Control strategies for *Rhagoletis mendax* disrupt host-finding and ovipositional capability of its parasitic wasp, *Diachasma alloeum*

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Abstract

Diachasma alloeum (Muesebeck) is a braconid parasitoid of *Rhagoletis mendax* Curran and *Rhagoletis pomonella* (Walsh). Laboratory choice tests using a Y-tube olfactometer compared behavioral responses of *D. alloeum* to various olfactory stimuli. More than 25% of naïve *D. alloeum* females, never having experienced *R. mendax* or blueberries, were 'innately' attracted to volatiles emitted from uninfested blueberry fruit. Experience with *R. mendax*-infested blueberry fruit approximately doubled the proportion of *D. alloeum* that subsequently responded to volatiles from uninfested blueberry fruit and decreased the time required to elicit this behavior compared with naïve wasps. Volatiles emanating from infested blueberry fruit, that were oviposited into by *R. mendax* 16–19 d prior to the assay, attracted four and two times more naïve and experienced *D. alloeum*, respectively, than uninfested fruit or fruit one day post-*R. mendax* oviposition. Although parasitoids were found to be attracted to blueberry volatiles in the lab, we hypothesized that insecticide applications targeting *R. mendax* could interfere with the wasps' normal host-finding behavior. In field studies, blueberry fruit treated with the neonicotinoid insecticide imidacloprid (Provado 1.6 F) and the kaolin-clay particle film Surround WP, eliminated parasitization of *R. mendax* by feral *D. alloeum*. Blueberry fruit treated with the kaolin-clay particle film was equally attractive to *D. alloeum* as untreated and infested fruit. But, the use of kaolin clay prevented wasps from ovipositing into berries after alighting. The results of this study imply that current management tools for *R. mendax* may negatively impact the importance of *D. alloeum* as a biocontrol agent of *Rhagoletis* flies.
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1. Introduction

To overcome the challenges of host location within patchy and complex environments, parasitoids exploit chemical (Price et al., 1980; Read et al., 1970; Sandlan, 1980; Vet, 1983), acoustic (Cade, 1975; Glas and Vet, 1983) or visual (Wäckers and Lewis, 1999; Henneman, 1996; Henneman et al., 2002) signals derived from the herbivore host, the herbivore's host-plant or the interaction between the feeding herbivore and its host-plant. Chemical stimuli emitted by the host-plant of the herbivore are thought to be particularly important for long-range detection of infested plants by parasitoids (Geervliet et al., 1994). Moreover, many plant species are stimulated to produce and emit specific volatile compounds shortly after herbivores feed upon them (Dicke et al., 1990; Turlings et al., 1990). These chemicals, produced as a specific response to herbivore feeding damage, are released directly from the damaged tissues and systemically from undamaged tissues of damaged plants (Röse et al., 1996).

The above described foraging capabilities of parasitic wasps can be disrupted by the use of insecticidal control strategies. For example, the foraging ability of the wasp *Microplitis croceipes* Cresson is drastically reduced after application of the neonicotinoid, imidacloprid, and the carbamate, aldicarb (Stapel et al., 2000). This reduced flight response and foraging ability is likely caused by sublethal effects following feeding bouts on extrafloral nectar of insecticide-treated cotton (Stapel et al., 2000). Insecticides can also have a negative impact on wasps' ability to perceive host-odor cues. Specifically, the orientation behavior of *Aphidius ervi* (Haliday) to host odor is disrupted by the pyrethroid, lambda-cyhalothrin (Desneux et al., 2000), and by triazamate (Desneux et al., 2004). Identifying the potential impact of insecticidal control strategies on foraging ability of parasitic wasps is critical to understanding the compatibility of chemical and biological control tactics.

Diachasma alloeum (Muesebeck) is a braconid parasitoid known to specifically attack two species belonging to the genus *Rhagoletis*. It occurs in hawthorn, *Crataegus mollis* Scheele, and apple, *Malus domestica* Borkhausen, in the northeastern USA and Canada, attacking the third larval instar of the apple maggot fly, *Rhagoletis pomonella* (Walsh) (Glas and Vet, 1983; Stelinski and Liburd, 2005). In addition, *D. alloeum* attacks a sibling species of *R. pomonella*, the blueberry maggot fly, *Rhagoletis mendax* Curran during the second larval instar (Liburd and Finn, 2003; Stelinski et al., 2004). In Michigan and the eastern regions of the United States, *R. pomonella* and *R. mendax* are key pests of apples and blueberries, respectively, with a zero tolerance policy on the part of the fruit industry to maggot infestation (Liburd et al., 1999). Parasitization rates of *R. mendax* larvae by *D. alloeum* collected from abandoned blueberry plantings in Michigan are high, ranging from 30 to 50% (Stelinski et al., 2004). These rates are higher than those recorded for *R. pomonella*, which range from 0.1 to 20.1% (Cameron and Morrison, 1977; Maier, 1982; Rivard, 1967).

Feral *D. alloeum* exploit chemical cues emitted by host-infested blueberry fruit in highbush blueberries, *Vaccinium corymbosum* L. (Stelinski et al., 2004). Specifically, *D. alloeum* preferentially alight and spend more time on blueberry fruit infested with *R. mendax* larvae compared with uninfested blueberry fruit. Volatiles released by *R. mendax*-infested blueberries may emit specific olfactory cues mediating attraction of female *D. alloeum* (Stelinski et al., 2004).

Recent regulations (Food Quality Protection Act) forecast reduced prevalence of broad-spectrum insecticides, such as organophosphates, for control of *Rhagoletis* species (Anonymous, 1996). Therefore, the effectiveness of 'reduced-risk', non-organophosphate insecticides, including imidacloprid and kaolin-clay particle film, has been evaluated for both *R. pomonella* (Reissig, 2003; Pelz et al., 2005) and *R. mendax* (Liburd et al., 2003; Pelz et al., 2005). These recently developed control tactics may feature prominently as management tools of *Rhagoletis* pests in the near future. Their impact on *D. alloeum*, an important biological control agent of both *R. mendax* and *R. pomonella*, requires investigation.

We describe here laboratory and field experiments designed to further elucidate chemically mediated behaviors of *D. alloeum* involved in microhabitat selection within blueberries. The objectives of the laboratory experiments were to determine: (1) whether volatiles emitted from uninfested, newly infested (1 d post-infestation), or aged (16–19 d post-infestation) blueberries are attractive to *D. alloeum*, and (2) whether previous experience with host-infested blueberries increases subsequent responses of *D. alloeum* to host-related volatile stimuli. We hypothesized that management strategies targeting *R. mendax* could affect microhabitat selection by *D. alloeum*. Thus, the objective of the field experiments was to determine whether applications of the insecticide (imidacloprid, Provado 1.6 F) or the kaolin-clay particle film (Surround WP), recently evaluated and proven effective against *R. mendax* (Liburd et al., 2003), impact host-finding and oviposition behavior of *D. alloeum*.

2. Materials and methods

2.1. Insect source

R. mendax were reared from larval collections of infested fruit of unsprayed blueberries from the abandoned plantation described below. Flies were reared according to the protocol outlined in Liburd et al. (2003). Prior to testing, flies were maintained in aluminum screen-Plexiglas cages ($30 \times 30 \times 30$ cm) (BioQuip, Gardenia, CA) and supplied with water and food (enzymatic yeast hydrolysate and sucrose) (ICN Biomedicals Inc, Costa Mesa, CA). Adults were kept at 24 °C, 55–60% RH, under a 16:8 (L:D) photocycle.

Three weeks after removal of *R. mendax* puparia from 4 °C (diapause), *D. alloeum* began emerging from ca. 40% of puparia. The parasitoids had been previously identified by R.A. Wharton (Texas A&M University) (Stelinski et al., 2004) and voucher specimens were deposited at Michigan State University (A.J. Cook Arthropod Research Collection). *D. alloeum* were segregated by sex and maintained in 1 L plastic cages containing a 5% sucrose solution in plastic cups with dental cotton wicks protruding from their lids. Photocycle and temperature conditions were identical to those described above for *R. mendax*.

2.2. Y-tube olfactometer studies

Choice tests comparing behavioral responses of *D. alloeum* to various olfactory stimuli were conducted

in a horizontal, glass Y-tube olfactometer (stem length 25 cm, arm length 12.5 cm, internal diameter 1.5 cm). Experiments were conducted at 24 °C with a light intensity of 800–1000 lx generated by two fluorescent bulbs (Lumchrome model 1XC, 40 W) mounted 1.5 m above the olfactometer. Assays ran between 1230 and 1500 h, a period when *D. alloeum* are highly active in the field (Stelinski et al., 2004). All wasp used in Y-tube tests were 3–4 d post-emergence. Data recorded were: (1) number of female *D. alloeum* contacting the end of a particular arm of the Y-tube olfactometer, (2) time interval required to reach end of a Y-tube arm, (3) and "latency time" as previously defined by Mbata et al. (2004). Typically, wasps exhibited random walking limited to the first 1.5 cm of the Y-tube stem during the 30–45 s prior to introductions of odor stimuli. Directly after an introduction of a stimulus, wasps exhibited arrestment, followed by upwind orientation after a short time interval. "Latency time" is defined as that time interval from wasp arrestment, which usually took place directly upon introduction of an olfactory stimulus into an arm of the Y-tube, until commencement of directed upwind movement by a wasp within the olfactometer.

In behavioral olfactometer assays, female *D. alloeum* were presented with: (1) uninfested blueberries (previously enveloped within plastic bags in the field to prevent oviposition by feral *R. mendax* females as described below), (2) newly infested blueberries that were oviposited into by laboratory-reared and mated *R. mendax* females 24 h prior to the assay, and (3) aged-infested blueberries that were oviposited into by laboratory-reared and mated *R. mendax* 16–19 d prior to the assay. Blueberries were infested by *R. mendax* in the field by placing 15 mated *R. mendax* females into bags enveloping blueberry clusters for 24 h as described below. Fruit treatments used in the olfactometer were assayed between 50 and 180 min after picking berries from the field. Berries of each treatment were randomly selected and inserted individually into a 1 L flask connected via Tygon tubing to one arm of the Y-tube. Glass marbles were placed into a second 1 L flask connected to the other arm of the Y-tube. Immediately after test stimuli were inserted, carbon-filtered and humidified air (100 ml/min) was delivered via Tygon tubing through each flask and into each arm of the Y-tube. Treatment pairs tested in choice tests were: (1) uninfested berry versus marble, (2) newly infested berry versus marble, (3) aged-infested berry versus marble, (4) newly infested berry versus uninfested berry, (5) newly infested berry versus aged-infested berry.

Two treatment 'types' of female *D. alloeum* were tested in the olfactometer. 'Naïve' wasps were 3–4 d old females kept in 1 L plastic cups and never exposed to blueberry fruit or *R. mendax* larvae after emergence. 'Experienced' wasps were 3–4 d old females maintained in 1 L plastic cups containing 15–20 d old *R. mendax*-infested blueberries for 24 h prior to the assay. Fifteen replicates of two *D. alloeum* females (naïve or experienced) were inserted into the olfactometer and assayed per treatment. Observations were conducted for 4 min on each group of wasps assayed.

2.3. Field sites

Field studies were conducted in an unmanaged (2.6 ha) plantation of highbush blueberry, *V. corymbosum* L. (Variety: Jersey) that has not received pesticide applications for >5 years and an organically managed (3.7 ha) plantation of the same variety described by Stelinski et al. (2004) and Stelinski and Gut (2004). Both sites are located less than 4.5 km apart in Fennville, MI, USA. These blueberry plantings were chosen because they were known to harbor high *R. mendax* populations with >45% of picked berries containing developing larvae in 1999 and 2000 (Liburd and Stelinski, 1999; Stelinski et al., 2004). In addition, parasitization of *R. mendax* by *D. alloeum* was relatively high at both sites with *D. alloeum* emerging from more than 50% of collected puparia in 1999 and 2000 (Stelinski et al., 2004).

2.4. Field studies (fruit infestation)

Thirty-five blueberry fruit clusters were selected for field studies before *R. mendax* emergence (June 24). Each cluster consisted of ca. 20–35 blueberries. All clusters were approximately 15 cm from the uppermost bush; this location within the blueberry bush canopy has been found to be the most effective position for trapping blueberry maggot (Liburd et al., 2000). At this stage of the season, blueberry fruit was still green and unripe. Experimental bushes were flagged and selected clusters were individually enveloped with 1 L translucent plastic bags that had been punctured with a pin multiple times. Bags were positioned around blueberry fruit clusters such that berries did not directly contact the bag surface. This bagging technique has been shown to prevent feral *R. mendax* females from ovipositing into blueberries (Stelinski et al., 2004). In addition, this technique has been determined not to interfere with normal berry development and ripening (Stelinski et al., 2004).

On 22 July, each of the 35 bagged blueberry clusters were infested with 10 laboratory-reared, mated *R. mendax* according to the protocol outlined by Stelinski et al. (2004). Introduced *R. mendax* were left in the bags for 24 h and then removed. Of the 35 blueberry clusters infested by *R. mendax*, 30 were randomly chosen for the field study. These selected 30 clusters were randomly assigned to six treatments, each replicated five times. Treatments were applied 24 h after fruit was infested by *R. mendax* and prepared as described in Liburd et al. (2003). The treatments were: (1) untreated berry clusters with the bag removed after *R. mendax* infestation, (2) untreated berry clusters with bag present after

R. mendax infestation, (3) berry clusters treated with the kaolin clay Surround WP (Engelhard Co., Iselin, New Jersey, USA) at a rate of 6 g in 100 ml of water and with the bag removed after treatment, (4) berry clusters treated with kaolin clay as in treatment 3 but with bag replaced after treatment, (5) berry clusters treated with imidacloprid (Provado 1.6 F, Bayer, Kansas City, Missouri, USA) at a rate of 100 μl in 160 ml of water with bag removed after treatment, (6) berry clusters treated with imidacloprid as in treatment 5 but with bag replaced after treatment. The treatments were prepared with an oil surfactant (Sun ultra, Sun Refining and Marketing Company, Philadelphia, Pennsylvania, USA) at 1% v/v (surfactant/water). Treatments were applied to berry clusters with an atomizer at recommended field rates by proportionally scaling down large quantity application recipes. Both kaolin clay and imidacloprid treatments were reapplied biweekly after the initial application date. Koalin clay was also reapplied, as needed, after rain events.

At the end of the growing season (10 August), ripe blueberries from each treatment cluster (majority of berries turned blue around stem) were picked and kept separate according to treatment. All berries from each replicate were then placed over 0.5 cm mesh hardware cloth allowing *R. mendax* larvae to exit the fruit and drop into containers filled with vermiculite (Stelinski and Liburd, 2001). The vermiculite was sifted and *R. mendax* puparia were collected and placed into cold storage for 140 d at 4 °C. Pupal development was initiated by removing puparia from cold storage and placing them into an environmental chamber at 24 °C, 55–60% RH, under a 16:8 (L:D) photocycle. Approximately 1 month afterwards, *R. mendax* and *D. alloeum* began to emerge from collected puparia. Emergence data for both species were collected over a 1 month period thereafter.

2.5. Field studies (direct observation)

Direct visual observations began 13 d after blueberry clusters were infested with *R. mendax* and continued thereafter on every second day until day 23. Observations were conducted between 1230 and 1530 h. Observers rotated among the five replicates of treatments 1, 3, and 5 described above (with bags removed after treatments were applied), conducting approximately 15 min observational bouts per location.

Observed events were spoken into a hand-held microcassette audio recorder by an investigator sitting or standing 0.75 m from the treatment clusters. Data recorded were: (1) number of female *D. alloeum* alighting on berry clusters, and (2) number of female *D. alloeum* ovipositing into berries. We attempted to collect observed *D. alloeum* with an aspirator after they oviposited and before they left experimental berry clusters. We estimate to have captured 65% of all visitors. Captured *D. alloeum* were taken to the laboratory to confirm identification.

2.6. Statistical analysis

Analyses of numbers of wasps responding to treatments within the Y-tube olfactometer were carried out using the G statistic (Sokal and Rohlf, 1981) with the Proc GENMOD procedure in SAS (SAS Institute, 2000). Data for duration to reach odor sources and latency time were transformed to $\ln(x + 1)$ (which normalized the distributions) and then subjected to analysis of variance (ANOVA). Comparisons of mean numbers of *R. mendax* puparia retrieved from infested berry treatments and mean numbers of *D. alloeum* and *R. mendax* that emerged from recovered puparia were made with ANOVA and Fisher's protected least significant difference (LSD) procedure (SAS Institute, 2000). Field data were square-root transformed $[(x + 0.5)^{1/2}]$ before analysis. In all cases, significance level was $\alpha < 0.05$.

3. Results

3.1. Y-tube olfactometer

Significantly more female *D. alloeum* (naïve or experienced) oriented to and contacted the source of volatiles emanating from uninfested, newly infested (1 d after infestation), and aged-infested (16–19 d after infestation) blueberries compared with marbles (surrogate blueberries) in the Y-tube olfactometer (Table 1). In assays that directly compared attractiveness of uninfested versus newly infested blueberries, nearly equal numbers of naïve or experienced female *D. alloeum* approached each type of fruit (Table 1). However, significantly more naïve or experienced female *D. alloeum* oriented to and contacted the source of volatiles from aged-infested compared with newly infested blueberries (Table 1).

Table 1
Proportions (Mean ± SE) of naïve or experienced female *Diachasma alloeum* contacting odor sources in Y-tube olfactometer

Treatment pairs	Proportion contacting source		
	Naïve		Experienced
Uninfested berry	0.26 ± 0.04a[a]	*	0.43 ± 0.04a
Marble	0.03 ± 0.01b	NS	0.03 ± 0.02b
Newly infested (1 day) berry	0.23 ± 0.03a	*	0.43 ± 0.04a
Marble	0.03 ± 0.01b	NS	0.03 ± 0.01b
Aged (17 day) Infested berry	0.87 ± 0.03a	NS	0.83 ± 0.03a
Marble	0.02 ± 0.01b	NS	0.05 ± 0.02b
Newly infested berry	0.20 ± 0.04a	*	0.33 ± 0.03a
Uninfested berry	0.20 ± 0.03a	*	0.36 ± 0.02a
Newly infested berry	0.07 ± 0.02b	*	0.13 ± 0.02b
Aged (17 day) infested berry	0.80 ± 0.04a	NS	0.70 ± 0.04a

NS indicates lack of significance.

[a] Pairs of means in the same column followed by the same letter are not significantly different and paired values within rows for naïve and experienced wasps marked with an asterisk are significantly different ($P < 0.05$, G^2 test of homogeneity).

Significantly more experienced *D. alloeum* females oriented to and contacted the source of volatiles from uninfested and newly infested blueberries compared with naïve wasps orienting to these same odor sources (Table 1). However, there was no significant difference between the proportions of naïve versus experienced *D. alloeum* orienting to and contacting aged-infested berries (Table 1).

D. alloeum (naïve or experienced) oriented to and contacted the source of volatiles from aged-infested blueberries in significantly less time compared with counterparts orienting to uninfested or newly infested blueberries (Table 2). Furthermore, experienced female *D. alloeum* contacted uninfested and newly infested blueberries significantly faster than naïve counterparts (Table 2). However, the mean time required for naïve or experienced *D. alloeum* to contact the source of volatiles from aged-infested blueberries was not significantly different (Table 2). The latency time for experienced female *D. alloeum* was significantly shorter compared with naïve wasps when orienting to and contacting uninfested blueberries (Table 2).

3.2. Field studies (fruit infestation data)

The mean number of *R. mendax* puparia collected from infested blueberries of each treatment (with and without bags enveloping clusters) was nearly equal (Table 3). Significantly more *D. alloeum* emerged from untreated infested blueberries without bags compared with infested blueberries treated with either kaolin clay or imidacloprid (Table 3). In addition, significantly fewer *R. mendax* emerged from untreated and infested blueberries without bags compared with those treated with either kaolin or imidacloprid (Table 3). The proportion of viable puparia that resulted in fly emergence did not differ between un-bagged berries treated with either kaolin clay or imidacloprid and those treated identically but enveloped in bags (Table 3). Emergence of *R. mendax* was significantly reduced through parasitization by *D. alloeum* only in those berries which were left untreated and un-bagged. The number of puparia that did not result in either *R. mendax* or *D. alloeum* emergence (unviable) did not differ between treatments (Table 3).

3.3. Field studies (observational data)

The mean number of female *D. alloeum* observed alighting on untreated and infested blueberries (4.6 ± 1.9/day) was not statistically ($F = 0.4$; $df = 2$, 15; $P > 0.05$) different from to the mean number observed alighting on kaolin-treated and infested blueberries (5.5 ± 2.2/day). However, only one female *D. alloeum* landed on an imidacloprid-treated and infested blueberry throughout the observation period (Fig. 1A). Of the 28 female *D. alloeum* observed alighting on untreated and infested blueberries, 15 were observed ovipositing into those berries soon after landing (Fig. 1B). However, only one of the 33 female *D. alloeum* alighting

Table 2
Mean (±SE) time to reach odor sources and latency time (s) of *Diachasma alloeum* females in Y-tube olfactometer

Treatments	Time to contact source (s)			Latency time (s)		
	Naïve		Experienced	Naïve		Experienced
Uninfested berry	121.6 ± 8.6a[a]	*	95.6 ± 9.3a	19.7 ± 2.1a	*	11.5 ± 1.5a
Newly infested berry	121.2 ± 9.2a	*	84.8 ± 8.1a	18.5 ± 1.8a	NS	13.6 ± 1.7a
Aged (17 day) infested berry	67.7 ± 8.6b	NS	59.6 ± 8.5b	10.1 ± 2.3b	NS	6.2 ± 1.4b

NS indicates lack of significance.
[a] Means in the same column followed by the same letter are not significantly different and paired values within rows for naïve and experienced wasps marked with an asterisk are significantly different ($P < 0.05$, ANOVA followed by Tukey's multiple comparisons test).

Table 3
Numbers (Mean ± SE) of *Rhagoletis mendax* puparia retrieved from infested berry treatments and Mean (±SE) numbers of *Diachasma alloeum* and *R. mendax* emerging from recovered puparia

Treatment	Number of *R. mendax* puparia	Number of emerged *D. alloeum*	Number of emerged *R. mendax*	Number of non-viable *R. mendax* puparia (no emergence)
Untreated infested berries without bag	11.2 ± 1.5a[a]	4.8 ± 1.0a	6.0 ± 0.7b	0.3 ± 0.2a
Untreated infested berries with bag	11.8 ± 1.2a	0.0 ± 0.0	10.8 ± 1.1a	1.1 ± 0.4a
Kaolin-sprayed infested berries without bag	11.7 ± 1.5a	1.2 ± 0.5b	10.0 ± 1.3a	0.5 ± 0.3a
Kaolin-sprayed infested berries covered with bag	11.5 ± 1.9a	0.0 ± 0.0	10.7 ± 1.8a	0.8 ± 0.3a
Imidacloprid-sprayed infested berries without bag	12.2 ± 1.8a	0.3 ± 0.2b	11.2 ± 1.7a	0.7 ± 0.3a
Imidacloprid-sprayed infested berries covered with bag	12.0 ± 1.5a	0.0 ± 0.0	11.5 ± 1.6a	0.5 ± 0.2a

[a] Means in the same column followed by the same letter are not significantly different ($P < 0.05$, ANOVA followed by LSD test).
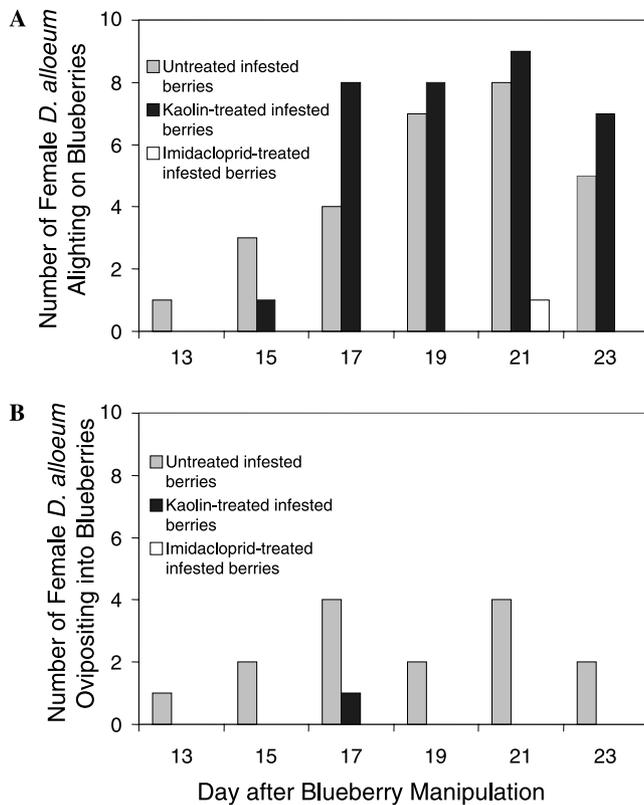
Fig. 1. (A) Numbers of female *D. alloeum* observed alighting on: (1) untreated *R. mendax*-infested blueberries, (2) kaolin-treated and *R. mendax*-infested blueberries, (3) imidacloprid-treated and *R. mendax*-infested blueberries. (B) Numbers of female *D. alloeum* observed ovipositing into: (1) Untreated *R. mendax*-infested blueberries, (2) kaolin-treated and *R. mendax*-infested blueberries, (3) imidacloprid-treated and *R. mendax*-infested blueberries. Observations were conducted on every second day 13–23 d post-*R. mendax* infestation.

on kaolin-treated and infested blueberries was observed ovipositing; the rest left berries ca. 10 s after landing. Furthermore, the single *D. alloeum* female alighting on an imidacloprid-treated berry left that berry within 5 s after landing and did not oviposit.

4. Discussion

The braconid parasitoid, *D. alloeum*, is a host specialist, restricted to the tephritid sibling species, *R. mendax* and *R. pomonella*. In our laboratory assays, slightly more than 25% of naïve *D. alloeum* females, never having experienced their host *R. mendax* or blueberries, were 'innately' attracted to volatiles emitted from uninfested blueberry fruit. Specialist parasitoids are known to exhibit innate responsiveness to olfactory stimuli associated with their host or host habitat (Mbata et al., 2004; Vet, 1983; Waage, 1979). Experience with *R. mendax*-infested blueberry fruit approximately doubled the proportion of *D. alloeum* that subsequently responded to volatiles from uninfested blueberry fruit. In addition,

experienced *D. alloeum* oriented to volatiles from uninfested blueberries and contacted the source of these volatiles in much less time compared with naïve wasps. Behavioral plasticity or learning, where previous experience enhances responsiveness to semiochemicals, is a well documented characteristic of parasitic wasps (Vet et al., 2002). Furthermore, learning associated with experiencing host-related chemical cues, such as was observed here for *D. alloeum*, occurs in many species of specialist parasitoids (Arthur, 1971; de Jong and Kaiser, 1991; Kaiser and Cardé, 1992; Simons et al., 1992; Vet, 1983; Vet and van Opzeeland, 1984).

Feral *D. alloeum* are maximally attracted to *R. mendax*-infested blueberries 17–21 d post-*R. mendax* oviposition (Stelinski et al., 2004). The current results suggest that naïve wasps are highly attracted to blueberry fruit infested by *R. mendax* for at least 16 d, and that this attraction is not further influenced by experience. In contrast, attraction to uninfested fruit is approximately doubled by prior experience. At 16 d post-infestation, the majority of *R. mendax* have likely reached the second larval instar (Lathrop and Nickels, 1932; Nuenzig and Sorensen, 1976). Perhaps at this stage, larval feeding damage induces berries to produce specific volatile cues that maximally attract *D. alloeum* or volatiles from accumulating frass become maximally attractive (Cortesero et al., 1997; Röse et al., 1997; Steinberg et al., 1993). Enhanced attraction of parasitoids to habitats damaged by host-infestation is a well-documented and reviewed phenomenon (Bragg, 1974; Camors and Payne, 1972; Monteith, 1964; Turlings and Wäckers, 2004; Vet and Dicke, 1992; Vinson, 1985) and appears to be highly influential in microhabitat selection by *D. alloeum*.

Parasitic wasps are known to exhibit attraction to the epideictic (host marking) pheromones of tephritid fruit flies (Hoffmeister and Gienapp, 1999). Both *R. mendax* and *R. pomonella* mark their host fruit with an epideictic pheromone directly after ovipositing (Prokopy, 1981). In the current study, uninfested blueberry fruit kept isolated from *R. mendax* and blueberry fruit oviposited into by *R. mendax* 24 h prior to the behavioral assay were equally attractive to *D. alloeum*. These results do not support the hypothesis that *D. alloeum* exploits the epideictic pheromone of *R. mendax* as a long-range stimulus. However, further work should address whether this epideictic pheromone is exploited as a contact stimulus.

In the field, pre-treatment by both the neonicotinoid insecticide, imidacloprid, and the kaolin-clay particle film, Surround WP, applied to selected blueberry fruit in otherwise untreated plantings, eliminated parasitization of *R. mendax* by feral *D. alloeum*. During observations of imidacloprid-treated fruit, only a single *D. alloeum* briefly (ca. 5 s) alighted on berries, without ovipositing, suggesting that volatilization of imidacloprid repelled *D. alloeum*. This hypothesis is corroborated by a previous study, which demonstrated that solutions of imidacloprid

(500–1000 ppb) repel honey bees (*Apis mellifera*) (Bortolotti et al., 2003). In contrast, the number of *D. alloeum* observed alighting on kaolin-treated berries was slightly higher, but not statistically different from the number alighting on untreated fruit. However, kaolin treatment suppressed parasitization of *R. mendax* by *D. alloeum*. Wasps were observed alighting on fruit, but only a single observed wasp attempted oviposition. Therefore, blueberry fruit treated with the kaolin-clay particle film was not less attractive to *D. alloeum*, but prevented wasps from ovipositing into berries after alighting. Kaolin clay is a known insect ovipositional and feeding deterrent (Glenn et al., 1999; Knight et al., 2000) and is thought to inhibit activity of *R. mendax* on treated blueberries (Liburd et al., 2003). Thus, it is not surprising that kaolin clay, applied to blueberries, impaired normal oviposition by *D. alloeum*.

It is interesting that *R. mendax*-infested blueberries treated with kaolin clay, which appeared bright white in color, attracted *D. alloeum* as effectively as untreated infested berries of natural color. This suggests that the olfactory cue emitted by infested blueberries is more important than a visual cue for attracting female *D. alloeum*. *Diachasmimorpha juglandis* (Muesebeck) can distinguish between uninfested and infested walnuts, in the early stages of infestation (3–4 d after oviposition by *Rhagoletis* host), using olfactory cues (Henneman et al., 2002). However, this species can also use visual cues alone to identify infested fruit in the later stages of infestation (Henneman et al., 2002). Damaged walnuts are distinctly different in appearance (darkened) compared with undamaged walnuts, and this difference is known to influence fruit selection by *D. juglandis* (Henneman et al., 2002). In contrast, *R. mendax*-infested blueberries are virtually indistinguishable from uninfested berries during the stage when berries are maximally attractive (15–21 d post-*R. mendax* oviposition) to *D. alloeum* (Stelinski et al., 2004) and thus visual cues are unlikely to play an important role in host finding for this species in blueberries.

Parasitization of *R. mendax* by *D. alloeum* can be extremely high in unmanaged blueberry plantations in Michigan (>50%); however, rates decrease to ca. 2% in commercially managed blueberries receiving broad-spectrum insecticide sprays (Stelinski et al., 2004). In certain cases, the presence of *D. alloeum* cannot be detected under heavy management regimes (Stelinski, personal observation). The current study has shown that two newly evaluated and effective management tools for *R. mendax* (imidacloprid and Surround WP), thought to be more selective (Liburd et al., 2003), eliminate parasitization of *R. mendax* by *D. alloeum*. Thus, the effect of this potentially important biocontrol agent of *R. mendax* might be negated in plantings treated with these more selective and environmentally friendly controls and is likely heavily impacted in plantings sprayed with broad-spectrum compounds such as organophosphates or carbamates. Recent studies have shown that imidacloprid-treated spheres, baited with an ammonium lure, attract and kill *R. mendax* (Stelinski and Liburd, 2001; Stelinski et al., 2001). In small plot trials, deployment of such spheres suppressed *R. mendax* infestation equally well to applications of the organophosphate insecticide Guthion (Stelinski and Liburd, 2001). It is unlikely that these attracticidal devices, specifically attractive to *R. mendax*, impact *D. alloeum* populations (Liburd et al., 2004). Therefore, implementation of insecticide-treated spheres for *R. mendax* control may better serve biorational management strategies for *R. mendax*, which aim to conserve natural enemies such as *D. alloeum*.

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