

Ovipositor characteristics differ between two parasitoids (Hymenoptera, Figitidae) of *Drosophila suzukii* (Diptera, Drosophilidae) in an adventive landscape

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Academic editor: Elijah Talamas | Received 8 September 2022 | Accepted 31 January 2023 | Published 17 February 2023

<https://zoobank.org/D4BF1C35-568A-4116-8890-B83B85D2B923>

Citation: Earley NG, Abram PK, Lalonde RG, Moffat CE (2023) Ovipositor characteristics differ between two parasitoids (Hymenoptera, Figitidae) of *Drosophila suzukii* (Diptera, Drosophilidae) in an adventive landscape. Journal of Hymenoptera Research 95: 13–30. <https://doi.org/10.3897/jhr.95.89678>

Abstract

Different ovipositor characteristics among parasitoid species that share similar niches are associated with different wasp life histories and selective pressures. The length of wasp ovipositors, for example, can determine the accessibility of hosts that feed at different depths within food substrates. Two parasitoids, *Ganaspis brasiliensis* and *Leptopilina japonica* (Hymenoptera, Figitidae), which attack *Drosophila suzukii* (Diptera, Drosophilidae) in their native range, have been investigated for their suitability for the global biological control of the small fruit pest. Despite their sympatry in microhabitat, the parasitoids have differing host ranges, and *D. suzukii* parasitism rates by each parasitoid species appear to depend on the fruit species occupied by the host species. Adventive populations of both parasitoids have been detected in the Pacific Northwest of Canada and the United States where they can be found parasitizing *D. suzukii* larvae in crop and non-crop fruits. We dissected and measured the ovipositors of parasitoids reared from three species of fresh fruits at three sites in southwestern British Columbia, Canada, and investigated the influence of parasitoid species, fruit type, and collection site on ovipositor characteristics. We found that ovipositor length differed markedly between the two parasitoid species and between sites while ovipositor width, and stoutness, differed only between the two parasitoid species, but did not vary among sites or fruit hosts. We discuss how ovipositor morphology traits could be associated with differences in life history and host ranges in the two parasitoid species.

Keywords

biological control, competition, *Ganaspis brasiliensis*, *Leptopilina japonica*, morphology, niche partitioning, spotted-wing drosophila

Introduction

Parasitoid reproductive success depends on access to and successful exploitation of available hosts. For parasitoid Hymenoptera, the morphology of ovipositors used to lay their eggs into or onto hosts, is critical for the effective exploitation of hosts within structural niches (Le Ralec et al. 1996; Quicke et al. 1999; Vilhelmsen and Turrisi 2011). One way that insect herbivores can reduce attack by parasitoids is by feeding deep within the confines of plant structures like galls, fruits, or tree trunks. Select parasitoid species are able to circumvent this type of host defence by accessing their hosts earlier in host development in smaller plant structures (a phenological adaptation, e.g. Weis and Abrahamson 1985), while others overcome this defence using long ovipositors that can access hosts deep inside larger plant structures (a morphological adaptation, e.g. Weis and Abrahamson 1985). Hosts can further escape pressures imposed by previously associated parasitoids by occupying larger plant structures like cultivated fruits (e.g. Feder 1995) or feeding on earlier phenological stages of their host plants (e.g. ripening as opposed to rotting fruit). Some of these shifts in host feeding behaviour could engender costs for the host, which would be balanced against the benefit of decreased parasitism risk (Gratton and Welter 1999). In addition, these differences in feeding niche could hypothetically spur the evolution of modified ovipositors in parasitoids that allow them to effectively access hosts in these novel environments (Vermeij 1999; Sivinski et al. 2001; Sivinski and Aluja 2003).

Constraints imposed by parasitoid ovipositor length can affect the success of biological control of non-native pests such as the olive fruit fly *Bactrocera oleae* Rossi (Diptera: Tephritidae) in the California olive agricultural system (Sime et al. 2007; Wang et al. 2008, 2009). Here, larger fruits are preferred by growers and this bias in cultivar choice has been shown to prevent certain parasitoids (Hymenoptera: Braconidae) with shorter ovipositors from accessing their hosts (Sime et al. 2007; Wang et al. 2008, 2009). Parasitoid species with shorter ovipositors are less effective at controlling the pest larvae in larger fruits, while those species with longer ovipositors can access host larvae more effectively in larger fruits (Sime et al. 2007; Wang et al. 2008, 2009). Parasitoids in this system with longer ovipositors also have broader host ranges; a consideration that complicates the biological control of *B. oleae* in an agricultural system that selects for larger fruits (Sime et al. 2007; Wang et al. 2008, 2009). Thus, the success of a biological control programme could be reduced by physical barriers to parasitism.

The behaviour of host larvae can also influence the success of parasitism, by evading detection by parasitoids or by allowing larvae to escape parasitism when first encountered by a parasitoid (Gross 1993; Kacsoh et al. 2013; Robertson et al. 2013; Singh et al. 2015). Larvae living in semi-concealed environments may escape

parasitism by diving deeper into their developmental substrate (van Lenteren et al. 1998). In order to parasitize mobile hosts that attempt to escape parasitism, Figitidae (Hymenoptera) that parasitize semi-concealed dipterous larvae have an ovipositor clip (Buffington 2007), a morphological feature that restrains host larvae prior to their envenomation (van Lenteren et al. 1998). *Drosophila* Fallén (Diptera: Drosophilidae) larvae have differing foraging behaviours within their developmental substrate where some (e.g. *D. melanogaster* Meigen) feed relatively close to the surface while others (e.g. *D. suzukii* Matsumura), that typically develop in deeper substrates such as ripening fruits, dive deeper into the substrate more frequently and for longer (Kim et al. 2017). Differential diving behaviours of *Drosophila* larvae could have implications for parasitoid access to hosts.

Two parasitoid wasp species, *Leptopilina japonica* Novković & Kimura and *Ganaspis brasiliensis* Ihering (Hymenoptera: Figitidae), have been proposed as candidate classical biological control agents for the small fruit pest *D. suzukii* (Lee et al. 2019). These parasitoids have recently been detected in North America, specifically in southwestern British Columbia (BC), Canada (Abram et al. 2020) and northwestern Washington State, USA (Beers et al. 2022). *Leptopilina japonica* has the broader host range of the two parasitoids and successfully parasitizes several drosophilid species in four genera in laboratory trials (Kimura and Novković 2015; Girod et al. 2018b; Daane et al. 2021). The lineage of *G. brasiliensis* recorded in BC and Washington State (“G1”; Beers et al. 2022) has a narrower host range, primarily attacking flies in the subgenus *Sophophora* Sturtevant in laboratory trials (Girod et al. 2018b; Giorgini et al. 2019; Daane et al. 2021). These parasitoids are adventive in the fruit growing regions of southwestern BC but have evidently not dispersed into the province’s interior (Abram et al. 2022a, b).

The sympatry of parasitoids exploiting the same host in the same habitat can lead to competition between parasitoids, which typically manifests as either competition between adult parasitoids for mating or oviposition resources (extrinsic competition) and/or competition between immature parasitoids competing for host resources (intrinsic competition) (Harvey et al. 2013; Ode et al. 2022). The inability of a developing parasitoid to compete in intrinsic competition within the host can be mitigated by extrinsic competition by the adult, and vice versa (Hood et al. 2021). Extrinsic and intrinsic competition may influence niche partitioning by parasitoids that exploit the same host in the same habitat by influencing their exploitation of hosts in unrecognized microhabitats (e.g. Heatwole et al. 1964; Heatwole and Davis 1965). The competitive interactions between *L. japonica* and *G. brasiliensis* are beginning to be characterized. While *G. brasiliensis* avoids hyperparasitizing hosts (Wang et al. 2019), *L. japonica* does not avoid such intrinsic competition (Böttinger et al. 2019) and is a superior intrinsic competitor in hyperparasitized larvae than is *G. brasiliensis* if hyperparasitism occurs within 24 hours of the initial parasitism event (Wang et al. 2019). Therefore, extrinsic competition by *G. brasiliensis* through the exploitation of host larvae in media that is not accessible to ovipositing *L. japonica* could facilitate host partitioning in this system.

In a field survey of fresh fruit collections in southwestern BC, *L. japonica* and *G. brasiliensis* made up 67.2% and 32.0% of the larval parasitoid community, respectively (Abram et al. 2022a). In the adventive range, *L. japonica* appears to cause higher percent parasitism of *D. suzukii* in larger fruits (e.g. strawberries, *Fragaria* spp.; blackberries, *Rubus* spp.) whereas *G. brasiliensis* appears to impose higher percent parasitism than *L. japonica* in smaller fruits (e.g. elderberries, *Sambucus* spp.; Abram et al. 2022a). In fresh fruit collections in China, *G. brasiliensis* was better able to parasitize *D. suzukii* in smaller fruits than in larger fruits with a parasitism rate that was higher in *Sambucus adnata* Wallich than in *Rubus foliosus* Weihe or *Rubus niveus* Thunberg, respectively (Giorgini et al. 2019). Abram et al. (2022a) observed differences in larval *D. suzukii* attack by adventive parasitoids that were similar to those observed in California olives (Sime et al. 2007; Wang et al. 2008, 2009), where higher parasitism rates were observed in parasitoid species with broader host ranges, and these tended to be associated with larger fruits. Additionally, Fellin et al. (2023) found that the G1 lineage of *G. brasiliensis* that was released to control *D. suzukii* in Italy have significantly shorter ovipositors than the adventive Italian *L. japonica*. It is possible that different ovipositor characteristics may affect host-choice and facilitate coexistence through niche partitioning (Price 1972) in the *D. suzukii* biological control system.

The objective of this study was to quantify differences in ovipositor characteristics of *L. japonica* and *G. brasiliensis* in BC, Canada. First, based on previously described associations between host range breadth and ovipositor length in other parasitoid guilds, we predicted that *L. japonica* would have longer ovipositors than *G. brasiliensis*, as longer ovipositors may be more efficient for indiscriminately parasitizing larvae regardless of their species, or level of prior parasitism. Additionally, we predicted that the apparently more specialized *G. brasiliensis* would have significantly stouter ovipositors (ovipositor width/ovipositor length) than do *L. japonica*. Stouter ovipositors may be better able to puncture fruits at an earlier stage of ripeness with *D. suzukii* developing inside; thus, further reducing the probability of mortality through lethal intra- and interspecific competition not only spatially (within a fruit) but also temporally (over the ripening period of a fruit).

Methods

Field sampling

Our samples were taken from fresh fruit collections by Abram et al. (2022a) at field sites throughout the lower mainland of southwestern BC, Canada, from late May to late October, 2020 (Fig. 1). Fruit collections at a site began when the first ripe fruit of the target fruit species or variety were available to pick and ended when fruit was scarce and remaining fruit was rotten or desiccated. We collected samples of three fruit species (cultivated blackberry, *R. fruticosus* agg. L.; Himalayan blackberry, *R. armeniacus* Focke; and red elderberry, *S. racemosa* L.). The number of berries collected was not constant for each host plant because fruit size varied from plant to plant and the aim

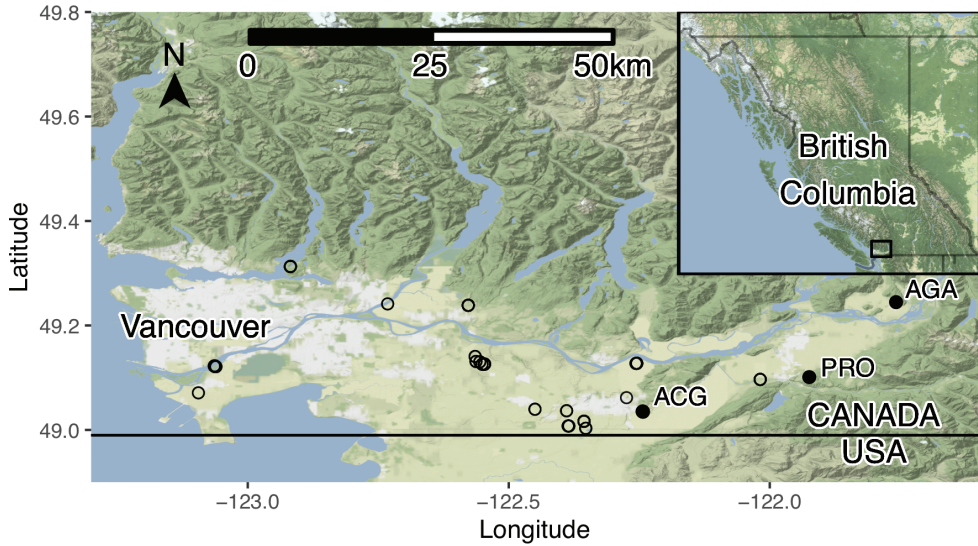


Figure 1. Map of sites in the lower mainland, British Columbia, Canada, where fresh fruits were sampled and insect inhabitants were reared and identified throughout the growing season of 2020. Solid circles with site IDs represent collection sites where the ovipositors of a subsample of female figitid emergents were removed and measured. Open circles represent all collection sites in Abram et al. (2022b). The box in the inset shows where the study area is located in British Columbia, Canada. WGS84 projected map made in ggmap v.3.0.0 (Kahle and Wickham 2013) with map tiles by Stamen Design, under CC BY 3.0.

of each collection was to collect a similar net fruit weight. See Abram et al. (2022a) for more detailed collection procedures, but briefly, we made approximately weekly fresh fruit collections at sites over the fruiting period of a mixture of cultivated and non-cultivated plants from a range of habitat types (e.g. community garden, experimental farm, mid-elevation forest). We did not record individual fruit characteristics (size, weight, depth, etc.).

Fruit rearing

Fruit samples were held in ventilated $12 \times 12 \times 8$ cm plastic containers (Ziploc, SC Johnson, Racine, WI) as described in Abram et al. (2022b) and monitored every 2–3 days for adult *D. suzukii*, *L. japonica*, and *G. brasiliensis* emergence. Due to SARS-CoV-2 pandemic restrictions, samples were reared in uncontrolled rearing rooms where temperatures ranged from 15 °C to 25 °C (ACG rearing room: 19 °C, 21 °C, 20 °C; PRO and AGA rearing room: 15 °C, 25 °C, 22 °C; minimum, maximum, and mean temperatures, respectively). Held at constant temperature, the developmental extremes of *D. suzukii* are close to 10 and 30 °C (Tochen et al. 2014) and *L. japonica* and *G. brasiliensis* have been shown to complete development between 17.2 and 27.5 °C (Hougardy et al. 2019). Abram et al. (2022a) reported no evidence for parasitoids having entered diapause and the PRO and AGA rearing room only dropped below 17 °C for 2 days. See Abram et al. (2022b) for more detailed rearing procedures.

Dissections

We selected wasps from fruit collections with high female emergence of both parasitoid species at locations with collections of *Rubus armeniacus* Focke, *R. fruticosus* agg. L., or *Sambucus racemosa* L. These collections were chosen to limit potential variation in collection date as many collections had few females of both species. All females from each selected collection were dissected but only those specimens that yielded measurable ovipositors were included in our data. We selected wasps from three sites in southwestern BC: *i*) Abbotsford Community Garden, Abbotsford (ACG), *ii*) Agassiz Research and Development Centre, Agassiz (AGA) and *iii*) Promontory Park, Chilliwack (PRO) (Table 1). We identified wasps emerging from fresh fruit collections based on scutellar plate morphology as the shapes of the scutellar plates of *L. japonica* and *G. brasiliensis* are distinctive enough to determine species identity (see Abram et al. 2020, 2022a, b). Representative vouchers of both species were identified by M. Buffington (USDA-ARS) and are deposited in the National Insect Collection, National Museum of Natural History, Smithsonian Institution, Washington D.C (Abram et al. 2022a, b).

We point-mounted, labelled, and photographed the identified wasps, removed the metasoma, and dissected out ovipositors using insect pins and fine-tipped forceps. We photographed ovipositors through a dissecting microscope at 50× magnification and glued the ovipositor to collection paper affixed to the specimen pin. We measured ovipositor characteristics from the photographs using the straight-line tool, the freehand line tool, and the measure function in ImageJ v1.53a (Schneider et al. 2012) to transform pixels to mm (Lue 2017). All measurements were calibrated to the ocular micrometer. We measured ovipositor length along the inside curve of each ovipositor from the base of the egg sac to the tip of the ovipositor (Fig. 2). We measured the width of each ovipositor where the picture was the clearest nearest the middle. We calculated ovipositor stoutness as the quotient of the ovipositor's width divided by its length.

Tibial length measurements

To test whether some intraspecific variation in ovipositor length could be due to a positive association with body size (Niklas 1994), we measured hind tibia length as a proxy of body size (Van Alpen and Thunnissen 1983; West et al. 1996; Nicol and Mackuer 1999) of a random subset of wasps from both species, and from all sites, *post hoc*. We removed the left hind leg from point mounted specimens and photographed the inside of the tibia bent at approximately 90° at the femorotibial joint through a dissecting microscope at 80X magnification. We measured the tibia from the photographs using the straight-line tool and the measure function in ImageJ v1.53a (Schneider et al. 2012). We measured the straight line distance from the base of the tibial spur to the far edge of the femorotibial joint (Fig. 3).

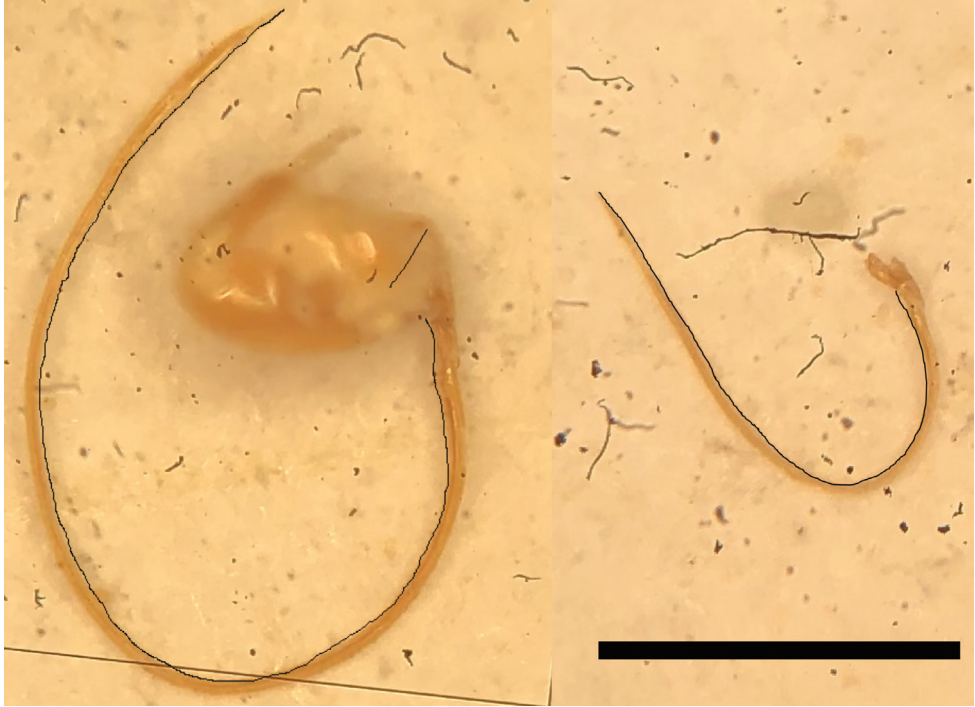


Figure 2. Comparison of ovipositors dissected out of female *L. japonica* (left) and *G. brasiliensis* (right) collected in southwestern British Columbia, Canada. Images were taken through a dissecting microscope at $\times 50$ magnification. Scale bar represents 0.5mm.

Table 1. The number of measured ovipositors dissected from parasitoids reared from collections of fresh fruits at different sites in British Columbia, Canada.

Collection site	Plant species	Collection date	Wasp species	Number dissected
Abbotsford Community Garden, Abbotsford	<i>S. racemosa</i>	2020-06-22	<i>L. japonica</i>	2
		2020-07-10	<i>L. japonica</i>	6
	<i>R. armeniacus</i>	2020-07-24	<i>G. brasiliensis</i>	7
			<i>L. japonica</i>	4
		2020-08-07	<i>G. brasiliensis</i>	5
			<i>L. japonica</i>	3
Promontory Park, Chilliwack	<i>S. racemosa</i>	2020-07-24	<i>L. japonica</i>	3
			<i>G. brasiliensis</i>	8
	<i>R. armeniacus</i>	2020-08-31	<i>L. japonica</i>	7
			<i>G. brasiliensis</i>	7
		2020-09-07	<i>L. japonica</i>	2
			<i>G. brasiliensis</i>	2
Agassiz RDC, Agassiz	<i>R. fruticosus</i>	2020-07-25	<i>L. japonica</i>	5
			<i>G. brasiliensis</i>	4

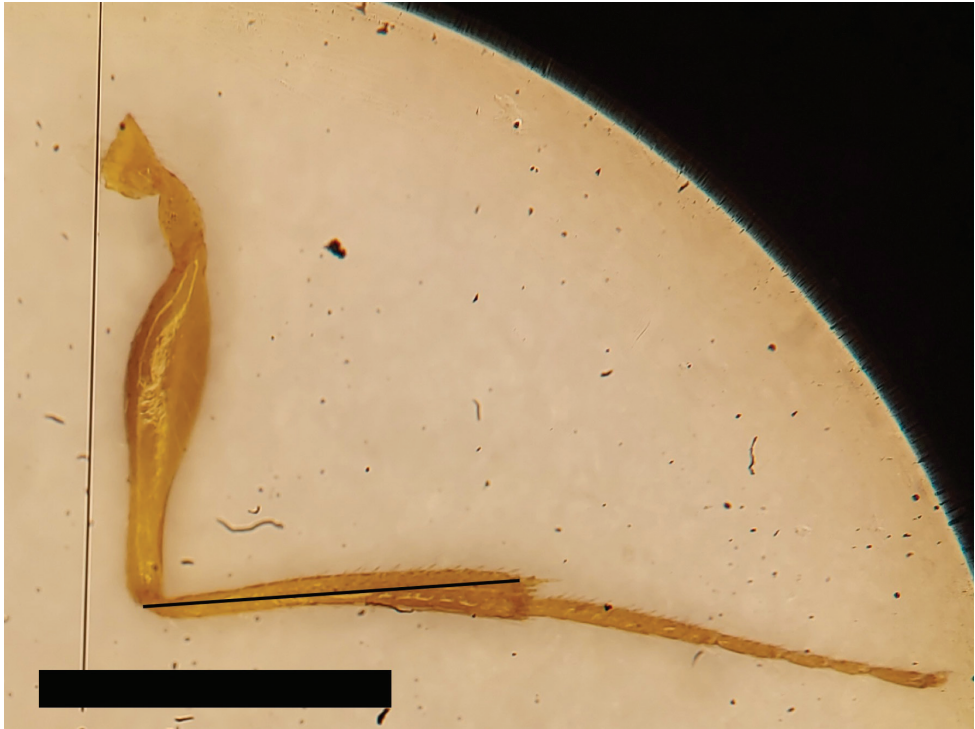


Figure 3. Left hind leg of a female *L. japonica* collected in southwestern British Columbia, Canada. We measured tibia length as the straight line distance of the inside of the tibia from the base of the tibial spur to the far edge of the femorotibial joint. Image taken through a dissecting microscope at $\times 80$ magnification. Scale bar represents 0.5 mm.

Statistical analysis

We investigated the influence of wasp species (*G. brasiliensis*, *L. japonica*), collection site (ACG, PRO), and fruit collected (red elderberry, Himalayan blackberry) as candidate categorical predictors for the response variables ovipositor length, ovipositor width, and ovipositor stoutness using Gamma family generalized linear models (GLMs) estimated using F-tests. We used Gamma distributed GLMs because the linear model variance across the predictors were unequal. In all cases, we inspected residual plots to assess adequacy of model fit. We did not consider interactions between covariates in our statistical analyses. Since the AGA site had different fruit species from the other two sites, we removed specimens collected from the AGA site from our GLM analyses. For specimens collected from the AGA site we fit two sample t-tests with ovipositor length, ovipositor width, and ovipositor stoutness as response variables and wasp species (*G. brasiliensis*, *L. japonica*) as categorical predictors for each response variable with Bonferroni-adjusted alpha. We also investigated associations between tibia length on ovipositor length within each of the two parasitoid species using generalized linear models with Gaussian error distributions. We conducted all statistical analyses in R software, version 4.0.3 (R Core Team 2020). See Suppl. material 1 for the data used to perform these analysis.

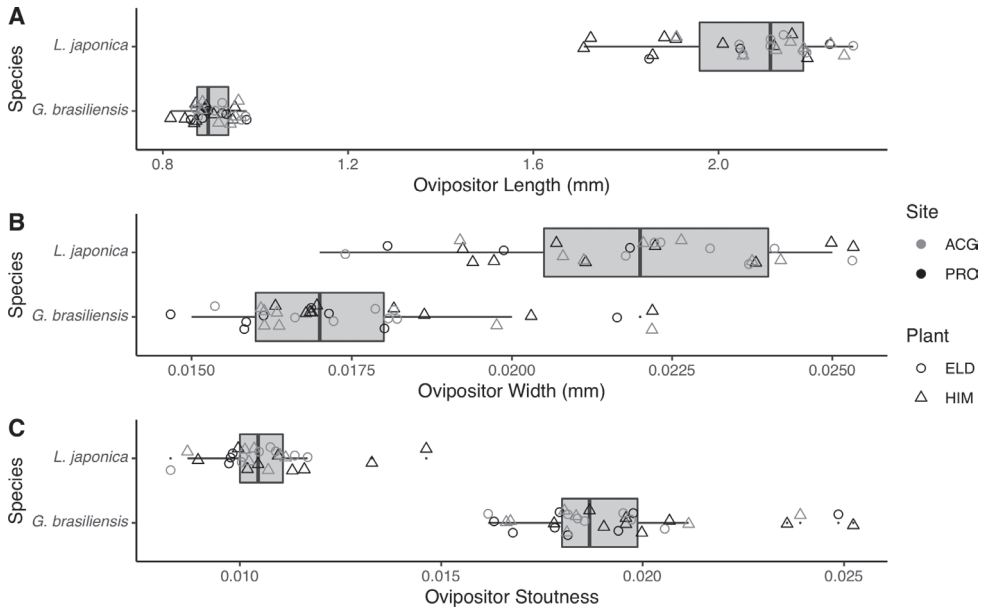


Figure 4. **A** Ovipositor length, **B** ovipositor width, and **C** ovipositor stoutness measured from representative samples of *L. japonica* (N=27) and *G. brasiliensis* (N=31) reared from *D. suzukii* infested fruit collected in southwestern British Columbia in 2020. Black markers represent specimens reared from fruits collected at Promontory Park, Chilliwack (PRO), while gray markers represent specimens reared from fruits collected at the Abbotsford Community Garden, Abbotsford (ACG). Circular markers represent specimens reared from collections of fresh red elderberry (*Sambucus racemosa* L.; ELD), while triangular markers represent specimens reared from collections of fresh Himalayan blackberry (*Rubus armeniacus* Focke; HIM). Vertical lines in boxes represent the first, second, and third quartiles while whiskers represent the 1.5 inter-quartile range.

Results

Ovipositor length

For specimens reared from fruits collected at the ACG and PRO sites, *L. japonica* had 77.8% longer ovipositors than *G. brasiliensis* (Fig. 4A, $F_{1,55} = 2882.36$, $P < 0.0001$) and wasps, regardless of species, collected from the ACG site had 14.3% longer ovipositors than those collected from the PRO site ($F_{1,55} = 14.24$, $P < 0.0001$). Fruit species was not associated with any differences in ovipositor length ($F_{1,54} = 1.27$, $P = 0.26$). For specimens reared from fruits collected at the AGA site, *L. japonica* had 79.6% longer ovipositors than *G. brasiliensis* ($t_{6,67} = -34.9$, $P < 0.0001$).

Ovipositor width

For specimens reared from fruits collected at the ACG and PRO sites, *L. japonica* had 21.9% wider ovipositors than *G. brasiliensis* (Fig. 4B, $F_{1,56} = 61.65$, $P < 0.0001$). Fruit species and collection site were not associated with any differences in ovipositor width

($F_{1,54} = 0.65$, $P = 0.42$ & $F_{1,54} = 0.34$, $P = 0.56$, respectively). For specimens reared from fruits collected at the AGA site, *L. japonica* had 16.9% wider ovipositors than *G. brasiliensis* ($t_{5,81} = -2.55$, $P = 0.0447$).

Ovipositor stoutness

For specimens reared from fruits collected at the ACG and PRO sites, *G. brasiliensis* had 58.2% stouter ovipositors than *L. japonica* (Fig. 4C, $F_{1,56} = 341.7$, $P < 0.0001$). Fruit species and collection site were not associated with any differences in ovipositor stoutness ($F_{1,54} = 2.35$, $P = 0.13$ & $F_{1,54} = 1.61$, $P = 0.21$, respectively). For specimens reared from fruits collected at the AGA site, *G. brasiliensis* had 65.3% stouter ovipositors than *L. japonica* ($t_{4,42} = 7.76$, $P < 0.0001$).

Tibia length

Ovipositor length was positively correlated with tibia length (Fig. 5) for both *L. japonica* ($F_{1,13} = 145.5$, $P < 0.0001$) and *G. brasiliensis* ($F_{1,12} = 10.9$, $P < 0.007$).

Discussion

We demonstrated that ovipositor length, width, and stoutness differed between *L. japonica* and *G. brasiliensis* in southwestern BC. Our findings are consistent with our predictions that *G. brasiliensis* have significantly shorter and stouter ovipositors than do *L. japonica*. Additionally, our findings align with those of Fellin et al. (2023) as *G. brasiliensis* in both studies had shorter ovipositors than *L. japonica*.

The absolute difference in ovipositor lengths between the *G. brasiliensis* and *L. japonica* is most relevant for determining how far their ovipositors could penetrate into substrates harboring their shared hosts, regardless of the intraspecific relationship between hind tibia length and ovipositor length. Our findings align with those in the California olive biocontrol system (Sime et al. 2007; Wang et al. 2008, 2009); in both systems multiple parasitoids attack hosts in the same developmental substrate (Giorgini et al. 2019; Wang et al. 2021) and those parasitoids with broader host ranges have longer ovipositors than do those parasitoids with more restricted host ranges that have shorter ovipositors.

The intraspecific variation in *G. brasiliensis* and *L. japonica* ovipositor characteristics is associated with variation in intraspecific body size. This may be driven by environmental factors such as fruit characteristics or rearing temperature. Although fruit type did not appear to influence ovipositor characteristics in our study, fruit characteristics (size, weight, depth, ripeness, etc.) may influence host size (Poças et al. 2022) and host size can affect parasitoid size (Nicol and Mackauer 1999; Harvey et al. 2004). Additionally, temperature variation can cause size variation in insects (Atkinson 1994) and thus could be a source of variation in our results.

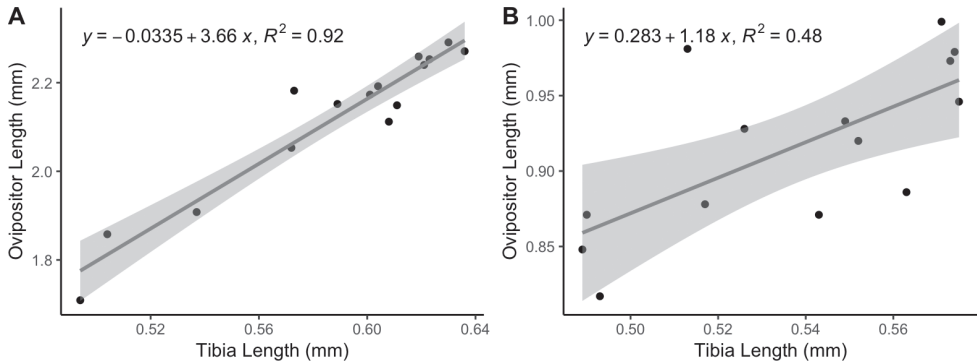


Figure 5. Relationship between ovipositor length and tibia length for a random subset of **A** *L. japonica* (N = 15) and **B** *G. brasiliensis* (N = 14). Trendlines represent the linear regression and shading represents the 95% confidence interval.

The interspecific variation in ovipositor length that we quantified here may be associated with different host searching behaviours (e.g. Compton et al. 2009). The oviposition behaviour of a subset of *Leptopilina* and *Ganaspis* species were described by Vet and Bakker (1985); they showed that the *Leptopilina* species in their study search for hosts using a “walk and probe” method where they walk over likely host substrate while constantly probing it with their ovipositors and rarely stopping. Conversely, *Ganaspis* species were shown to search for hosts using a “walk then probe” method where they take prolonged walks then stop and stand still for extended time periods before probing (Vet and Bakker 1985). The apparently more methodical host searching technique used by the *Ganaspis* species, when compared to the *Leptopilina* species described by Vet and Bakker (1985) may be related to their ability to sense hosts, likely through host movement, within the substrate. Vet and van Alphen (1985) assessed the importance of vibrotaxis (host vibrational cues), ovipositor searching, and antennal searching in a suite of *Leptopilina* and *Ganaspis* species and found that the *Ganaspis* investigated relied entirely on vibrotaxis. Conversely, the *Leptopilina* species investigated by Vet and van Alphen relied primarily on ovipositor searching, while vibrotaxis and antennal searching were used to limited and varying degrees depending on the *Leptopilina* species. Assuming that, like its congeners (Vet and van Alphen 1985), *G. brasiliensis* relies on vibrotaxis to locate high quality (i.e. unparasitized, early instar) hosts in their oviposition substrate, we speculate that *G. brasiliensis* is less likely to find hosts deeper in their oviposition substrate, so a longer ovipositor may have less utility. Future work should assess the importance of vibrotaxis, ovipositor searching, and antennal searching for *L. japonica* and *G. brasiliensis* while searching for *D. suzukii* larvae.

Leptopilina japonica larvae generally outcompete *G. brasiliensis* in *D. suzukii* hosts that have been parasitized by both parasitoids within 24 hours (Wang et al. 2019). Because of this, *G. brasiliensis* may find competitor free space by attacking *D. suzukii* larvae that are present in less ripe fruits. A stouter ovipositor may, for example, facilitate oviposition by *G. brasiliensis* through tougher fruit skins of undamaged fruits as a

stouter ovipositor could support puncturing fruit with a higher required penetration force without the ovipositor buckling (Vincent and King 1995; Quicke et al. 1999; Cerkvenik et al. 2017) although Figitids searching for hosts in damaged fruits are known to wander inside of fruits through holes made by other insects (e.g. Guimarães and Zucchi 2004). Abram et al. (2022a) found that parasitism by the parasitoids in southwestern BC typically lagged behind *D. suzukii* presence in fruits by approximately two weeks, and that *L. japonica* appeared earlier in the ripening season in collections of *R. armeniacus*, while *G. brasiliensis* appeared earlier in the ripening season in collections of *S. racemosa*. However, Abram et al. (2022a) did not account for variation in fruit ripeness within samples so their dataset is likely inadequate to describe the effect of fruit ripeness on parasitism by either species. In nature, *D. suzukii* oviposit into and develop from wild fruits of differing size, depth, and ripeness, which can be present simultaneously (e.g. Ulmer et al. 2022).

The relationship between fruit characteristics and the relative abundance of larval parasitoids of *D. suzukii* has not yet been quantified. The apparent affinity of *L. japonica* for larger fruits and *G. brasiliensis* for smaller fruits observed in fruit collections from both China (Giorgini et al. 2019) and BC (Abram et al. 2022a) invites further research. In the laboratory, *D. suzukii* larvae spend more time diving in fruits than do *D. melanogaster* Meigen larvae (Kim et al. 2017), which could be a behavioural response by *D. suzukii* to pressures imposed by the specialist *G. brasiliensis* and could present an open niche for *L. japonica* to exploit with its longer ovipositor. Future research should investigate how *L. japonica* and *G. brasiliensis* search for host larvae in fruits, the depths at which they parasitize host larvae, and the relationship between parasitoid ovipositor characteristics and fruit characteristics like fruit diameter, depth, and skin thickness. Future work should also focus on the parasitoid communities within *D. suzukii* infested fruits throughout the fruit's phenology (from ripening through decomposition) as *D. suzukii* infestation opens novel niches for other frugivorous insect species (e.g. Chamberlain et al. 2020) and by extension their associated parasitoids.

The narrow host range of *G. brasiliensis* has led to its recent approval for field release in the United States of America (Beers et al. 2022). *Leptopilina japonica*, however, has not yet been approved for release in the USA due to its broader host range. This presents an interesting opportunity to identify the realized niche of *G. brasiliensis* in the presence of the superior intrinsic competitor *L. japonica* where both species co-occur compared to the realized niche of *G. brasiliensis* where it is released for biological control. In its introduced range, the poorer intrinsic competitor (s. str. Harvey et al. 2013; Ode et al. 2022) will not be subject to the competitive pressures of *L. japonica*. This competitive release could impact the parasitoid's realized niche wherein *G. brasiliensis* may be better able to make use of its fundamental niche in North America and may result in differing ovipositor characteristics between populations that are and are not in direct competition with *L. japonica*.

The clear differences in ovipositor length, width, and stoutness between *G. brasiliensis* and *L. japonica* demonstrated in our study, and the apparent use of

semiochemical cues for competitive avoidance by *G. brasiliensis* but not *L. japonica* (Böttinger et al. 2019; Wang et al. 2019) offers an interesting opportunity to investigate the evolutionary drivers of these biological and ecological differences. Such insights are likely important for our understanding of the usefulness of *G. brasiliensis* and *L. japonica* for the biological control of *D. suzukii* globally.

Acknowledgements

We thank T. Hueppelsheuser, M. Franklin, J. Sherwood, E. Grove, and P. Eraso for their efforts in fruit collection and insect rearing. We thank D. Iritani and Y. Watanabe for their help with microscopy and C.-H. Lue and M. Buffington for their wisdom regarding ovipositor measurements. Finally, we thank C. Cock, T. Nelson, and J. Sherwood for their technical support, D. Ensing for his support with model selection, and W. Wong for his participation in brainstorming ovipositor questions and future research. This research (funding to P.K.A., C.E.M., N.G.E.) is part of Organic Science Cluster 3, led by the Organic Federation of Canada in collaboration with the Organic Agriculture Centre of Canada at Dalhousie University, supported by Agriculture and Agri-Food Canada's Canadian Agricultural Partnership-AgriScience Program. P.K.A. and C.E.M. were also supported by funding from Agriculture and Agri-Food Canada, A-BASE #2955. N.G.E. was additionally supported by Graduate Student Fellowships from the University of British Columbia-Okanagan.

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Supplementary material I

Raw data from figitid collections and ovipositor and tibia measurements

Authors: Nathan G. Earley, Paul K. Abram, Robert G. Lalonde, Chandra E. Moffat

Data type: table (excel file)

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Link: <https://doi.org/10.3897/jhr.95.89678.suppl1>