

Comparative Antennal and Behavioral Responses of Summer and Winter Morph *Drosophila suzukii* (Diptera: Drosophilidae) to Ecologically Relevant Volatiles

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Abstract

Drosophila suzukii (Matsumura) (Diptera: Drosophilidae) is a devastating global pest of berry crops and cherries. Little is understood about its biology during the winter in northern temperate regions, including potential resources that it may utilize during this period. In this study, olfactory and behavioral responses of female *D. suzukii* to six volatiles (methionol, acetic acid, linalool, bornyl acetate, isoamyl acetate, and geosmin) were evaluated separately for electroantennogram (EAG) and behavioral assays between summer and winter morphs. Results of EAG indicated that isoamyl acetate, acetic acid, and geosmin elicited significantly higher olfactory responses from the antennae of female summer morph *D. suzukii* compared with those of female winter morph *D. suzukii*. Winter morph *D. suzukii* showed reduced antennal response to the volatiles overall. Geosmin and bornyl acetate elicited significantly different behavioral responses from the two morphs in no-choice laboratory behavioral assays. T-maze behavioral assays with geosmin further revealed that summer morphs had a significant aversion, while winter morphs showed no significant aversion to geosmin. Overall, we demonstrate that responses of the two seasonally induced morphs to environmental stimuli are different, and future studies are justified to further understand how these physiological and behavioral differences may contribute to improved pest management of *D. suzukii*.

Key words: spotted wing drosophila, electroantennogram, volatile, winter morph

First discovered on mainland United States in 2008, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) has rapidly spread to become a devastating global pest of berry and cherry crops in the Americas and Europe (Walsh et al. 2011, Cini et al. 2012, Deprá et al. 2014, Asplen et al. 2015). Unlike other species of *Drosophila*, female *D. suzukii* have a large, serrated ovipositor that enables them to penetrate the skin of undamaged and ripening fruit and lay eggs inside (Walsh et al. 2011, Atalla et al. 2014). The feeding larvae degrade the fruit, causing significant crop losses (Bolda et al. 2010, Farnsworth et al. 2017). Current management programs rely primarily on regular applications of conventional broad-spectrum insecticides (Van Timmeren and Isaacs 2013, Haye et al. 2016, Diepenbrock et al. 2017), which are not only unsustainable, but also have negative impacts on beneficial arthropods and disrupt integrated pest management programs (Desneux et al. 2007, Biondi et al. 2012). Moreover, little is understood about the seasonal biology and behavior of *D. suzukii*, particularly during cold periods (Asplen et al. 2015), knowledge that is necessary to effectively implement control strategies.

To adapt and survive in certain temperate climates, insects have evolved a number of physiological mechanisms for coping with

the effects of low temperature, such as reproductive diapause, cold hardiness, and improved tolerance to environmental stressors such as extreme cold (Kimura 1988, Hoffmann et al. 2003, Teets and Denlinger 2013). Several species of *Drosophila* overwinter in cold climates as adults under leaf litter where they are protected from extreme cold temperatures by a layer of snow (Hoffmann et al. 2003, Stephens et al. 2015). Their overwintering strategy may include reproductive diapause, limiting their reproduction to favorable conditions, and directing their resources to winter survival (Hoffmann et al. 2003, Wallingford and Loeb 2016). Additionally, environmental signals such as shorter day lengths and decreasing temperatures can trigger seasonal adaptations such as rapid cold-hardening or gradual acclimation experienced during development (Lee et al. 1987, Teets and Denlinger 2013). Developmentally acclimated flies would be the most prepared to survive the starvation and freeze stress associated with overwintering (Wallingford and Loeb 2016).

Drosophila suzukii has a wide climatic range and is now found on nearly every continent (Calabria et al. 2012, Deprá et al. 2014, Asplen et al. 2015). Phenotypic plasticity in response to seasonal changes produces separate morphs of *D. suzukii* that survive in a range of temperatures and environmental conditions, including low

or freezing temperatures (Stephens et al. 2015, Jakobs et al. 2015, Shearer et al. 2016). The winter morph has a darker pigmented body and longer wings than the summer morph (Stephens et al. 2015, Shearer et al. 2016). In the laboratory, this morph can be induced when larvae are subjected to colder temperatures and shorter day lengths (Shearer et al. 2016). Additionally, winter morphs of *D. sukuzii* have various upregulated and downregulated genes compared with summer morphs of *D. sukuzii* that are responsible for female diapause, synthesis of cryoprotectants and chitin, and metabolic processes, among others (Shearer et al. 2016, Wallingford and Loeb 2016).

Our current knowledge of the winter morph of *D. sukuzii* is limited to physiological changes and oviposition capacity (Stephens et al. 2015, Jakobs et al. 2015, Shearer et al. 2016, Wallingford and Loeb 2016). As temperatures in northern regions begin to decline in the late fall and winter months, captures of *D. sukuzii* in traps decline until captures are extremely low or zero. After January, typically no winter morph *D. sukuzii* are captured in traps, and the first summer morphs are not captured in traps in late May or June in Michigan and northern regions (D. Kirkpatrick, unpublished data). Understanding differences in response to cues between the two morphs could potentially lead to improved monitoring during the winter and early spring periods and inform studies of early season biology.

While attractive volatiles for summer morphs of *D. sukuzii* for use in trapping have been identified (Cha et al. 2012, 2013; Revadi et al. 2015), there is a lack of information regarding volatiles that might be important for overwintering behavior. To better understand the influence of volatiles between these morphs, we evaluated 1) antennal responses to various odorants using electroantennogram (EAG), 2) morphology and number of olfactory sensory neurons using a scanning electron microscope (SEM), and 3) behavioral responses in no-choice and T-maze bioassays to determine if there was a differential response to volatiles between summer morphs and winter morphs of *D. sukuzii*. Differential response to odorants may help optimize traps that are morph-dependent, but also allow us to better understand the overwintering behavior of *D. sukuzii*.

Materials and Methods

Drosophila sukuzii Colonies

Laboratory-reared female adult *D. sukuzii* were used in all experiments. The laboratory colony was initially obtained from field-collected flies in 2015 and was maintained as summer morphs on *D. sukuzii* solid food diet (Dalton et al., 2011) in 50-ml polystyrene vials (Genesee Scientific, San Diego, CA) and held in a growth chamber at 24°C, 45% R.H., and 16:8 (L:D) h photoperiod.

The winter morph *D. sukuzii* colony was reared from the summer morph colony. Thirty adult summer morphs were held in vials with diet in a growth chamber at 24°C for 3 d. The adult flies were then removed from the vials, and the vials containing eggs and young larvae were placed in another growth chamber at 10°C, 45% R.H. and 12:12 (L:D) h photoperiod (Shearer et al. 2016). Winter morph flies eclosed from pupae in these vials after an average of 42 d.

All flies used in the experiments were 3–7 d old and sexually mature. Flies were lightly anesthetized with CO₂ to facilitate handling, sorting by sex, and counting.

Odorants

Odorants were purchased from Sigma–Aldrich (St. Louis, MO) and were of the highest purity available (>95%). Odorants used for experiments were isoamyl acetate (W205508-SAMPLE-K), geosmin

(G5908-1ML), methionol (318396-5G), linalool (51782-1ML), bornyl acetate (45855-1ML-F), and acetic acid (64-19-7). Paraffin oil (18512-1L) was used as a solvent for all chemicals except acetic acid, where deionized water (DI) water was used as a solvent. Test odorants isoamyl acetate, geosmin, methionol, linalool, and bornyl acetate were diluted in paraffin oil to obtain three concentrations (10⁻¹, 10⁻², and 10⁻³) and the control solution was paraffin oil alone. The test odorant acetic acid was diluted in DI water to obtain three concentrations (10⁻¹, 10⁻², and 10⁻³) and the control solution for this odorant was DI water alone. Acetic acid, methionol, and isoamyl acetate were selected for their known attraction to summer morph *D. sukuzii* (Cha et al. 2012, Revadi et al. 2015). Both bornyl acetate (derived from pine) and linalool (derived from flowers) were evaluated because of their potential as a sugar source when fruit is not available (sap from pines in the fall, and nectar from flowers in the spring). Geosmin (derived from soil-borne bacteria) was selected because it had previously been evaluated for summer morphs of *D. sukuzii* as a potential deterrent (Wallingford et al. 2016) and due to the potential for soil and leaf litter to be an overwintering site for *D. sukuzii*.

Electroantennogram Recordings

Antennal receptivity of adult female *D. sukuzii* to synthetic compounds was determined by EAG. The procedure for EAG is similar to that previously described for *Drosophila melanogaster* by Ayer and Carlson (1992). Flies were held during recording in a 200- μ l pipette tip. The fly was inserted into the pipette tip using a mouth aspirator with the head of the fly toward the small end of the tip. The small end of the tip was trimmed such that the antennae and head emerged from the end of the tip, and a second cut was made to trim the pipette tip approximately 1 mm behind the fly. To prevent the fly from crawling out backward, clay was placed in the large end of the cut tip, so that it touched the posterior end of the fly. The fly was then placed on a microscope slide with the head next to a coverslip that was mounted on the microscope slide. The antennae were maneuvered, so that they lay flat on the stack of the coverslip. To stabilize one of the antennae further, a glass capillary tube was used to hold the second antennal segment in place. The third antennal segment was centered under a stereomicroscope (Nikon SMA645, Japan) that was mounted on a vibration isolation table. Reference and recording electrodes were glass capillaries (World Precision Instruments, 1B100F-4), pulled such that tips were $\leq 1 \mu$ m in diameter, and filled with *Drosophila* Ringer's solution (in mM): NaCl 100, KCl 5, MgCl₂ 20, CaCl₂ 0.15, HEPES 5, Sucrose 115, Trehalose 5. The reference electrode was inserted into the contralateral eye. Using a stereomicroscope (Nikon SMA645, Japan), the recording electrode was then brought into contact with the posterior aspect of the third antennal segment and advanced until stable electrical contact was established. The electrodes made electrical contact with a high impedance amplifier (World Precision Instruments, DAM 50) via silver/silver-chloride wires. The output signal of the amplifier was fed into a computer via a digitizer (Axon CNS molecular devices, Digidata 1440A). The electrical signal was collected using Axoscope 10.4 software and measured and analyzed by using Clampfit 10.4 software. Odor stimuli were presented from Pasteur pipettes holding solutions of chemicals in paraffin oil or DI water on filter paper. An aliquot of 50 μ l of solution was dropped on a 1.27-cm filter paper strip placed in the shaft of a Pasteur pipette. The tip of a Pasteur pipette was placed through a hole in a tube that carried a humidified air stream over the fly. Compressed air was controlled by a solenoid that created puffs of odorant through the pipette. A minimum of 10 females of each morph was used in these

tests. Flies were starved for 2 h prior to use in experiments. All EAG experiments were performed from 10:00 to 14:00 h at 21–22°C and 45–60% R.H.

Scanning Electron Microscope Photos

Summer and winter morph flies were placed in a –80°C freezer for 5 min. After defrosting, the flies' heads were cut off with a small syringe and then fixed at 4°C for 1–2 h in 4% glutaraldehyde buffered with 0.1 M sodium phosphate at pH 7.4. Following a brief rinse in the buffer, samples were dehydrated in an ethanol series (25%, 50%, 75%, 95%) for 45 min at each gradation. Samples were critical point dried in a critical point dryer (EM CPD300, Leica Microsystems, Vienna, Austria) using carbon dioxide as the transitional fluid. The heads were mounted on aluminum stubs with heads facing up using carbon tape (Ted Pella, Inc., Redding, CA) and were then coated with osmium (≈ 10 nm thickness) in a NEOC-AT osmium chemical vapor deposition coater (Meiwafosis Co., Ltd., Osaka, Japan). Samples were examined and photos were taken in a JEOL 6610LV (tungsten hairpin emitter) scanning electron microscope (JEOL Ltd., Tokyo, Japan).

Using the 250 \times magnification SEM photos of the third antennal segment, the total antennal area was calculated by tracing the circumference of the antennae using ImageJ. The numbers of ab4 and ab6 small basiconic sensilla were counted (de Bruyne et al. 2001) within a 40- \times 60- μ m area (approximately where the recording electrode was placed for the EAG studies) on the anterior of region 4 in the third antennal segment using the 600 \times magnification photos (Fig. 1). The ab4 basiconic sensilla are responsible for detection of the geosmin compound (Stensmyr et al. 2012), but it is difficult to visually distinguish from the ab6 basiconic sensilla (Venkatesh and Singh 1984); therefore, all small basiconic sensilla were counted and considered together.

No-choice Test Behavioral Bioassay

No-choice tests were conducted to elucidate behavioral responses of *D. suzukii* to volatiles. For behavioral assays, 20 ml glass scintillation vials (RPI Corp., Mount Prospect, IL) with a 3-D printed (Ultimaker 3, Cambridge, MA) plastic lids having 1-cm diameter opening in a funnel shape to facilitate capture of the flies were used as traps. Furthermore, 3.79-liter glass jars covered with a piece of mesh material to facilitate airflow were used as bioassay arenas. The bioassay arenas were placed in a room with 45–60% R.H. and 22°C. A piece of filter paper moistened with water was placed at the bottom of each bioassay arena to provide humidity inside. A vial trap containing either 1 ml of solution (concentration 10^{-3}) or the control was

also placed at the bottom of the arena. A yeast and sugar solution (83% DI water, 14% sugar, 3% baker's yeast) was used as a positive control, because it is routinely used as bait for trapping *D. suzukii* (Burrack et al. 2015). Twenty female flies were held in the assay room to acclimate to the conditions in a Petri dish with a moist piece of filter paper in the bottom and were starved for 2 h before each experiment. The Petri dishes were placed in the bottom of the glass arena with the lids were on, and with the mesh covering the top of the arena, the tops of the Petri dishes were removed to allow the flies to emerge from the Petri dishes on their own. Traps were assessed after 24 h by counting the number of flies caught in each of the vial traps.

T-maze Behavioral Bioassay

T-maze assays were used to further elucidate stimulatory or deterrent effects of geosmin on summer and winter morphs *D. suzukii* following Stensmyr et al. (2012) with some modifications. Thus, a T-maze was constructed as follows: two 1,000- μ l pipette tips were cut 2.5 cm from the narrow end and 0.5 cm from top. They were inserted into the bottom of two 1.5-ml microcentrifuge tubes with 2 mm removed from the tapered end. These two assembling units were connected using 4 cm of tubing (Tygon, E-3603, Akron, OH). In the middle of the tubing, a small hole was made and a 1,000- μ l pipette tip (cut 0.5 cm from the narrow end) was inserted into the tubing via the opening. A 0.8 \times 3.2 mm piece of filter paper was inserted between the pipette tip and the tip of microcentrifuge tube, so flies in the tubing could not make direct contact with the filter paper. A solvent or test compound (5 μ l) was applied to the filter paper after the assembly. After the compound was applied, 10 female flies were gently introduced into the long pipette tip, and the opening was closed with a piece of cotton in the end of the pipette tip to keep the flies inside. In both microcentrifuge tube lids, a small hole was made using a syringe needle to facilitate airflow to maintain a concentration gradient for each tested compound. Trials were run in a room with 45–60% R.H. and 22°C. The numbers of flies entering the trap were counted after 24 h. The response index (RI) was calculated following the equation of Stensmyr et al. (2012) and calculated as $(O-C)/T$, where O is the number of flies in the baited arm, C is the number of flies in the control arm, and T is the total number of flies used in the trial. The resulting index ranges from –1 (complete avoidance) to 1 (complete attraction) (Stensmyr et al. 2012).

Statistical Analyses

EAG response data were normalized with response subtracted from the respective control solution and analyzed using a two-way analysis of variance. The antennal area and number of small basiconic

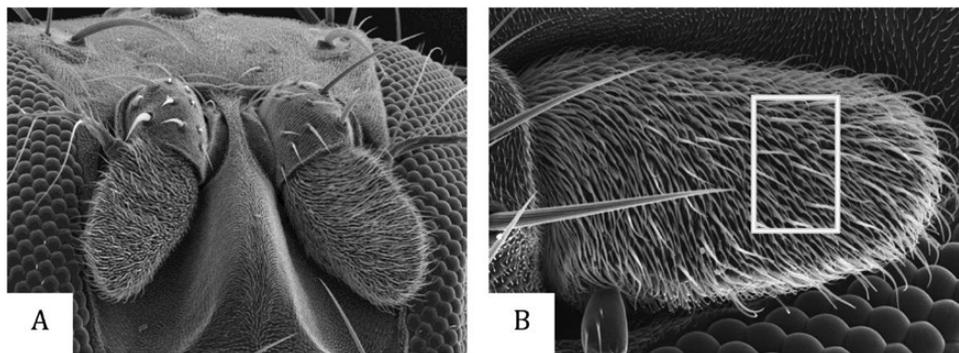


Fig. 1. Scanning electron microscope (SEM) photographs of the third antennal segments of *Drosophila suzukii*. Photograph (A) shown at 250 \times and photograph (B) at 600 \times magnification. Photograph (B) shows the 40 \times 60 μ m area (white box) used to count the number of small basiconic sensilla on the third antennal segment of summer and winter morphs.

sensilla satisfied normality assumptions using a Levene's test and were compared using analysis of variance. Data from both behavioral experiments satisfied assumptions of normality using a Levene's test and were analyzed using a two-way analysis of variance. Data from the no-choice tests were analyzed using a generalized linear mixed model with a Poisson distribution. The T-maze data were analyzed using the RI calculation with a generalized linear model. Tukey's HSD was used for all post hoc comparisons. Data were analyzed using R (3.3.3, R Core Team, R Foundation for Statistical Computing, Vienna, Austria).

Results

Electroantennogram Recordings

Using dose-response curves, we determined the appropriate concentration for each odorant tested to be 10^{-2} , except for acetic acid, where a 10^{-1} concentration was also included. We found that summer morph response was elevated compared with winter morphs across four of the volatiles tested (Fig. 2). This was significantly different for geosmin ($F_{1,18} = 4.9, P = 0.03$), isoamyl acetate ($F_{1,18} = 4.5, P = 0.04$), and acetic acid 10^{-1} ($F_{1,18} = 14.8, P = 0.001$). Acetic acid 10^{-2} response was higher for summer morphs, but not significantly different ($F_{1,18} = 4.1, P = 0.05$). Likewise, methionol elicited a larger response in summer morphs, but not significantly so ($F_{1,18} = 3.5, P = 0.07$). There were no differences found between morphs for either bornyl acetate ($F_{1,18} = 0.06, P = 0.8$) or linalool ($F_{1,18} = 0.04, P = 0.8$).

Scanning Electron Microscope Photos

The area of the third antennal segment was similar between summer morphs (13.1 ± 0.9 mm) and winter morphs (13.2 ± 0.8 mm) ($F_{1,7} = 0.001, P = 0.98$). There were numerically more small basiconic sensilla found on summer morph antennae (8.6 ± 1.2) compared with winter morph antennae (6.2 ± 0.6), but these were statistically similar ($F_{1,7} = 3.36, P = 0.1$).

No-choice Test Behavioral Bioassay

Winter morph *D. sukukii* exhibited a significantly higher response to the volatiles geosmin ($F_{1,14} = 9.36, P = 0.008$) and bornyl acetate ($F_{1,14} = 4.9, P = 0.04$) compared with summer morphs in no-choice

behavioral bioassays (Table 1). There was no significant difference between the morphs across any of the other tested volatiles.

T-maze Behavioral Bioassay

The summer morph flies exhibited a negative response and the winter morph flies showed a positive response to geosmin (Fig. 3). When the RI was compared with zero (no response), summer morphs had a significant avoidance to geosmin ($F_{1,11} = 7.3, P = 0.01$). However, response from winter morphs was not significantly different from zero ($F_{1,11} = 0.01, P = 0.95$).

Discussion

Electroantennography and the recording from receptor cells is a commonly used technique that has been previously used to explore *D. sukukii* antennal responses to individual volatile chemicals (Cha et al. 2012, Abraham et al. 2015, Revadi et al. 2015). This study reports the use of EAG for screening volatile chemicals that mediate attraction and drive behaviors between two different seasonally induced morphs of *D. sukukii*. EAG has also been used to previously compare several types of *Drosophila* mutants (Störtkuhl et al. 1999, Kain et al. 2008), but to our knowledge, this is the first time that differences in response to volatiles between seasonally induced insect morphs have been evaluated using any type of electrophysiological technique.

In our EAG study, both summer and winter morphs showed an antennal response to all volatiles tested, suggesting that both morphs have the same type of olfactory sensory neurons and receptors present for these compounds. However, winter morphs had a consistently reduced response to most volatiles when compared with summer morphs. This could provide insights into behavioral strategies used between the two morphs for locating resources and successful overwintering sites. Winter morphs are thought to be quiescent during the winter, and a reduction in the capacity to respond to environmental stimuli and cold winter stress would likely benefit their survival during this period. EAG is excellent for quickly assessing the receptive range of an insect's antenna and evaluates the sum of the electrical response of the activated olfactory sensory neurons on the entire antenna (Olsson and Hansson 2013). Despite being a commonly used electrophysiological technique for evaluating olfactory reception, EAG has potential shortcomings and is subject to change depending on the connection strength, insect vitality, and position of the electrode (Olsson and Hansson 2013). For a more quantitative measurement of the olfactory response, single-sensillum recording is an electrophysiological technique that can target specific receptor sites and could be used in the future to further evaluate the difference in response to volatiles between the two morphs of this species.

Geosmin is a volatile compound produced by a select number of fungi, bacteria, and cyanobacteria (Stensmyr et al. 2012). It is typically recognized as a warning sign for the presence of toxic compounds for insects, and negatively affects behaviors such as attraction, feeding, and oviposition (Becher et al. 2010, Stensmyr et al. 2012). Geosmin is a known repellent for some species of *Drosophila* (Stensmyr et al. 2012), and has been previously evaluated as a repellent for *D. sukukii* summer morphs (Wallingford et al. 2016, Wallingford et al. 2017). We found that while the numbers of ab4 and ab6 small basiconic sensilla, where the ab4 and ab6 olfactory sensory neurons are located in other *Drosophila* (De Bruyne et al. 2001), were numerically lower on winter morph antennae compared with summer morphs, there was no statistical difference in the number of basiconic sensilla between morphs. This small reduction could be responsible for the change in EAG response, and

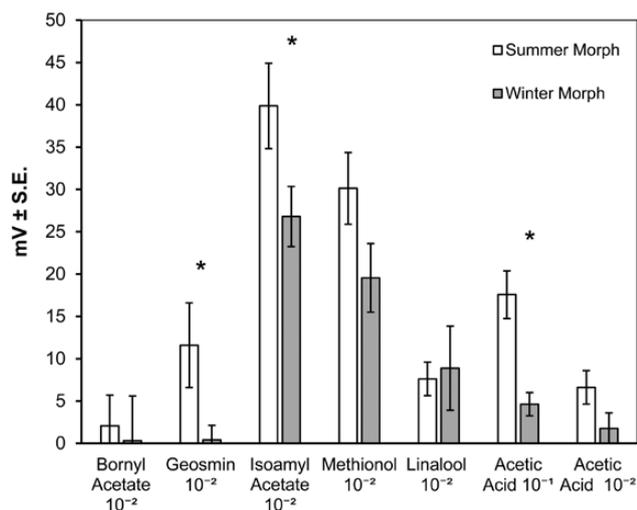


Fig. 2. Mean (\pm S.E.) summer morph (white bars) and winter morph (dark gray bars) *D. sukukii* response to volatiles in EAG assays. Asterisks indicate a significant difference between morphs, within each volatile tested ($P < 0.05$).

Table 1. Average percent response (\pm S.E.) and associated statistical values by winter and summer morph *D. suzukii* in a no-choice test behavioral bioassay to nine different volatiles

Treatment	Summer morph mean (%)	Winter morph mean (%)	$F_{1,14}$ value	<i>P</i> -value
DI water	22.2 \pm 5.4	27.2 \pm 7.7	0.28	0.61
Paraffin oil	6.7 \pm 1.7	15.6 \pm 4.4	3.82	0.07
Yeast sugar	47.2 \pm 7.5	38.3 \pm 7.8	0.68	0.42
Geosmin	17.2 \pm 3.2	35.6 \pm 4.9	9.36	0.008
Bornyl acetate	10 \pm 1.9	20.6 \pm 4.5	4.90	0.04
Linalool	9.4 \pm 3.7	7.2 \pm 1.5	0.37	0.55
Acetic acid	17.2 \pm 5.5	20.5 \pm 6.6	0.17	0.68
Methionol	20.5 \pm 4.1	10.5 \pm 2.8	4.23	0.05
Isoamyl acetate	18.3 \pm 3.7	17.2 \pm 5.2	0.03	0.86

Bold *P*-values indicate significantly different means between the two morphs.

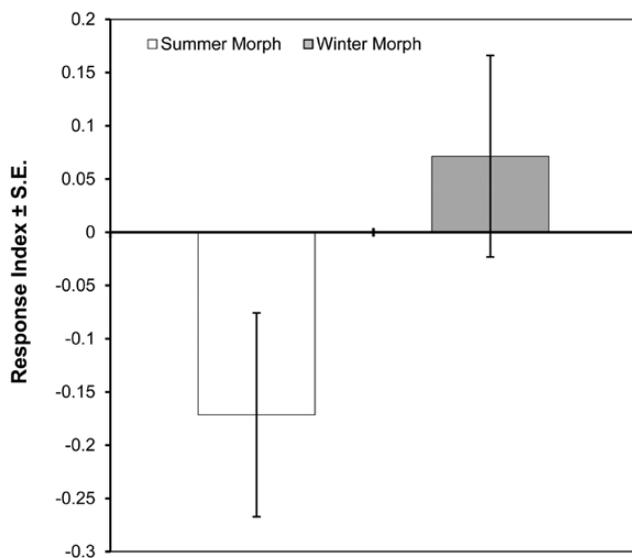


Fig. 3. Response index (RI) of summer morph (white bar) and winter morph (gray bar) *D. suzukii* (\pm S.E.) in a T-maze assay with a choice between geosmin (concentration 10^{-3}) and paraffin oil control.

further exploration is needed on the effect of the possible reduction of these basiconic sensilla. In our EAG study and the no-choice behavioral assay, there was a significant difference in response to geosmin between the two morphs. Thus, geosmin was the only volatile further evaluated in the T-maze behavioral bioassay to evaluate potential repellency to geosmin. In the T-maze assay, we found that there was a slight aversion to geosmin by summer morphs, and no significant response (aversion or attraction) to geosmin by winter morphs (Fig. 3). In a study by Wallingford et al. (2017), it was found that geosmin did not repel summer morphs and did not reduce the density of *D. suzukii* on treated fruit, but acted as an ovipositional deterrent. This is one possible explanation for why a higher aversion to geosmin was not seen from summer morphs in either type of behavioral bioassay, despite a strong relationship between other species of *Drosophila* and repellency, which is typically highly conserved among flies (Stensmyr et al. 2012). Other types of avoidance behaviors, such as avoidance to CO_2 , are not conserved in all *Drosophila* species and have been adapted to suit *D. suzukii* (Pham and Ray 2015). Low aversion to geosmin may also help explain why some summer morphs were found in vials containing geosmin in the no-choice assays, though still numerically less than winter morphs. Certain behaviors could be further adapted to suit winter morphs of *D. suzukii* and could offer a distinct evolutionary advantage for successful overwintering for winter morphs of *D. suzukii* by not

avoiding volatiles that can potentially indicate overwintering sites. The landscape and fruit present when summer morphs are active and searching for suitable hosts are very different compared with the landscape for a winter morph *D. suzukii* searching for a suitable overwintering site. Further studies evaluating differential responses between the two morphs are warranted, particularly with geosmin.

Our no-choice behavioral assay indicated a significantly higher response from winter morphs to bornyl acetate compared with summer morphs (Table 1). No difference with bornyl acetate was found in our EAG studies and in fact, very low response to this compound was observed with both morphs (Fig. 2). Interestingly, field studies have found high populations of *D. suzukii* in pine trees during the fall, but we are not currently aware of what drives this attraction. While there was a reduced response from winter morphs to many of the compounds tested using EAG, fewer differences were noted in our behavioral studies. We expect that *D. suzukii* behavior is highly plastic dependent on factors such as climate, temperature, humidity, resource availability, and fruit stage, among others. Therefore, we would expect to see greater difference between morphs when compared in their natural environmental settings. Other insects that produce a distinct seasonal morphs for winter survival also exhibit shifts in behavior, such as increased takeoff and flight capacity of winter morph cabbage whitefly, *Aleyrodes proletella* (Iheagwam 1977), longer wings for greater dispersal from orchards in the fall of winter morph pear psylla, *Cacopsylla pyricola* (Oldfield 1970), and migration from peaches and interrupted parthenogenesis of the overwintering green peach aphid, *Myzus persicae* (Blackman 1974).

Additional studies could further elucidate preference for specific resource signals within and between morphs with two-choice or multi-choice experimental behavioral assays. Lower temperatures experienced by winter morphs in the field may also be important for observing representative behavior in behavioral bioassays. We also emphasize the need for further evaluation of the behavioral responses to these compounds in a field or semi-field study, relating spatial and temporal dependent factors to these morphs. Moreover, these morphs were lab-reared under static temperature and photoperiod conditions, and on a resource rich diet, which could have implications for the physiological and behavioral responses to environmental stimuli.

There are important management implications for increased knowledge on the winter morph of *D. suzukii*, particularly because we expect that the surviving populations of this pest are quite low given low spring adult captures, similar to orchards with pear psylla populations where peak spring densities were often considerably lower than peak fall densities of the overwintering form (Horton et al. 1992). This provides a unique opportunity to exploit their winter or spring resources and further reduce the surviving population

by targeting the winter morphs for control after the population bottleneck. Our findings indicate that seasonally induced morphs of *D. suzukii* have behavioral adaptations that likely help them to locate appropriate resources for successful overwintering in northern climates. Further defining the overwintering requirements for this pest will allow us to better understand their seasonal phenology and make appropriate management decisions.

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