J Insect Behav (2010) 23:419–430
DOI 10.1007/s10905-010-9225-9

Oviposition Marking Behavior of *Diachasma alloeum*, (Hymenoptera: Braconidae), Parasitizing *Rhagoletis pomonella*, (Diptera: Tephritidae)

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Revised: 4 August 2010 / Accepted: 10 August 2010 /
Published online: 18 August 2010

Abstract *Diachasma alloeum* (Muesebeck) (Hymenoptera: Braconidae) is a solitary larval endoparasitoid attacking *Rhagoletis* (Diptera: Tephritidae) species. *Rhagoletis pomonella* (Walsh) mark the surface of fruit after oviposition with an oviposition marking pheromone (OMP) which deters conspecific female flies. Herein we demonstrate that female *D. alloeum* wasps reared from either apple or hawthorn race *R. pomenella* larvae also deposit an OMP that reduces oviposition by conspecific female wasps. Significantly fewer wasps accepted fruit that had received prior wasp oviposition and OMP or OMP alone without oviposition compared with control fruit for a minimum of 7 days on both fruit types. Rinsing fruit with a 50% ethanol solution appeared to remove the OMP rendering fruit more acceptable for oviposition than marked fruit that was not rinsed. Wasps of each host race were able to detect and avoid the OMP of the sister race and fruit substrate type did not affect wasp response to the pheromone. The possibility of an internal marker deposited during oviposition is also discussed.

Keywords *Rhagoletis pomonella* · *Diachasma alloeum* · oviposition behavior · oviposition marking pheromone · apple · hawthorn

Introduction

Female insects belonging to the orders Coleoptera, Diptera, Homoptera, Hymenoptera, Lepidoptera, Neuroptera, and Orthoptera are known to deposit chemicals on or near their site of oviposition directly after egg laying (Prokopy 1981a, b; van Lenteren 1981; Roitberg and Prokopy 1987; Landolt and Averill 1999; Nufio and Papaj 2001). These chemicals have been defined as oviposition marking pheromones (OMPs) (Corbet 1971; Prokopy 1981a, b; Hoffmeister and Roitberg 2002). OMPs

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serve to inform conspecifics of previously utilized hosts and thereby prevent deposition of future eggs into a less suitable host (Prokopy 1981a; Hoffmeister and Roitberg 2002).

Rhagoletis pomonella (Walsh) (Dipera: Tephritidae) is a North American insect pest that infests hawthorn (*Crataegus mollis* Scheele) and has recently expanded its host range to utilize domesticated apple (*Malus domestica* Borkh.) (Bush 1966). The apple and hawthorn races of these flies have unique behavioral, physiological, and genetic differences (Feder et al. 1994; Linn et al. 2004, 2005; Dambroski et al. 2005). *Diachasma alloeum* is a stenophagous, solitary endoparasitoid attacking third-instar *R. pomonella* larvae infesting either apple or hawthorn and are specifically limited to a subset of the *R. pomonella* group including the blueberry maggot, *R. mendax* Curran, and the snowberry maggot, *R. zephyria* Snow (Wharton and Marsh 1978). *D. alloeum* females preferentially locate fly infested fruit for oviposition based on vibrational (Glas and Vet 1983) and olfactory (Stelinski et al. 2006) cues. Genetic, physiological and behavioral data indicate that populations of *D. alloeum* are also becoming reproductively isolated and are preferentially attracted to odors of their natal fly's host fruit (Forbes et al. 2009). After locating fruit, female wasps typically inspect the fruit surface and may probe the fruit by inserting their ovipositor. Glas and Vet (1983) reported that female *D. alloeum* exhibited three types of ovipositor probes while attacking *R. pomonella* in hawthorn: 1) a short probe where the ovipositor was immediately retracted; 2) a longer probe with regular insertion and removal of the ovipositor into the puncture hole without complete retraction; and 3) a probe which lasted for 1–4 min with the insect being motionless for the entire duration. It was concluded that 'type 3' probing resulted in oviposition. These probes most likely allow the wasp to locate a host larva within the fruit and perhaps determine if the larva has been previously parasitized. Directly after completion of 'type 3' probing, females walk on fruit while dragging their ovipositor on the fruit surface and deposit a clear liquid, which was termed 'excreting' behavior by Glas and Vet (1983).

The ovipositor dragging behavior of *D. alloeum* that parasitize the blueberry maggot, *R. mendax,* has been previously investigated (Stelinski et al. 2004, 2007). Blueberries marked by *D. alloeum* emerging from *R. mendax* were rejected for oviposition by conspecific female wasps and the behavioral effect lasted for at least 7 days following deposition of the marking pheromone. In the present study, we demonstrate that *D. alloeum* females, originating from either apple or hawthorn-infesting *R. pomenella*, also deposit an OMP directly following oviposition. Female wasps reject their 'natal' and 'non-natal' fruits marked with the OMP and thereby do not deposit eggs into a previously utilized resource. Furthermore, we demonstrate cross-recognition of the OMP between wasp host races.

Materials and Methods

Insects

R. pomonella flies and *D. alloeum* wasps were obtained from apple and hawthorn fruits infested with parasitized and un-parasitized *R. pomonella* larvae in Fennville,

MI, USA (42° N, 86° W) in 2005–2007. The apple orchard was abandoned and located ~1.4 km away from a stand of 18 hawthorn trees (Stelinski and Liburd 2005). Mature *R. pomonella* larvae exiting from infested apple or hawthorn fruit were allowed to pupate in moist vermiculite (Liburd et al. 1998). Puparia were placed in cold storage at 4°C for 140 days for diapause development and then transferred to an environmental growth chamber set at 24°C and 55–60% relative humidity (RH) with a photocycle of 16:8 h (L:D). Adult *R. pomonella* and *D. alloeum* began to emerge approximately 4–5 and 5–6 weeks after removal from cold storage, respectively. Flies and wasps originating from apple or hawthorn fruits were maintained separately in aluminum screen-Plexiglas cages (30×30×30 cm) (Bio-Quip, Rancho Dominguez, CA). Adult flies were provided with enzymatic yeast hydrolysate (ICN Biomedicals, Costa Mesa, CA) and water and wasps with a 5% sucrose solution.

Infestation of Apple and Hawthorn Fruits by *R. pomonella*

The protocol used for infesting apple or hawthorn fruits was similar to that described by Stelinski et al. (2007). In the respective field sites, selected unripe and uninfested apple (June 20th–30th) and clusters of 3–6 hawthorn fruit (August 15th–30th) were wrapped with 1-L nylon mesh bags to prevent feral *R. pomonella* from laying eggs into these fruits. This procedure is known to prevent egg laying into blueberries by female *R. mendax* and does not interfere with normal fruit development (Stelinski et al. 2006). Ten laboratory-reared and mated *R. pomonella* females from either apple or hawthorn were released into the bagged fruit (August 25th–30th for apple; September 15th–18th for hawthorn) and given 24 h for oviposition prior to removal (Stelinski et al. 2006). Infested apple and hawthorn fruits were bagged for approximately 3–4 weeks to allow for development of *R. pomenella* to the third larval instar and remained on the trees prior to use in behavioral assays.

Laboratory Assays of Wasp Behavior on Apple or Hawthorn Fruit

Experiments were conducted to test hypotheses that: 1) naïve female *D. alloeum* avoid depositing eggs into apple or hawthorn fruits infested by *R. pomonella* that had previously received oviposition and ovipositor dragging from a conspecific female wasp compared with controls; and 2) that this behavior is mediated by an OMP deposited during dragging and not by other stimuli associated with oviposition. *D. alloeum* females obtained from the apple race of *R. pomonella* were tested with fly-infested apples while those obtained from the hawthorn race of *R. pomonella* were tested with fly-infested hawthorns unless stated otherwise.

The test procedure was similar to that described by Stelinski et al. (2007). Stems containing field infested fruits (apple or hawthorn) were removed from trees and were inserted into floral aquapiks. Each aquapik with fruit was placed into a 1-L translucent plastic container and closed with a perforated lid. Fruit picked for testing were used within 3 d of removal from the field. Both pre-assay treatment manipulations and subsequent behavioral assays were performed using sexually mature (7–10-d-old) female wasps that were maintained in cages in groups of 15–30 from eclosion until use in assays.

Female wasp behavior observed in all assays was similar to that previously described for *D. allouem* wasps attacking *R. mendax* developing in blueberry fruit (Stelinski et al. 2007). After alighting, female wasps spent approximately 1 min walking on fruit while intermittently drumming their antennae on the fruit surface. Approximately 15% of the females observed landing on fruit left unmarked fruit within 30 s without probing with their ovipositor. The remainder (85%) briefly (1–4 s) probed with their ovipositor approximately 9 times in such a manner that the ovipositor bowed inward toward the abdominal ventrum. Of those wasps that probed in this manner, 100% subsequently probed such that the ovipositor bowed outward from the abdominal ventrum and was inserted below the fruit skin. The latter type of probing lasted approximately 75 s and is assumed to have resulted in oviposition. Following oviposition, all observed females dragged their ovipositor across the fruit surface depositing a clear liquid. On average, female wasps made 11 circles around the circumference of fruit while dragging their ovipositor, which required approximately 1 min.

The objective of Experiment 1 was to determine the effect of female *D. alloeum* oviposition and fruit marking on the oviposition behavior of subsequent conspecific females. The pre-assay manipulation consisted of allowing a single naïve female *D. alloeum* wasp to oviposit and drag its ovipositor on the surface of a single fruit. A fly-infested fruit that was not exposed to *D. alloeum* was the control treatment. Thereafter, naïve female *D. alloeum* were presented sequentially and in random order with either control or *D. alloeum*-marked fruit to determine acceptance or rejection of fruit for oviposition. Females will oviposit in an acceptable fruit usually within 10 s of the initial probing event; wasps leave fruit that are unacceptable within ca. 2–5 s after landing. Experiment 2 was conducted to differentiate between the effect of wasp oviposition without subsequent marking and marking without oviposition. Female *D. alloeum* were allowed either to oviposit into fruit without subsequent marking or to mark fruit without oviposition. This was achieved by aspirating females off of fruit immediately after oviposition and gently transferring them onto new fruit. The transferred females would then drag their ovipositors on the clean fruit. The marking behavior of transferred wasps did not appear different from wasps that were not interrupted during oviposition. Naïve female *D. alloeum* were presented sequentially and in random order with fruit that had: 1) received oviposition but no marking; 2) received marking but no oviposition; and 3) no exposure to *D. alloeum.*

In Experiment 3, the duration of the behavioral effect of the wasp OMP was determined by preparing fruit with *D. alloeum* oviposition and marking or fruit with oviposition but no mark as described above. Fruit were then maintained in floral aquapicks in an environmental chamber for 7 days prior to initiation of the bioassays at the insect rearing conditions described above. *D. alloeum* females were then presented sequentially and in random order with fruit that had: 1) oviposition and marking; 2) oviposition without marking; or 3) untreated control fruit.

In Experiment 4, we confirmed the chemical nature of the mark deposited by wasps following oviposition. Test fruit were prepared by: 1) allowing female *D. alloeum* to oviposit and drag their ovipositor on a single fruit surface, or 2) transferring females as above after oviposition and then allowing them to mark on a clean fruit that did not receive oviposition. Within 30 min of these treatment

manipulations, a portion of the marked fruit was washed with 50% ethanol solution. Rinsed fruit were air dried for 1 h prior to assays. Following treatment manipulations, female *D. alloeum* were presented sequentially and in random order with: 1) solvent rinsed fruit that had received *D. alloeum* oviposition and marking; 2) solvent rinsed fruit that had received *D. alloeum* marking only; 3) unrinsed fruit that had been marked only (positive control); 4) or fruit infested with *Rhagoletis* larvae but not exposed to *D. alloeum* females (negative control).

To further confirm the chemical nature of the wasp OMP, in Experiment 5 the pheromone was harvested from marked fruit by solvent rinsing and artificially re-applied to unmarked fruit for testing. *D. alloeum* were allowed to oviposit and mark fly-infested fruit (either apple or hawthorn). Thirty marked fruit (separately for apple and hawthorn) were washed with 5 ml of the 50% ethanol solution. Fly-infested fruit with no exposure to *D. alloeum* were either sprayed with ~2.7 ml of the fruit wash solution or with the same amount of 50% ethanol alone. Sprayed fruit were air dried for 1 h prior to assays and presented to naïve female *D. alloeum* sequentially and in random order.

Given that *D. alloeum* mark and inspect the surface of fruit concealing their larval host, in Experiment 6 we tested the hypothesis that recognition of the OMP by wasps is context dependent; i.e. apple wasps detect their pheromone on apple but not hawthorn and vice versa. Apple or hawthorn fruit were treated with the 50% ethanol extract of pheromone harvested from either apple- or hawthorn origin wasps. Following treatment manipulations, female *D. alloeum* were presented with: 1) apple or hawthorn fruit treated with apple-origin wasp OMP versus an untreated control fruit of the same type; or 2) hawthorn or apple fruit treated with hawthorn-origin wasp OMP versus the same type of untreated control fruit. Experiments on apple and hawthorn were conducted separately. The proportions of oviposition attempts into untreated (control) apple and hawthorn fruit were nearly identical in each experiment and thus these data were combined as a single control for subsequent comparison among treatments (see Results).

In Experiment 7, we tested the hypothesis that cross-recognition of the OMP occurs between the two wasp host races. As above, apple or hawthorn fruit were treated with the 50% ethanol extract of pheromone harvested from either apple- or hawthorn-fly origin wasps. Following treatment manipulations, female *D. alloeum* were presented with: 1) apple fruit treated with either apple- or hawthorn-origin wasp OMP versus an untreated control; or 2) hawthorn fruit treated with hawthorn- or apple-fly origin wasp OMP versus an untreated control. Experiments with apple-fly origin and hawthorn-fly origin wasps were conducted separately with their respective fruit of origin.

All the experiments were conducted at the temperature and RH conditions described above and observations for each test run were conducted for 5 min after introduction of the test wasp. A minimum of 25 naïve females were tested only once in each experiment.

Statistical Analysis

The number of female *D. alloeum* accepting fruit for oviposition for treated and control apple or hawthorn fruit was converted to percentages. For each experiment,

significant differences were determined between the percentages of female wasps choosing treated versus respective control fruit by logistic regressions at α=0.05.

Results

Laboratory Assays of Wasp Behavior on Apple and Hawthorn Fruit Infested by *R. pomonella*

A significantly (Wald χ^2=20.6 and 15.9, df=1, P=0.0001 and < 0.0001) lower percentage of female *D. alloeum* accepted apple or hawthorn fruit infested with *R. pomonella* larvae of the same host race that were previously oviposited into and marked (ovipositor dragging) by conspecific wasps compared with unmarked control fruit (Fig. 1a, b). Previous oviposition without marking by female wasps on fly-infested apple or hawthorn fruit did not significantly affect the oviposition behavior of subsequent wasps (Wald χ^2=0.11 and 0.09, df=1, P=0.73 and 0.75,), but deposition of the OMP by female wasps without associated oviposition significantly decreased (Wald χ^2=21.6 and 21.4, df=1, P=< 0.0001 and < 0.0001) the percentage of *D. alloeum* accepting apple or hawthorn fruit (Fig. 1c, d). A significantly (Wald χ^2=4.4 and 12.1, df=1, P=0.03 and 0.0005) lower percentage of female wasps deposited eggs into infested apple or hawthorn fruit that received oviposition and marking 7 days earlier than control fruit or fruit that had received an oviposition with no associated marking (Wald χ^2=1.0 and 0.8, df=1, P=0.32 and 0.37; Fig. 1e, f).

The results of the experiments in which the OMP was selectively removed are shown in Fig. 2. For apple, rinsed fruit that received an oviposition and mark as well as unrinsed fruit that had been wasp-marked only were significantly less acceptable for oviposition (Wald χ^2=6.3 and 20.6, df=1, P=0.01 and P=<0.0001; Fig. 2a) than control fruit. In contrast, apple fruit that were marked only and rinsed with the ethanol solution was as acceptable to subsequent female wasps as control fruit (Wald χ^2=1.1, df=1, P=0.28; Fig. 2a). The results for hawthorn fruit were similar in that significantly fewer females (Wald χ^2=16.5, df=1, P=< 0.0001) accepted fruit that was marked and not rinsed compared with fruit that was marked and subsequently rinsed (Fig. 2b). However, fruit that received oviposition and marking that was rinsed with the solvent solution was equally acceptable to females as compared with fruit that did not receive oviposition or marking (untreated control) (Wald χ^2=0.3, df=1, P= 0.56; Fig. 2b) or fruit that was marked without oviposition and rinsed (Wald χ^2=2.5, df=1, P=0.33; Fig. 2b).

Apple or hawthorn fruit that was not previously exposed to *D. alloeum* but sprayed with the 50% ethanol rinsate from wasp marked fruit received significantly fewer oviposition attempts by female wasps compared with fruit sprayed with 50% ethanol alone (for apple Wald χ^2=12.0, df=1, P=0.0005; for hawthorn Wald χ^2= 16.5, df=1, P<0.0001; Fig. 2c, d).

Oviposition by apple fly-origin wasps was significantly reduced by apple-fly origin OMP on both apples and hawthorns (for apple Wald χ^2=14.3, df=1, P=0.0005; for hawthorn Wald χ^2=12.8, df=1, P<0.0005; Fig. 3a); however, there was no noticeable difference in the effect of the pheromone between the two substrates (Fig. 3a). Similarly, oviposition by hawthorn-fly origin wasps was significantly reduced by

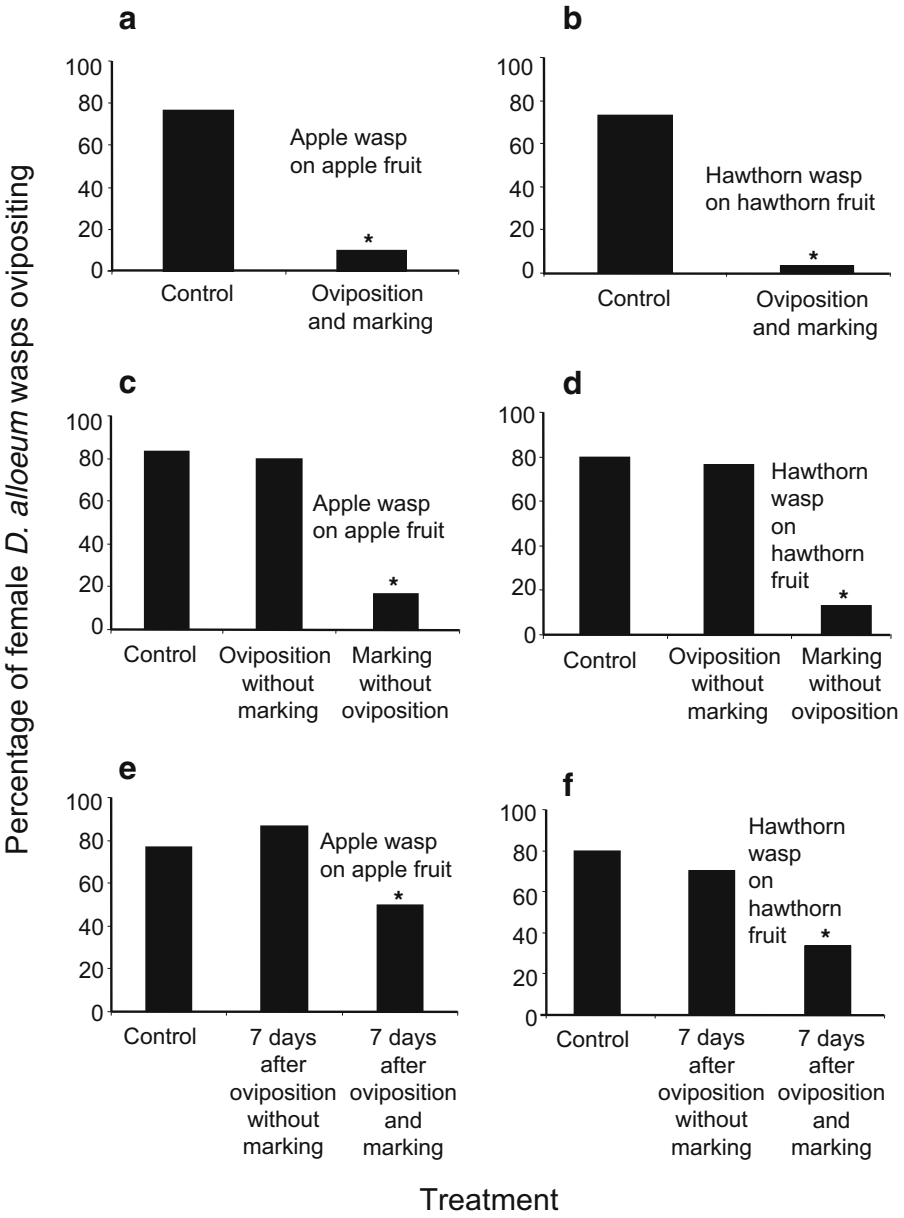

Fig. 1 Percentage of female *D. alloeum* wasps, obtained from apple or hawthorn race of *R. pomonella*, ovipositing into apple (a,c,e) or hawthorn (b,d,f) fruit. **a, b** Control refers to unmarked apple or hawthorn fruit infested with *R. pomenella*; treatment apple or hawthorn fruit received prior *D. alloeum* oviposition and marking. **c, d** Control-as above; treatment apple or hawthorn fruit either received oviposition but no marking or marking but no oviposition. **e**, **f** Control-as above; treatment apple or hawthorn fruit received oviposition with no marking or marking but no oviposition 7 days prior to assay. Asterisk indicates a significant reduction in the percentage of *D. alloeum* wasps ovipositing on treatment fruit compared to control fruit according to logistic regression ($P<0.05$)

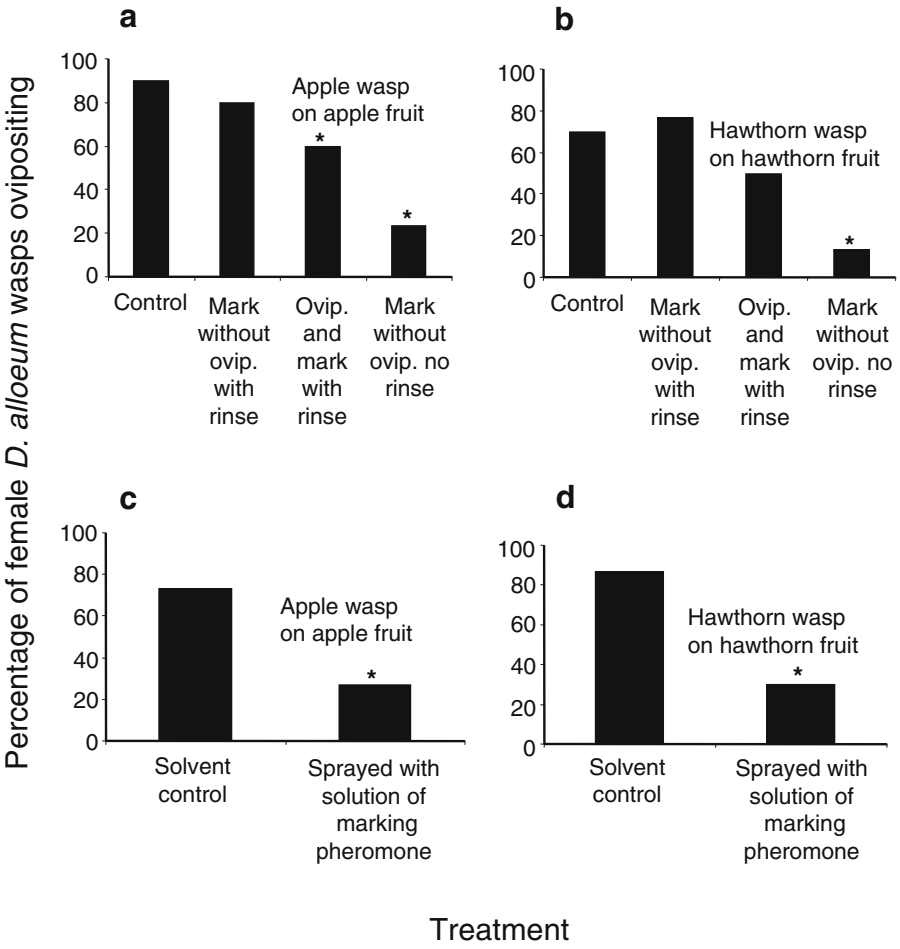

Fig. 2 Percentage of female *D. alloeum* wasps, obtained from apple or hawthorn race of *R. pomonella*, ovipositing into apple (a,c) or hawthorn (b,d) fruits. **a, b** Control refers to unmarked apple or hawthorn fruit infested with *R. pomenella*; treatment apple or hawthorn fruit with *D. alloeum* marking only and with rinse, oviposition and marking and with subsequent rinse, or marking only without rinse. **c, d** Solvent control refers to unmarked apple or hawthorn fruit infested with *R. pomenella* and sprayed with 50% ethanol solution; treatment apple or hawthorn fruit infested with *R. pomenella* and sprayed with 50% ethanol solution of *D. alloeum* OMP. Asterisk indicates a significant reduction in the percentage of *D. alloeum* wasps ovipositing on treatment fruit compared to control fruit according to logistic regression ($P<0.05$)

hawthorn fly-origin OMP on both hawthorns and apples (for hawthorn Wald $\chi^2=18.7$, df=1, $P=0.0001$; for apple Wald $\chi^2=17.1$, df=1, $P<0.0001$; Fig. 3b), but there was no observed difference in the effect on the two types of fruits (Fig. 3b).

Oviposition by apple fly-origin wasps was significantly reduced by both apple fly- and hawthorn fly-origin OMPs on apples (for apple wasp OMP Wald $\chi^2=21.3$, df=1, $P=0.0001$; for hawthorn wasp OMP Wald $\chi^2=16.2$, df=1, $P<0.0001$; Fig. 3c); however, there was no noticeable difference between the effect of the two pheromone types on apple (Fig. 3c). Similarly, oviposition by hawthorn fly-origin wasps was significantly reduced by both pheromone types (for hawthorn wasp

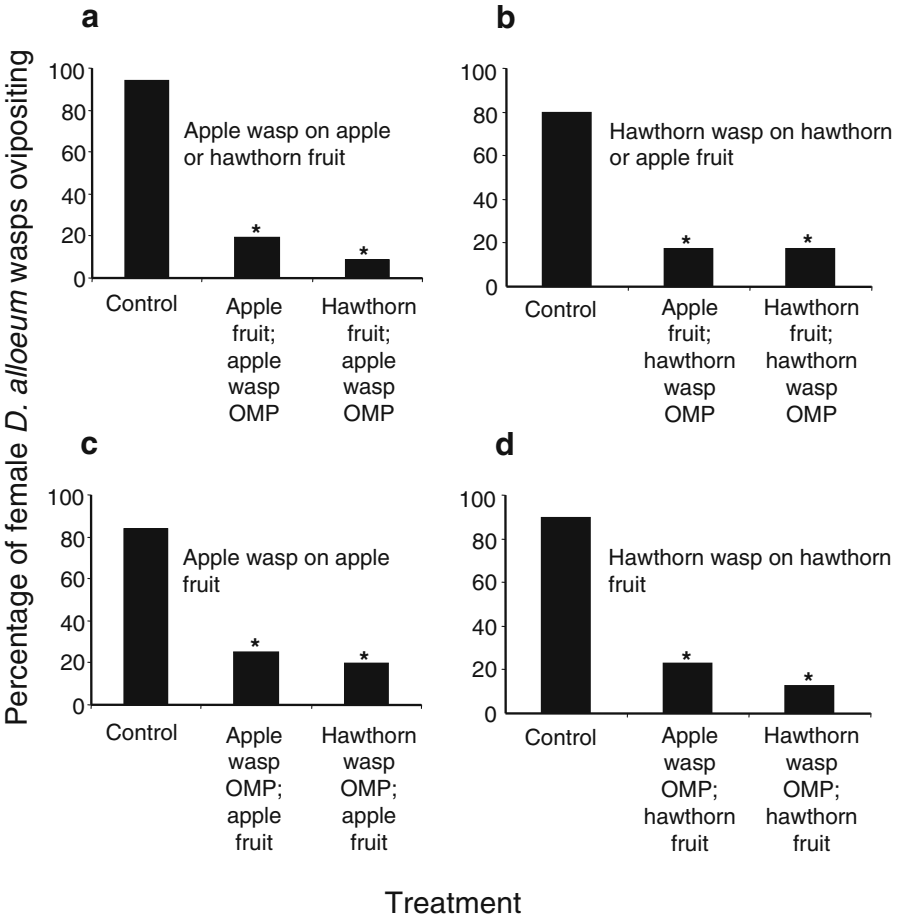

Fig. 3 Percentage of female *D. alloeum* wasps, obtained from apple or hawthorn race of *R. pomonella*, ovipositing into apple or hawthorn fruit. **a, b** Control refers to unmarked apple and hawthorn fruit infested with *R. pomenella.* Since the proportions of oviposition attempts into untreated (control) apple and hawthorn fruit were nearly identical, these data were combined as a single control for comparison with the two fruit substrate treatments. Treatment apple or hawthorn received apple or hawthorn OMP and wasps were tested on two different fruit types with the same OMP. **c, d** Control refers to unmarked apple and hawthorn fruit infested with *R. pomenella*; treatment apple or hawthorn received apple or hawthorn OMP and wasps were tested on the same fruit type with two different OMPs. Asterisk indicates a significant reduction in the percentage of *D. alloeum* wasps ovipositing on treatment fruit compared to control fruit according to logistic regression ($P<0.05$)

OMP Wald $\chi^2=14.4$, df=1, $P=0.0005$; for apple wasp OMP Wald $\chi^2=13.0$, df=1, $P<0.0005$; Fig. 3d) on hawthorns and there was no observed difference in the effect of the two pheromones on this fruit surface (Fig. 3d).

Discussion

Our results indicate that *D. alloeum* females, emerging from both apple and hawthorn race *R. pomonella*, deposit an OMP during 'excreting' behavior (Glas and

Vet 1983) subsequent to oviposition into their larval host concealed within fruit. The effect on conspecific female wasps is rejection of OMP-marked fruit for oviposition. The effect lasts for at least 7 days after marking. The chemical nature of this externally applied pheromone was demonstrated given that it can be removed with solvent and reapplied to clean fruit in solution causing female wasps to reject such fruit as though they had been marked naturally. Oviposition marking behavior and rejection of marked fruit by *D. alloeum* has also been described in the wasp host race parasitizing the blueberry maggot, *R. mendax* (Stelinski et al. 2007).

Oviposition marking pheromones are released into, onto or near larval resources during or after oviposition by numerous phytophagous and entomophagous insects (van Lenteren 1981; Roitberg and Prokopy 1987; Nufio and Papaj 2001; Hoffmeister and Roitberg 2002). These pheromones can inform individuals of an occupied host and deter oviposition into a sub-optimal resource. Recognition of an OMP helps to avoid self-superparasitism of a resource. If the receiver is a conspecific and it responds by rejecting the previously exploited resource, then both sender and receiver benefit by reducing superparasitism and potentially increasing the reproductive fitness of both individuals (Hoffmeister and Roitberg 2002).

Hymenopteran parasitoids, which typically lay their eggs on or into the host larval body, have evolved unique strategies for marking their hosts. When the host larva of the parasitoid is concealed within a substrate such as fruit, the marking pheromone is applied externally on the surface of host-infested substrate either locally (e.g. Hoffmeister 2000) or globally on the entire surface depending on the host larvae's mobility (e.g. Holler et al. 1994; Hoffmeister and Roitberg 1997; Chow and Mackauer 1999). Since *R. pomonella* is concealed within apple or hawthorn fruit and can move away from the oviposition site, marking the fruit surface globally and externally, rather than locally and internally, is favored as it reduces the time required to assess the mark by both the marking individual and potential conspecifics that subsequently encounter the utilized resource.

D. alloeum attacks two host races of *R. pomonella* and preferentially oviposits into third-instar larvae, which require approximately 7 days to develop into the pupal stage that is not utilized by the wasps for oviposition. Marking of apple or hawthorn fruit by *D. alloeum* in pre-assay treatments resulted in significant rejection of marked fruits for up to 7 days. This finding is similar to that reported for *R. mendax* (Stelinski et al. 2007) and likely prevents conspecifics from laying eggs until the fly host pupates. The ability of both apple and hawthorn fly-origin *D. alloeum* to recognize and avoid their own OMP was not dependent on the context of the fly host fruit. Specifically, both wasp host races were equally capable of detecting their own OMP on apple and hawthorn fruit. These results suggest that detection of the OMP alone is sufficient to induce the behavioral effect without the need for detecting and integrating additional chemical or physical information unique to the fruit surface.

The possibility that *D. alloeum* also marks their fly host internally during ovipsition cannot be excluded. Significantly fewer (on apple) and numerically fewer (on hawthorn) female wasps accepted a fruit for egg laying when presented with fruit that received an oviposition but had the OMP removed by rinsing compared with fruit that did not receive oviposition but had the OMP removed by rinsing (Fig. 2a, b). This suggests that *D. alloeum* may deposit an internal marker into the fruit or into the fly larvae that may be detected during 'type 2' (*sensu* Glas and Vet 1983) ovipositor

probing. Of the three types of probing described by Glas and Vet (1983), the longest lasting probe ('type 3') was suggested to be associated with egg laying while the other two types were suggested to be associated with larval host location and acceptance. *D. alloeum* wasps parasitizing *Rhagoletis* larvae may use type 1 and 2 probes to detect the physical presence of fly larvae and/or a chemical signal present in the absence of an external OMP to differentiate between parasitized and unparasitized hosts. However, if an internal marker is used by female wasps in the absence of an external OMP to assess occupancy of a host, we would have expected to see an effect when females were presented with fruit that received oviposition, but was not subsequently marked (Fig. 1b, c). Since this did not occur, it is more plausable that our rinsing protocol did not completely remove the OMP, thus explaining the slightly reduced oviposition rates into such fruit.

Apple fly- and hawthorn fly-origin *D. alloeum* wasps are genetically unique host races characterized by distinct behavioral and physiological phenotypes (Forbes et al. 2009). One mechanism contributing to this insipient speciation event is host fidelity (*sensu* Feder et al. 1994). That is, both wasp host races exhibit attraction to the odor of the fruit that concealed their larval host and simultaneous repulsion from the fruit that concealed the host of their sister race (Forbes et al. 2009). This ecological barrier acts to prevent gene flow between the wasp host races. Furthermore, the length of diapause differs between the two host races, acting as a partial allochronic barrier to gene flow (Forbes et al. 2009). Although the two wasp host races are beginning to diverge into separate species in sympatry (Forbes et al. 2009), the current results indicate that the OMP in the two host races, and its behavioral effect, are identical. These data establish a unique, identical phenotype among the two wasp parasitoid host races that are tracking the sympatric speciation of their fly hosts (Forbes et al. 2009). This current behavioral baseline will allow future studies to determine if this OMP marking and recognition system diverges over time as these wasp host races assume species status.

Acknowledgements A previous version of the manuscript was improved by insightful comments from two anonymous reviewers.

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