Field Distribution of *Dasineura oxycoccana* (Diptera: Cecidomyiidae) Adults, Larvae, Pupae, and Parasitoids and Evaluation of Monitoring Trap Designs in Florida

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ABSTRACT Blueberry gall midge, *Dasineura oxycoccana* (Johnson), is a pest of cultivated blueberries throughout the world. Larvae feed and develop in developing leaf buds, and also in flower buds of rabbiteye blueberries, which causes buds to fall off the plant. These injuries can cause up to 80% yield loss in heavy infestations. As the larvae are protected from insecticides, adults must be targeted with foliar applications. Consequently, the detection of adults through an effective monitoring program is critical to time insecticide sprays against the blueberry gall midge. Understanding the distribution of the midge and its parasitoids is also important information for developing a more effective pest management program. A comparison of three monitoring trap types demonstrated that bucket emergence traps and clear panel traps captured similar numbers of midges, although the bucket trap is more sensitive at low population levels. Using bucket emergence traps, we found that nearly 80% of the midges collected pupated within 48 cm of the blueberry bush, suggesting that a targeted soil treatment may be a viable integrated pest management tactic that could be included in a midge management program. Traps and bud samples demonstrated that adult and larval midges and parasitoids were randomly distributed throughout the field in both years, with the exception of larval aggregation in early 2012. As parasitoid distribution is parallel to host occurrence within blueberry plantings, this increases the potential for biological control activities against the blueberry gall midge in fields that do not receive broad-spectrum insecticide applications.

KEY WORDS *Dasineura oxycoccana*, blueberry gall midge, *Vaccinium*, SADIE

Blueberry production in the United States was valued at US$781.8 million in 2012 ((USDA) U.S. Department of Agriculture 2013). Florida is the main producer of early season blueberries. Harvest of southern highbush blueberries, *Vaccinium corymbosum* L. × *Vaccinium darrowi* Camp, in Florida usually runs from early April through early June, but can begin as early as mid-March in warmer years. Although rabbiteye (RE) blueberries, *Vaccinium virgatum* Aiton, are grown mainly for u-pick and local sales in Florida, they are a major part of commercial production in Georgia, Alabama, and Mississippi.

Blueberry gall midge, *Dasineura oxycoccana* (Johnson), is a specialist on *Vaccinium* spp. that is native to eastern North America (Sampson et al. 2006). It is a pest of cultivated blueberries throughout North America (Steck et al. 2000). There has been some confusion as to whether blueberry gall midge and cranberry tipworm are the same species, but recent evidence indicates that they are cryptic species (Cook et al. 2011, Mathur et al. 2012, Fitzpatrick et al. 2013).

D. oxycoccana females lay their eggs in developing leaf buds. Injury to vegetative buds by larval feeding causes distortion, stunting, and even death of leaves. High levels of infestation can lead to a reduction in floral buds the following season (Lyrene and Payne 1992, Steck et al. 2000, Tewari et al. 2012). In RE blueberries, blueberry gall midge is particularly destructive because it also uses flower buds. High infestations of blueberry gall midge in RE blueberries can cause up to 80% yield loss (Lyrene and Payne 1992, 1995).

Insecticide applications are the main tactic used to manage the blueberry gall midge (Sampson et al. 2002). However, their effectiveness is limited because the larvae are protected in the buds (Lyrene and Payne 1995). Adults emerging to mate and lay eggs are the standard targeted stage. Larvae leave the bud and pupate in the soil. Knowing the distribution of the pupae in the soil as well as adults flying in the field could help to maximize control while minimizing the amount of insecticides used. Edge effects occur in small blueberry plots (Roubos and Liburd 2010), but it is not known whether areas of dense population, "hot spots," occur in larger plantings.

Monitoring is an essential component of any integrated pest management (IPM) program, and key to targeting adult blueberry gall midge emergence peaks

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with insecticide applications. Sarzynski and Liburd (2003) found that bud dissections and a technique allowing larvae to emerge from buds provide accurate estimates of populations in the field. Although useful for research purposes, neither technique is practical for growers. Adult emergence traps can detect adults before larval infestation of buds occurs (Roubos and Liburd 2010, Hahn and Isaacs 2012). Cook (2011) used a 30- by 30-cm sticky panel trap to monitor adult midges in Canada. It is not known which trap type (panel vs. bucket) is better for monitoring adult blueberry gall midges.

There are several naturally occurring parasitoids of the blueberry gall midge in blueberry and cranberry fields. Sampson et al. (2002) noted a 75% reduction in larval blueberry gall midge populations while parasitoids were active in the field. Known parasitoids include several undescribed *Synopeas*, *Platygaster*, and *Inostemma* species in the family Platygastridae. A Eulophid wasp, *Aprostocetus* sp., was thought to attack pupae (Sampson et al. 2006), but it is now known that it is a larval parasitoid (Sampson et al. 2013). In Mississippi, 30–40% midge mortality due to parasitism was recorded (Sampson et al. 2006). Similarly, in Florida, parasitism rates from 25 to 40% were recorded (Roubos and Liburd 2013). Much of the biology and ecology of these parasitoids is still unknown.

Our goals were to refine and improve the monitoring program for the blueberry gall midge and to determine the distribution of midge and parasitoids in an unsprayed RE blueberry planting. The specific objectives were threefold. The first objective was to compare the bucket emergence trap with a Plexiglas panel trap to determine the best trap for monitoring blueberry gall midges in blueberries in Florida. The second objective was to determine where blueberry gall midges pupate relative to blueberry bushes. This information could be used to target overwintering pupae and emerging adults. The final objective was to examine the distribution of the blueberry gall midge and its parasitoids within a blueberry planting. Again, this information can be useful when targeting treatments against the blueberry gall midge and it will provide information on whether the blueberry gall midge and/or its parasitoids are present in the planting from the beginning of the season.

Materials and Methods

Study Site. All of the experiments were conducted at an unsprayed organic blueberry farm (N 29.635121°, W 082.301427°) with a known infestation of blueberry gall midge in Gainesville, FL. Blueberry bushes were planted 1.5 m apart within rows (varying with missing bushes and secondary shoots) and row centers were 3.7 m apart.

Objective 1: Trap Comparisons. Three experimental plots were used for the purposes of replication; two plots, 320 m apart, in 2012, from 19 January to 1 March, and one in 2013, from 1 February to 22 February, >160 m from those in 2012. In each plot, the experimental design was randomized complete block with four rep-

Fig. 1. Trap types: (a) bucket emergence trap, (b) panel trap, and (c) modified panel trap. (Online figure in color.)

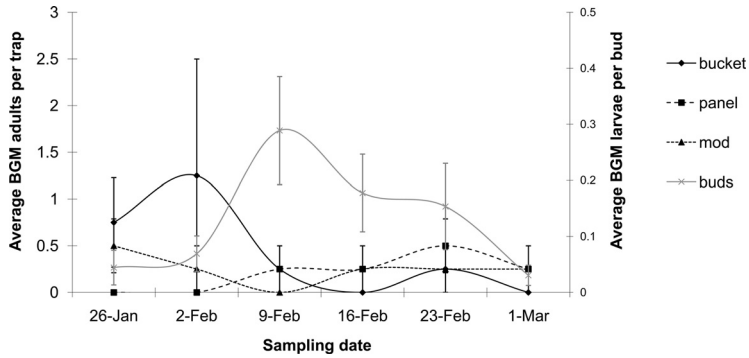

Fig. 2. Average blueberry gall midge adults per trap and larvae per bud in 2012 plot 1 on each sample date.

lications (blocked by variety) and three treatments (trap types). Treatments included: 1) bucket emergence trap (Roubos and Liburd 2010), 2) the 30- by 30-cm panel trap created by Cook (2011), and 3) a modified version of the panel trap. The bucket trap consisted of the bottom 13 cm of a 19-liter plastic bucket, 26 cm diameter with a 19-cm-diameter circle cut into the bottom. This hole was covered with a circular piece of Plexiglas, held in place with plastic, moveable tabs (Fig. 1a). The panel trap consisted of a single 30- by 30-cm Plexiglas panel attached to one side of a 1.4-m support post (Fig. 1b). The modified panel trap consisted of two 30- by 15-cm Plexiglas panels attached to either side of the support post (Fig. 1c). The Plexiglas panels and trap tops were sprayed with Tangle-Trap (The Tanglefoot Company, Grand Rapids, MI) that was allowed to air-dry for at least 24 h before use.

Bucket traps were placed on the soil surface ≈0.3 m from the trunk of a blueberry bush. Soil was piled over the edges of the traps to prevent midges from escaping. Plexiglas panel traps and modified panel traps were placed as close to the bushes as possible without touching any leaves. All three trap types were placed 10–15 m apart and rotated within the same replication each week to avoid location bias. Bucket trap tops and Plexiglas panels were replaced weekly and brought back to the Small Fruit and Vegetable IPM Laboratory, University of Florida, to count and sex captured adult blueberry gall midges.

To compare bud infestation with trap captures, 10–25 flower buds (development stage two or three, according to Spiers 1978) were collected each week from the two blueberry plants adjacent to each trap position. Leaf buds (stage two and three, according to NeSmith et al. 1998) replaced flower buds as the season progressed. Bud samples were placed in petri dishes with moistened filter paper and held in a growth chamber (Percival model I-35 LL, Percival Mfg. Co., Boone, IA) at 30 ± 2°C (day) and 20 ± 2°C (night) at a photoperiod of 14:10 (L:D) h. The bud samples were checked twice weekly for 2 wk, and the total number of emerged larvae was calculated per sample.

Objective 2: Distribution of Emerging Blueberry Gall Midges Relative to the Blueberry Bush. Bucket emergence traps, as described earlier, were deployed in three replicates 44–53 m apart. Each replicate consisted of five traps. Within the row, a trap was placed 4 and 16 cm from the outer shoots of a bush. Perpendicular to the row, a trap was placed 18, 34, and 51 cm from the same bush, extending nearly halfway across the lane.

The traps were moved weekly so a new area of soil was sampled each time, using the same configuration relative to a new bush. Trap lids were replaced weekly and brought back to the laboratory to count captured adult blueberry gall midges. This study was conducted for 5 wk in 2012 and for 3 wk in 2013 in January and February.

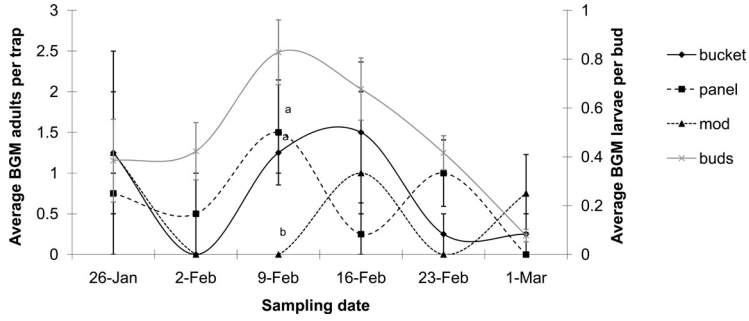

Fig. 3. Average blueberry gall midge adults per trap and larvae per bud in 2012 plot 2 on each sample date. Treatments with different letters are significantly different at $P \leq 0.05$.

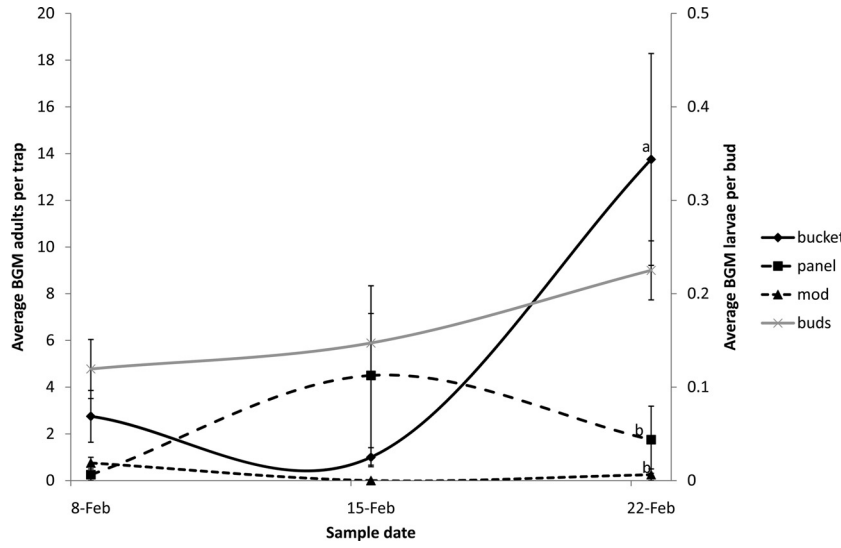

Fig. 4. Average blueberry gall midge adults per trap and larvae per bud in 2013 on each sample date. Treatments with different letters are significantly different at $P \leq 0.05$.

Objective 3: Adult and Larval Blueberry Gall Midges and Parasitoid Distribution. For this experiment, a 0.5-ha section of the blueberry farm was divided into a five by five grid pattern with 25 sample points. Each sample point corresponded to a point between two blueberry bushes. In each row, points were spaced 10 m apart. At each sample point, midge and parasitoid adults and larvae were sampled once every 2 wk (2012, from 19 January to 15 March) or once per week (2013, from 1 February to 22 February). The warm winter in 2013 necessitated collecting samples every week to obtain 3 wk of data.

Midge adults were sampled using petri dish emergence traps (Roubos and Liburd 2010). The petri dish trap consisted of a 3-liter plastic food container, painted white, with the bottom cut out and replaced by a 14-cm-diameter petri dish. The traps were moved after each sample date, so a new area of soil was sampled each time. The trap tops (petri dishes) were taken to the laboratory to count captured adult blueberry gall midges. Fresh petri dishes were used each time.

Midge larvae were sampled by collecting flower and/or leaf buds. In 2012, buds were dissected under

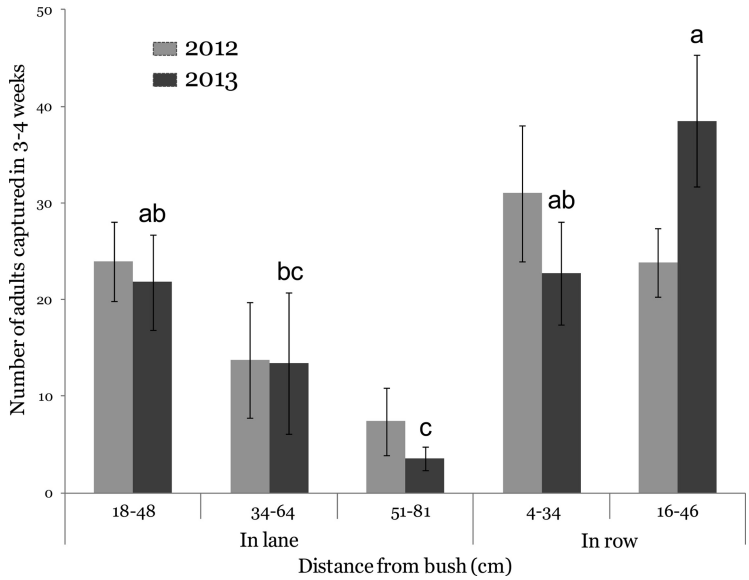

Fig. 5. Percentage of blueberry gall midges captured in bucket emergence traps at different locations relative to the blueberry bush (means ± SE). Bars with different letters are significantly different at $P \leq 0.05$.

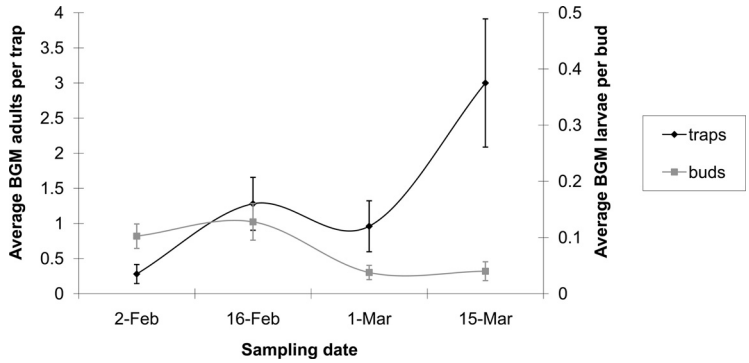

Fig. 6. Average blueberry gall midge adults per trap (left y-axis) and larvae per bud (right y-axis) on each sampling date during the 2012 distribution study.

a dissecting microscope. In 2013, buds were placed in petri dishes and midge larvae collected using the same procedure as in the trapping study. In both years, midge larvae were counted and placed on slides to determine parasitism.

Yellow sticky traps were used to monitor parasitoid adults. They were hung in the blueberry bush canopy ≈1.5 m above the ground. Traps were placed in the field for 2 d, just before midge sampling. All captured parasitoids were counted, placed in Histo-Clear for at least 24 h, and then transferred to 70% alcohol. They were identified to family using the method provided by Triplehorn and Johnson (2005). When possible, Eulophidae and Platygastroidea were identified to genus using the method provided by Schauff et al. (1997) and Rajmohana (2006), respectively.

To monitor parasitoid larvae, the midge larvae from the bud samples were compressed under a glass cover slip with a drop of water, and examined under a compound microscope to determine parasitism. Illustrations provided by Sampson et al. (2006) were used to determine the identity of the parasitoids.

Data Analysis. To compare the different trap types, the number of adult blueberry gall midges per trap was compared across treatment and time using repeated measures (SAS Institute 2010). If interaction with time was not significant, the least significant difference test was used to determine treatment differences. If interaction with time was significant, a one-way analysis of variance was used for each date, followed by least significant difference if $P \leq 0.05$.

To assess the distribution pattern of midge pupae, the total number of midges captured at each trap location relative to the central blueberry bush was calculated across weeks and then calculated as a percentage of the total captured in each replicate. To determine if the percent of midges at each trap location (square-root transformed) was affected by replicate and trap location, a factorial analysis of variance was conducted (SAS Institute 2010) with replicate treated as a random variable. Means were compared using a Student *t*-test of least squares means.

The distribution patterns of blueberry gall midge larvae and adults and its parasitoids were analyzed using SADIE analysis in SADIEShell v. 2.0 (Conrad 2008). The red-blue plots were visualized using ArcGIS 10.2 (ESRI 2013).

Results

Objective 1: Trap Comparisons. In 2012 plot 1, blueberry gall midge numbers were low (Fig. 2). There was no treatment × time interaction ($F = 0.8$, df = 10, 30, $P = 0.63$), so overall treatment effects were compared. There were no differences in average blueberry gall midges among trap types ($F = 0.51$, df = 2, 30, $P = 0.62$). Average blueberry gall midges per trap peaked on 2 February in the bucket traps, 1 wk before the peak in blueberry gall midge larvae per bud. In contrast, neither the panel trap nor the modified panel trap showed a peak in the average number of blueberry gall midges per trap.

Gall midge numbers were higher in 2012 plot 2 (Fig. 3). There was a significant treatment × time interaction ($F = 2.65$, df = 10, 30, $P = 0.02$), so treatments were compared on each date. There were significantly more blueberry gall midges per trap in the bucket and panel traps compared with the modified panel traps on 9 February ($F = 12.62$, df = 2, 11, $P = 0.007$). There were no differences among treatments on any other date (all $F \leq 4.38$, df = 2, 11, $P \geq 0.07$). The panel traps showed a peak in blueberry gall midge adults on 9 February, concurrent with the peak in average blueberry gall midge larvae per bud, and a week before the peak in the modified panel traps. The bucket traps showed a wider peak in blueberry gall midge adults between 9 and 16 February.

In 2013, there was significant treatment × time interaction ($F = 5.64$, df = 4, 12, $P = 0.009$), so treatments were compared on each sample date (Fig. 4). There were significantly higher numbers of blueberry gall midge in the bucket traps compared with both of the other traps on 22 February ($F = 8.96$, df = 2, 11, $P = 0.02$). There were no significant differences on either of the other sampling dates (both $F \leq 2.48$ 12.62, df = 2, 11, $P \geq 0.16$). There was no peak in average blueberry gall midge larvae during our sample period or in average blueberry gall midge adults collected from the bucket traps and panel traps. There appeared to be a peak in blueberry gall midge adults collected from the

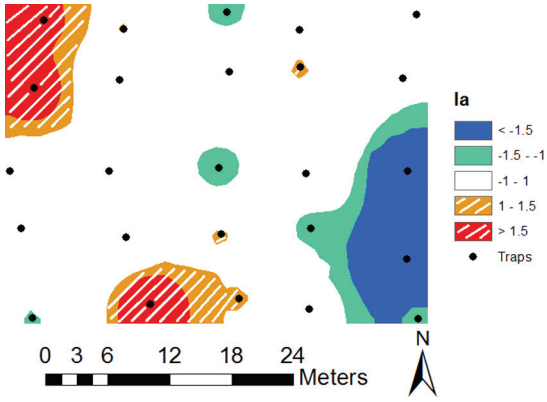

Fig. 7. SADIE red-blue plot showing blueberry gall midge larval aggregation on 2 February 2012. I_a values ≤ -1.5 or ≥ 1.5 are significant at $P \leq 0.05$. (Online figure in color.)

modified panel traps on 15 February, but the standard error is very large.

Objective 2: Distribution of Emerging Blueberry Gall Midges Within and Between Blueberry Rows. The number of midges captured in bucket traps declined rapidly with distance from the blueberry bush into the lane (Fig. 5). Trap location was significant in 2013 ($F = 5.4641$, $P = 0.0202$). The trap closest to the bush captured significantly more than the other traps, whereas the trap furthest from the bush captured significantly less.

Objective 3: Adult and Larval Blueberry Gall Midges and Parasitoid Distribution. In 2012, gall midge numbers were similar to those seen in plot 2 of the trapping study, with similar numbers of adult captures and bud counts (Fig. 6). Adults per trap were randomly distributed throughout the plot on each sample date (all $I_a \leq 1.15$, $P \geq 0.17$). Gall midge larvae per bud were aggregated on 2 February ($I_a = 1.38$, $P = 0.03$). Two aggregations of high numbers were present, one in the NW corner and the other on the west side of the southern border of the study area (Fig. 7). An aggregation of low numbers of midge larvae was present along the middle and lower east border of the study area. Larvae were randomly distributed on all other sample dates (all $I_a \leq 1.04$, $P \geq 0.34$).

Four genera of gall midge parasitoids were trapped in 2012 (Fig. 8): *Aprostocetus* (Eulophidae), *Inostemma*, *Platygaster*, and *Synopeas* (Platygastridae). Of these, only *Platygaster* was collected in high numbers, with a peak on 1 March. Few parasitoid larvae ($N = 18$) were collected in the study, so only the adult *Platygaster* data ($N = 117$) were subjected to SADIE analysis. *Platygaster* adults were distributed randomly throughout the plot on all sampling dates (all $I_a \leq 1.25$, $P \geq 0.09$).

In 2013, gall midge numbers were similar to those seen in the trapping study (Fig. 9). Both the adult and larval populations were randomly distributed throughout the plot (all $I_a \leq 1.03$, $P \geq 0.08$).

The same four genera of gall midge parasitoids were trapped in 2013 (Fig. 10). Of these, both *Platygaster* and *Synopeas* were collected in moderate numbers. There was no peak in any of the parasitoid populations. Few parasitoid larvae were collected ($N = 13$), so only the adult *Platygaster* and *Synopeas* data were subjected to SADIE analysis. *Platygaster* adults were distributed randomly throughout the plot on all sampling dates (all $I_a \leq 0.67$, $P \geq 0.22$). *Synopeas* adults were aggregated on 8 February ($I_a = 4.07$, $P = 0.02$) and randomly distributed on the remaining two sample dates (both $I_a \leq 0.81$, $P \geq 0.54$). The red-blue plot for 8 February (Fig. 11) shows that there was an area of aggregated low values in the northern part of the plot.

Discussion

Our data show that the bucket trap is the most effective trap in detecting low populations of blueberry gall midge. The numbers we collected in the bucket traps were similar to those seen by Roubos and Liburd (2010), except for the spike at the end of our 2013 sampling period.

The panel trap can be an effective trap but only when midge numbers are above 0.5 midges per trap. Cook (2011) found panel traps at bush height in cranberry to be a very effective monitoring tool, but caught few adult midges with them in blueberries. It is possible that the panel traps act as an emergence trap in lower-growing cranberry bogs where cranberry tip-

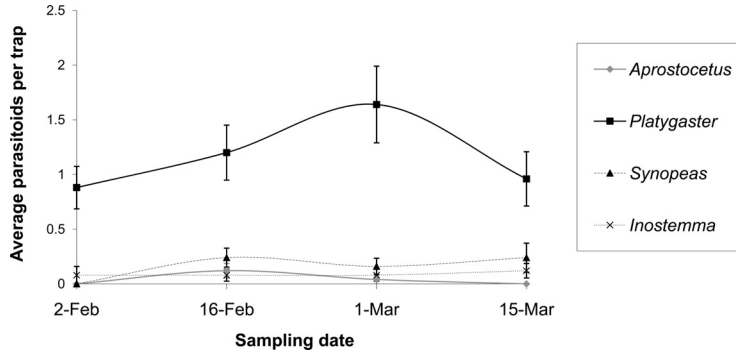

Fig. 8. Average parasitoid adults per trap on each sampling date in 2012.

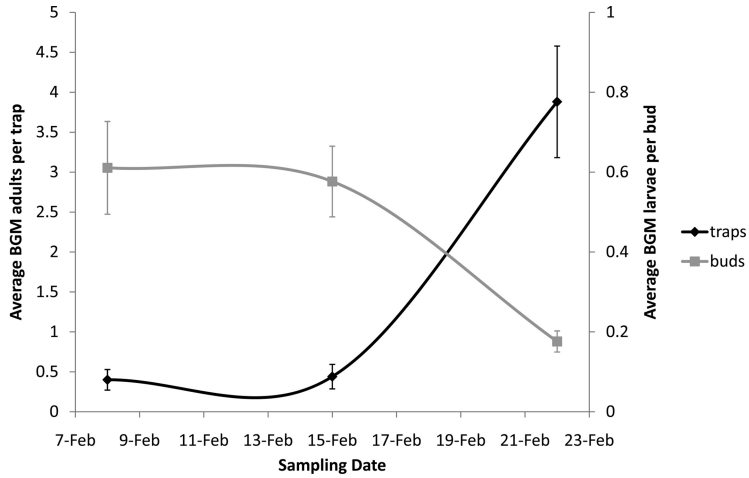

Fig. 9. Average blueberry gall midge adults per trap (left y-axis) and larvae per bud (right y-axis) on each sampling date during the 2013 distribution study.

worms pupate in plant tips until the last generation of the season (Fitzpatrick 2009). Therefore, panel traps may be more effective in lowbush blueberries, as compared with the RE blueberries we tested, which were >6 feet tall.

We found that most midges pupate very near the host plant, with 78% pupating within 48 cm of the plant. Therefore, monitoring traps and soil treatment for control of blueberry gall midge should be directed accordingly. Roubos and Liburd (2010) caught an average of 1.95 and 2.4 adults weekly with the bucket trap, which were placed 30 cm from the crown of the bush, ≈40–50 cm from the trunk. This is in agreement with our results, where we caught 3.2 and 1.5 adults on average weekly at 34 and 51 cm, respectively, from the crown of the bush and blueberry cane.

Both blueberry gall midge and its parasitoids tended to be randomly distributed throughout the plot. Similarly, *Dasineura brassicae* (Winnertz) and its parasitoids in oilseed rape and *Aphis gossypii* Glover and its predators, lady beetles, in chili showed similar spatial aggregation (Ferguson et al. 2004, Rahman et al. 2010). The study area was in the interior of a large blueberry field. Therefore, the lack of aggregation in the blueberry gall midge and *Platygaster* could have been due to a lack of edge effects. This is similar to the results obtained by Roubos and Liburd (2010). They found blueberry gall midge aggregation, in the form of edge effects, only in a small, isolated field. Ferguson et al. (2004) found that *D. brassicae* and its parasitoids were aggregated at field edges prediapause but not postdiapause. In contrast, midges collected from bucket emergence traps in Michigan blueberries appeared to be aggregated, although the data were insufficient for statistical analysis (Hahn and Isaacs 2012).

The two areas of high aggregation of blueberry gall midge larvae on the first sampling date in 2012 may have been areas where more buds of the appropriate stage were available. As the season progressed, buds became available throughout the field and the distribution became randomized. Further research is needed to substantiate this hypothesis.

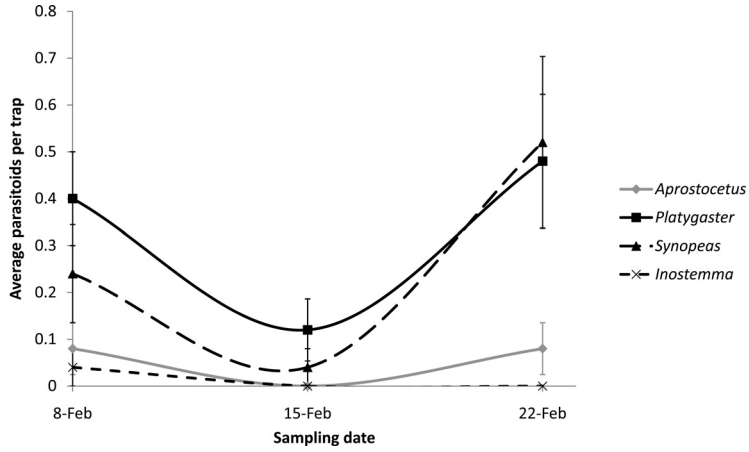

Fig. 10. Average parasitoid adults per trap on each sampling date in 2013.

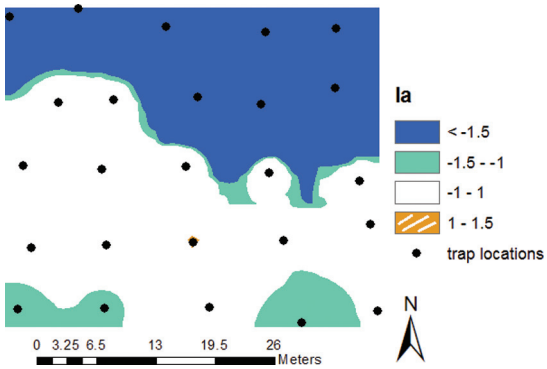

Fig. 11. SADIE red-blue plot showing *Synopeas* adult aggregation on 8 February 2013. I_a values ≤ -1.5 or ≥ 1.5 are significant at $P \leq 0.05$. (Online figure in color.)

The aggregation of low adult *Synopeas* population in 2012 was furthest from the true field edge. Therefore, it is possible that one or more *Synopeas* species came from outside the field and had not yet made their way across the plot by 8 February. This result may also be an artifact of the low numbers of captured *Synopeas.*

Few parasitoid larvae were observed in either year. Parasitism rates are reported to be 30–40% and sometimes higher (Sampson et al. 2002, 2006; Roubos and Liburd 2013). Properly mounting blueberry gall midge larvae so that parasitoid larvae can be seen and identified is very difficult. Rearing the parasitoids to adulthood may provide a more accurate result.

Platygastrid parasitoids were the most abundant parasitoids collected in both years. We only sampled until flower buds became scarce, so these parasitoids appear to parasitize blueberry gall midge larvae in flowers. The other parasitoids may become more common when leaf buds become more abundant. This is in agreement with Roubos 2009, who found that *Platygaster* and *Synopeas* are common in January and February when flower buds are abundant and *Aprostocetus* became more common as leaf buds increased in abundance in March. A more detailed survey, spanning at least two growing seasons, is needed to provide more information on parasitoid phenology.

The emergence of blueberry gall midge and its parasitoids from soil in the field interior early in the season when appropriate-stage buds were few suggests that at least part of the population remains in the field year round and is not moving in from outside populations. As no "hot spots" were detected, insecticide applications must be made to the whole field. Therefore, the use of insecticides that minimally affect parasitoids is very important. This also suggests that an off-season insecticide application targeting pupae may be an effective control measure.

Our research reveals several areas where blueberry gall midge management can be improved. Bucket emergence traps are an effective monitoring tool in detecting low populations of blueberry gall midges. However, as most growers lack the time to construct these traps, they need to be commercialized. Because blueberry gall midges pupate so close to the blueberry bushes, soil treatments may be an effective management tactic.

Acknowledgments

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