Biology and Efficacy of *Aprostocetus* (Eulophidae: Hymenoptera) as a Parasitoid of the Blueberry Gall Midge Complex: *Dasineura oxycoccana* and *Prodiplosis vaccinii* (Diptera: Cecidomyiidae)

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ABSTRACT In the southeastern United States, bud-infesting larvae of two gall midge species, *Dasineura oxycoccana* (Johnson) and *Prodiplosis vaccinii* (Felt), destroy from 20 to 80% of the rabbiteye blueberry crop, *Vaccinium virgatum* Aiton (syn. *V. ashei* Reade). These midge larvae are attacked by five species of parasitoid wasps. The most effective of these is the bivoltine eulophid *Aprostocetus* sp. nr. *marylandensis* (Eulophidae), whose adults constitute one-third of the gall midge parasitoids, active in both conventional and organic blueberry fields. Broods of *Aprostocetus* use several reproductive strategies to keep sole possession of their larval hosts. As solitary endoparasitoids as well as facultative hyperparasitoids, precocial larvae of *Aprostocetus* devour hosts organs along with any younger siblings and rival parasitoid broods. Although larger hosts are preferred, any sized larvae can be parasitized, which reduces brood congestion and infanticide. An *Aprostocetus* female spends an hour or more in a systematic hunt for hosts, during which time 40 to 100% of midge larvae encountered are parasitized. *Aprostocetus* females could have located hosts more quickly had they recognized host-feeding scars as cues. Even so, high rates of larval parasitism achieved by *Aprostocetus* may kill as many midges as insecticides do.

KEY WORDS biological control, Platygastridae, Eulophidae, *Vaccinium*, parasitism
use a more conventional approach to parental care. Females prevent the encapsulation of their eggs by ovipositing in organs where the host immune response is presumably weakest—in this case, the host’s midgut (Sampson et al. 2006). Among these stomach-inhabiting parasitoids, the tetrastichine eulophid, Aprostocetus sp., is notable for its high abundance, high parasitism rate, and most importantly, its early emergence. Although Aprostocetus emerges 1–2 wk later than D. oxyccocana does, these wasps may help protect blueberry buds from midge attack at a time when pollinator-toxic insecticides cannot be used.

Aprostocetus in blueberry fields closely resembles the Neotropical midge parasitoid, Aprostocetus marylandensis (Girault), a common tetrastichine of the U.S. Gulf Coast and Caribbean Islands. However, Aprostocetus associated with Cecidomyiidae on Vaccinium is principally Nearctic in distribution and reportedly occur in most if not all blueberry and cranberry growing regions throughout Canada and the United States (Crawford 1907, Dean 1911, Myers 1930, Breden 1939, Burks 1943, Barnes 1948, LaSalle 1994, Sampson et al. 2006, Fitzpatrick 2010, Peach et al. 2012). Much of what is known about Aprostocetus and other gall midge enemies in blueberry arises from research of southern U.S. populations. Here, Aprostocetus, unlike its univoltine midge hosts, is a bivoltine species. Females actively seek D. oxyccocana or P. vaccinii hosts for ≈200 d from March to November (Sampson et al. 2002). Their peak activity occurs in May and October, during which time females parasitize upwards of 6 to 40% of larval gall midges field-wide (Sampson et al. 2006). However, the efficiency of individual females to parasitize hosts may well exceed these earlier field-wide estimates (Sampson et al. 2006). To assess more fairly the biocontrol value of Aprostocetus, the wasp’s reproductive behavior and parasitism success were further explored in organic and conventional blueberry agro-ecosystems.

Materials and Methods

Aprostocetus Reproductive Behavior. Three-hundred potted rabbit eye blueberry plants V. virgatum (syn. V. ashei) inside a screenhouse at the U.S. Department of Agriculture-Agriculture Research Service (USDA-ARS) Thad Cochran Southern Horticultural Research Laboratory (Poplarville, MS), which being pesticide-free for 10 yr, supported natural populations of blueberry gall midge and blueberry tip midge. From April to May in this screenhouse, a jeweler’s lens was used to observe female Aprostocetus as they oviposit, rest, groom, and greet conspecifics. A stopwatch was used to measure the time female wasps spend on leaf buds. After female wasps departed, an observer placed leaf buds, presumably with parasitized hosts, inside plastic baggies and returned these samples in an ice-filled chest to laboratory for dissection.

Under magnification, leaflets were peeled away to expose host eggs and larvae. Host larvae were slide mounted and their parasitoid brood, if present, was revealed either by clearing tissues in 10% KOH solution for 30–60 min or by gently smearing host larvae on the slide. For each leaflet, the percentage of parasitized hosts was calculated, parasitoid eggs per host larva were counted, and the growth stage of each host larva determined. Midge eggs were identifiable by their distinctly spindle shape and brown tinge. Because neonates and eggs are similar in size, first instars (L1) were easily distinguishable from second instars (L2). Mature third instars (L3, prepupae) were the easiest to recognize. Midge prepupae of both species are bright orange (as opposed to white for L1 and L2) and bear a unique “Y”–shaped sclerite called a spatula, which aids in burrowing. Midge larvae often crawl around inside leaf buds to find undamaged tissue to eat. Therefore, a larva’s location (relative depth) inside a leaf bud was recorded. The outermost leaflet occurred at a relative depth of zero. Interior leaflets occur at relative depths of 0.4, 0.7, and 0.9, respectively—the smallest and innermost leaflet received a value of 1.0. Leaf bud damage inflicted by host larvae was measured as the average percentage of necrotic tissue per leaflet. For each leaflet, whether the bud was damaged or not, the number of capitate glandular trichomes was counted. Mean host parasitism at each leaflet layer was calculated as the percentage of midge larvae with at least one Aprostocetus egg inside them.

Before analysis of variance (ANOVA), data were transformed as follows: leaf damage (Log10), host density (Log10), parasitoid clutch size (Log10), and percentage parasitism [arcsine (sqrt)]. When transformations failed to restore normality, nonparametric Kruskal–Wallis ANOVA was alternatively used for data analyses (PROC NPAR1WAY, SAS Institute 1990). Abundance of host and parasitoid broods in blueberry leaf buds were modeled using the following equations developed by the authors using MathCad seven Modeling software (MathSoft, Inc., Cambridge, MA):

\[
H_i = a_i \exp \left[ -0.5(d - d_{\text{max}})/c_i \right]^2
\]

\[
e_i = np_i \cdot H_i \phi / i
\]

(0 ≤ i ≤ 3)

Where \(H_i\) is the number of host larvae of stadia (i) in the leaflet. \(a_i\) is the maximum host clutch size. \(d\) is relative depth from the bud surface. \(d_{\text{max}}\) is depth where most host larvae feed. \(c_i\) is a constant defining host larval distribution among inner leaflets. \(e_i\) is the number of parasitoid eggs distributed among host larval stages (or stadia, i). \(n\) is total parasitoid eggs laid. \(p_i\) is the likelihood of hosts of the ith size being parasitized. \(H_i\) is the number of suitable hosts. \(\Phi\) is the number of parasitoid eggs inserted into a host larva’s midgut (single or multiple attacks possible).

Parasitoid Surveys. Surveys of parasitoids covered ~1,000 ha of south Mississippi blueberry fields. Yellow sticky insect traps continually sampled for midge parasitoids from 30 March 2009 through 25 April 2009 during a time of peak host abundance. Eighteen blueberry fields were chosen for this survey: half were organic; the remaining fields were managed conventionally. Conventional berry producers with acreages...
>10 ha blueberry annually apply fertilizer and fungicides; and on occasion, synthetic insecticides for gall midge control (Braswell et al. 2009). The nine small “organic” fields (0.25–2.00 ha) differed from conventional ones in that synthetic pesticides had not been applied for at least 5 yr. The other nine fields were of the conventional type and measured 5–10 ha. In each of the 18 fields, 10 double-sided Pherocon AM traps (Treécé Inc, Salinas, CA) were placed in each field with one trap erected within the canopies of 10 randomly selected healthy blueberry bushes. Selected bushes occurred within an area of ∼0.25 ha. Traps were set out from 30 March to the 8 April and each recovered 18 d after their installation. GENMOD Procedure (SAS Institute 1990) compared Log10 (abundances) of parasitoid species within conventional and organic blueberry fields. Species composition was compared between parasitoid guilds of conventional and organic farms via two-way ANOVA and Tukey’s honestly significant difference (HSD).

Results

Feeding by gall midge larvae damaged as much of 20% of a leaf bud including inner trichome-rich leaflets. Most midge larvae inhabiting the first and second leaflet sections (Fig. 1; F = 3.84; df = 4, 460; P < 0.005) inflicted greater damage to the deepest second and third leaflets (F = 9.24; df = 4, 460; P < 0.0001). Most of the damage was attributable to first instar midges (Tukey’s HSD = 3.65; df = 441; P < 0.05). Hundreds of trichomes adorn the leaflets where gall midges actively feed. However, parasitoids did not optimize host searches by cuing in on these tiny plant organs. Instead, female Aprostocetus after landing on a leaf bud begin a 1-h long systematic search for host larvae, which involves a complex repertoire of stereotypical reproductive behaviors. Female Aprostocetus pause to bend their abdomens downward to position their ovipositors perpendicular to the leaf bud’s surface. They then lift their abdomens to unsheathe their stings (presting position), thrusting both abdomen and sting downward. If the sting (ovipositor) fails to contact a host larva, it is then retracted. The wasp then steps forward and repeats this stereotypical probing sequence 80 or more times at a tempo of ∼1–2 probes per min depending on host availability (r > 0.35; P < 0.05). Abdominal contractions by female wasps during these probing sequences signified oviposition (Fig. 2). The frequency of these oviposition gestures increased as females began probing into necrotic bud tissue, below which multiple host larvae were presumably being detected (F = 2.14; df = 1, 484; P < 0.05). Interestingly however, female Aprostocetus systematically hunt midge larvae without first narrowing their searches to visibly damaged portions (e.g., necrotic lesions) of the leaf bud (F = 0.41; df = 1, 16; P > 0.650). Instead, the oviposition rate of Aprostocetus was quite consistent, 1–2 eggs every 10 min, which resulted in 40–100% parasitism. Prepupal hosts suffered the highest rate of parasitism—100% (Figs. 1 and 2; F = 7.52; df = 1, 31; P < 0.05). The number of probes (stings) per bud or per minute, however, had little effect on overall percentage parasitism (F = 1.41; df = 1, 38; P > 0.240).

Fig. 1. Spatial distribution of host larvae, parasitism by Aprostocetus, and host parasite load (number of eggs per midge larva). Represented by circles are host eggs, by squares and the red horizontal bar are host first instar larvae (L1), by diamonds and the blue horizontal bar are second host instars (L2), and by triangles and the black horizontal bar are third host instars or prepupae (L3). Relative depth is shown on the x-axis. Also presented is the percentage leaf bud damage (necrosis) inflicted by feeding midge larvae at each relative leaflet depth.
However, a female Aprostocetus that fails to find suitable hosts within 15 min of landing will abandon the search and move onto the next leaf bud.

Because Aprostocetus eggs are enormous for such a tiny insect, a female wasp’s clutch size is constrained by host size. In fact, mature L3 host larvae are more likely to be detected by Aprostocetus (Fig. 1; \( F = 7.45; \text{df} = 1, 118; P < 0.0001 \)) and carry optimal loads of \( \approx 3 \) parasitoid eggs each (Kruskal–Wallis ANOVA; \( \chi^2 = 30.41; \text{df} = 2; P < 0.0001 \)). Approximately 40% of L1 larvae carried one parasitoid egg and 70% of L2s each carried \( \approx 2 \) eggs. Female wasps expressed no host preference for prepupal \( D. oxycocca \) (56% ± 11 parasitism, \( n = 25 \)) or \( P. vaccinii \) (56% ± 13 parasitism, \( n = 29 \), Kruskal–Wallis ANOVA; \( \chi^2 = 1.10; \text{df} = 1; P > 0.290 \)). A larger sample size was hoped for to test host preference in Aprostocetus; however, most mounted larvae (\( n = 113 \)) lacked a sutura, the shape of which is key to distinguishing between larvae of \( D. oxycocca \) and \( P. vaccinii \).

A blueberry leaf bud affords \( D. oxycocca \) and \( P. vaccinii \) little protection from Aprostocetus attack. In fact, any surviving larvae must deal with the other 67% of the parasitoids (Platygastridae) that frequent both conventional (\( \chi^2 = 1255.1; \text{df} = 7; P < 0.0001 \)) and organic blueberry fields (\( \chi^2 = 1078.9; \text{df} = 7; P < 0.0001 \)). Most of these other parasitoids were Synopeas sp., particularly the species with a stubby scutellar spine, which was befittingly given the moniker “short-spined Synopeas.” Platygaster sp., another common midge parasitoid, lacks a scutellar spine. Rarer but no less interesting species of Platygastridae also parasitized blueberry-infesting Cecidomyiidae (\( \chi^2 = 262.2; \text{df} = 1; P < 0.0001 \)). There were \( \approx 4\% \) of these incidental parasitoids included a long-spined species of Synopeas, a stomach parasitoid, and Inostemma, another undescribed species noteworthy for larvae infesting a host’s brain and central nervous system, CNS (Fig. 3).

A third of all parasitoids collected in the sticky traps were Aprostocetus, of which females outnumbered males by a factor of six (Fig. 3). In the 18 surveyed blueberry fields, parasitoid guilds were remarkably similar in species composition and abundance. The only noticeable difference in parasitoid community structure between the two farm types was that, in organic fields, an increase in Inostemma abundance (Tukey’s HSD; \( P < 0.01 \)) occurred along with a drop in the density of short-spined Synopeas (Fig. 3; Tukey’s HSD; \( P < 0.001 \)).

**Discussion**

We observed five undescribed species of midge-killing parasitoids common to both organic and conventional blueberry fields in the southern United States (Sampson et al. 2006). Four species are platygastrids, which account for 67% of blueberry gall midge and blueberry tip midge parasitoids. The two most abundant of these belong to the genera Synopeas sp. and Platygaster sp. Both genera have species that produce highly specialized cyclopiform first instar larvae that bear massive sickle-like mandibles, which they use to slay rival siblings. These “fighting” larvae, although large and formidable, are no match for the more benign looking hyperparasitic larvae of Aprostocetus. Aprostocetus larvae grow fast, kill, and in the end consume their hosts and any cohabiting parasitoid broods. Parasitoids of blueberry gall midges and blueberry tip midges also differed in host searching behavior. Platygastrid females, for instance, locate larval hosts by crawling inside leaf buds, a reproductive behavior not used by Aprostocetus. Female Aprostocetus instead hunt host larvae by methodically probing through leaf bud tissue using their long tactile ovipositors. Although the long ovipositors of Aprostocetus penetrate deep into bud tissue, the plant suffers no obvious harm. Only linear trails of tiny brown specks demarcate the path taken by host-searching Aprostocetus.

Initially, farm practices such as pruning and pesticide use were expected to alter parasitoid abundance in organic and conventional agro-ecosystems. Because of infrequent pruning in the organic experimental plots, there were proportionally fewer buds to support larval hosts. As a result, intensified competition for fewer hosts may have substantially reduced the abundances of some wasp species. Among these, were the rarest species that constituted no \( > 4\% \) of midge parasitoids including Inostemma and a long-spined species of Synopeas. Another Synopeas species, a short-spined wasp, was more abundant in the conventional blueberry fields where perhaps its habit of crawling deep inside leaf buds to find hosts limits its exposure to harmful insecticides.
Whether insecticides are applied or not, Aprostocetus sp. is one of three gall midge parasitoids that were consistently common to both organic and conventional blueberry fields. As mentioned earlier, the reproductive behavior of Aprostocetus differs significantly from that of platygastrids. Eulophid females do not require direct contact with their hosts during oviposition. Instead, they use their long ovipositors to probe for concealed midge larvae. Only the females appear to seek out midge habitat. Consequently, females were trapped or sighted six times more often than were males. One plausible reason why males
were so rare within blueberry canopies is that they engage in brief courtship immediately after emergence and then either die off or more likely move onto adjacent nectar hosts. Females of course have no choice but to visit blueberry leaf buds if they intend to find suitable ovipositional hosts. Adult parasitoids also outnumbered adult midges on the sticky traps. The highly vagile flight paths of female parasitoids, both Aprostocetus and the four species of Platygasteridae, resulted in catch rates that were three to four times those of adult midges. For measuring midge abundance, counting wasps on yellow sticky cards may be a more reliable and far less laborious method than dissecting individual leaf buds for larvae (Sampson et al. 2002, 2006; Sarzynski and Liburd 2003).

The abundance of cecidomyiid parasitoids such as Aprostocetus is often determined by the complex multitrophic interactions that wasps enter into with their plant and larval hosts. For example, blueberry plants may erect physical barriers of glandular trichomes that if dense enough could aid parasitism by impeding host movement. The converse might also be true whereby trichomes could actually hamper the foraging activities of parasitoids. Our initial hypothesis was that trichomes provide midge parasitoids with useful chemical and physical host-seeking cues (Sampson et al. 2002, Sarzynski and Liburd 2003). An interlocking lattice of leaflet trichomes was also thought to bar midge larvae from sensitive meristematic tissues and alert parasitoids to host locations. Blueberry trichomes certainly did not encumber or kill the tiny parasitoids, as those of other plants have sometimes done (Gross and Price 1988, Carrillo et al. 2008). Host-searching parasitoids, like Aprostocetus, easily traverse the dense fields of blueberry trichomes and sting deep enough to parasitize on the first visit 40% of first instar hosts and 100% of mature larvae. Therefore, it seems that trichomes play only a neutral role and provide no obvious benefit to either host or parasitoid (Begon and Mortimer 1986). Interestingly, wasps were observed “licking” and “antennating” trichomes. However, the significance of these behaviors remains unknown.

The nutritional value of these trichomes seems negligible given the clear absence of male parasitoids at blueberry leaf buds. The nutritional value of these trichomes seems negligible given the clear absence of male parasitoids at blueberry leaf buds.

Midge parasitoids, particularly Aprostocetus, actively hunt broods of both the blueberry gall midge and the blueberry tip midge. Females could have narrowly searched their host plants on successful parasitism by D. oxycoccana and P. vaccinii, an introduced pest of blueberries in southwestern Spain. Acta Hort. 715: 519–524.

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