Galvis, unpublished data). Dissection is also the only method currently available to document cases of multiple parasitism of a *Lygus* nymph, which we have infrequently observed during our research (Pawluk, Ibarra-Galvis, Roy, and Cárcamo, unpublished data). This type of information, along with the relative size of the parasitoid, which is important for detailed study of phenology, would be missed if we relied solely on the use of PCR screening.

Conclusion

Previous studies by Ashfaq *et al.* (2004) and Gariepy *et al.* (2005, 2008) established the superior accuracy of PCR screening over dissection or rearing of *Lygus* nymphs to estimate *Peristenus*

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parasitism. In the present study, we demonstrate that the advantage of the PCR method is specific to the estimation of parasitism in younger nymphs – in particular, second instars. For the largest fifth instars and even for fourth instars, dissection can provide similar estimates at a fraction of the cost and with fewer human resources. For detailed phenological studies requiring assessment of parasitism by all instars of the host, including very small ones, PCR or other molecular screening methods are clearly ideal. If the goal is to assess only baseline levels of parasitism for integrated pest management or before or after release of an exotic biocontrol agent, then repeated time sampling and dissection of the older fourth and fifth instars from all *Lygus* generations should suffice. Barcoding tools can be further combined to determine the species composition of dissected samples (kept in 95% ethanol or frozen) for fauna with accurate genomic inventories.

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References

Ashfaq, M., Braun, L., Hegedus, D., and Erlandson, M. 2004. Estimating parasitism levels in *Lygus* spp. (Hemiptera: Miridae) field populations using standard and molecular techniques. Biocontrol Science and Technology, **14**: 731–735.

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

Broadbent, A.B., Haye, T., Gariepy, T.D., Olfert, O., and Kuhlmann, U. 2013. *Lygus lineolaris* (Palisot), tarnished plant bug (Hemiptera: Miridae). Chapter 31. *In* Biological Control Programmes in Canada 2001–2012. *Edited by* P.G. Mason and D.R. Gillespie. CABI Publishing, Wallingford, Oxfordshire, United Kingdom. Pp. 221–227.

Broadbent, A.B., Mason, P.G., Lachance, S., Whistlecraft, J.W., Soroka, J.J., and Kuhlmann, U. 2002. Chapter 32. *Lygus* spp., plant bugs (Hemiptera: Miridae). *In* Biological Control Programmes in Canada, 1981–2000. *Edited by* P.G. Mason and J.T. Huber. CABI Publishing, Wallingford, Oxon, United Kingdom. Pp. 152–159.

Cárcamo H., Herle, C., Schwinghamer, T., Robinson, S., Reid, P., Gabert, K., *et al.* 2024. Revising economic injury levels for *Lygus* spp. in canola: the value of historical yield and insect data to improve decision making. Crop Protection, **176**: 106467.

Cárcamo, H., Huber, J., Larson, T., and Bourchier, R. 2008. Egg parasitoids of *Lygus* bugs in southern Alberta. Proceedings of the second international *Lygus* symposium, Asilomar Conference Grounds, Pacific Grove, California, April 15–19 2007. Journal of Insect Science, **8**: 49.

Cárcamo, H., Otani, J., Herle, C., Dolinski, M., Dosdall, L., Mason, P., *et al.* 2002. Variation of *Lygus* species assemblages in canola agroecosystems in relation to ecoregion and crop stage. The Canadian Entomologist, **134**: 97–111. https://doi.org/10.4039/Ent13497-1.

Carignan, S., Stewart, R.K., Godin, C., and Boivin, G. 2007. Parasitism activity of *Peristenus* spp. (Hymenoptera: Braconidae) on *Lygus lineolaris* (Hemiptera: Miridae) nymphs prior to the establishment of *P. digoneutis* in southwestern Quebec. Biocontrol Science and Technology, **17**: 623–633.

Clancy, D.W. and Pierce, H.D. 1966. Natural enemies of some *Lygus* bugs. Journal of Economic Entomology, **59**: 853–858. https://doi.org/10.1093/jee/59.4.853.

Craig, C.H. and Loan, C.C. 1984. *Lygus* spp., plant bugs (Heteroptera: Miridae). Chapter 14. *In* Biological Control Programmes against Insects and Weeds in Canada 1969–1980. *Edited by* J.S. Kelleher and M.A. Hulme. Commonwealth Agricultural Bureaux, Famham Royal, United Kingdom. Pp. 45–47.

Day, W.H. 1995. Biological observations on *Phasia robertsonii* (Townsend) (Diptera: Tachinidae), a native parasite of adult plant bugs (Hemiptera: Miridae) feeding on alfalfa and grasses. Journal of the New York Entomological Society, **103**: 100–106.

Day, W.H. 2007. Effect of host instar on measuring parasitism of *Lygus* spp. (Hemiptera: Miridae) nymphs by *Peristenus* spp. (Hymenoptera: Braconidae). Environmental Entomology, **36**: 1154–1158.

Dorman, S.J., Gross, A.D., Musser, F.R., Catchot, B.D., Smith, R.H., Reisig, D.D., *et al.* 2020. Resistance monitoring to four insecticides and mechanisms of resistance in *Lygus lineolaris* Palisot de Beauvois (Hemiptera: Miridae) populations of southeastern USA cotton. Pest Management Science, **76**: 3935–3944.

Erlandson, M., Braun, L., Baldwin, D., Soroka, J., Ashfaq, M., and Hegedus, D. 2003. Molecular markers for *Peristenus* spp. (Hymenoptera: Braconidae) parasitoids associated with *Lygus* spp. (Hemiptera: Miridae). The Canadian Entomologist, **135**: 71–83. https://doi.org/10.4039/n02-017.

Fernández, D.C., Laird, R., Herle, C., Goulet, H., and Cárcamo, H. 2018. Seasonality and species composition of *Peristenus* (Hymenoptera: Braconidae) species, and *Lygus* (Hemiptera: Miridae) species parasitism in southern Alberta, Canada. Biocontrol Science and Technology, **28**: 702–717.

Gariepy, T., Kuhlmann, U., Gillott, C., and Erlandson, M. 2008. A large-scale comparison of conventional and molecular methods for the evaluation of host–parasitoid associations in non-target risk-assessment studies. Journal of Applied Ecology, **45**: 708–715.

Gariepy, T.D., Kuhlmann, U., Haye, T., Gillott, C., and Erlandson, M. 2005. A single-step multiplex PCR assay for the detection of European *Peristenus* spp., parasitoids of *Lygus* spp. Biocontrol Science and Technology, **15**: 481–495.

Goulet, H. and Mason, P.G. 2006. Review of the Nearctic species of *Leiophron* and *Peristenus* (Hymenoptera: Braconidae: Euphorinae) parasitizing *Lygus* (Hemiptera: Miridae: Mirini). Zootaxa, **1323**: 11–118.

Haye, T., Kuhlmann, U., Goulet, H., and Mason, P.G. 2006. Controlling *Lygus* plant bugs (Heteroptera: Miridae) with European *Peristenus relictus* (Hymenoptera: Braconidae) in Canada: risky or not? Bulletin of Entomological Research, **96**: 1187–1196.

Loan, C.C. 1965. Life cycle and development of *Leiophron pallipes* Curtis (Hymenoptera: Braconidae: Euphorinae) in five mirid hosts in the Belleville district. Proceedings of the Entomological Society of Ontario, **95**: 1115–1121.

Menke, H.F. 1954. Indications of *Lygus* resistance to DDT in Washington. Journal of Economic Entomology, **47**: 1704–1705.

Otani, J. and Cárcamo, H. 2011. Biology and management of *Lygus* in canola. Prairie Soils and Crops, **4**: 142–153.

Soroka, J.J. and Murrell, D.C. 1993. The effects of alfalfa plant bug (Hemiptera: Miridae) feeding late in the season on alfalfa seed yield in northern Saskatchewan. The Canadian Entomologist, **125**: 815–824. https://doi.org/10.4039/Ent125815-5.

Seymour, L.M., Mowry, T.M., Day, W.H., and Barbour, J.D. 2005. Parasitism of *Lygus* spp. nymphs by the parasitoid wasp, *Peristenus howardi*, in the alfalfa seed-growing region of the Pacific Northwest. Journal of Insect Science, **5**: 144.

Smith, A.M., Rivard, B., Feng, J., and Cárcamo, H.A. 2019. Quantifying *Lygus* (Hemiptera: Miridae) damage in faba bean (Fabaceae) seeds using shortwave-infrared imaging. The Canadian Entomologist, **151**: 1442–1455. https://doi.org/10.4039/tce.2019.28.

https://doi.org/10.4039/tce.2024.24 Published online by Cambridge University Press

Snodgrass, G.L. 1996. Insecticide resistance in field populations of the tarnished plant bug (Heteroptera: Miridae) in cotton in the Mississippi delta. Journal of Economic Entomology, **89**: 1783–1790.

Snodgrass, G.L., Gore, J., Abel, C.A., and Jackson, R. 2009. Acephate resistance in populations of the tarnished plant bug (Heteroptera: Miridae) from the Mississippi River Delta. Journal of Economic Entomology, **102**: 1699–1707.

Tilmon, K.J., Danforth, B.N., Day, W.H., and Hoffmann, M.P. 2000. Determining parasitoid species composition in a host population: a molecular approach. Annals of the Entomological Society of America, **93**: 1640–1647.

Whistlecraft, J.W., Haye, T., Kuhlmann, U., Muth, R., Murillo, H., and Mason, P. 2010. A large-scale rearing method for *Peristenus digoneutis* (Hymenoptera: Braconidae), a biological control agent of *Lygus lineolaris* (Hemiptera: Miridae). Biocontrol Science and Technology, **20**: 1923–1937.

Zhang, Y.M., Ridenbaugh, R.D., and Sharanowski, B.J. 2017. Integrative taxonomy improves understanding of native beneficial fauna: revision of the Nearctic *Peristenus pallipes* complex (Hymenoptera: Braconidae) and implications for release of exotic biocontrol agents. Systematic Entomology, **42**: 596–608.

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