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Effect of *Drosophila suzukii* on Blueberry VOCs: Chemical Cues for a Pupal Parasitoid, *Trichopria anastrephae*

G. J. de la Vega[1,2] · **F. Triñanes**[1] · **A. González**[1]

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Abstract

Biocontrol agents such as parasitic wasps use long-range volatiles and host-associated cues from lower trophic levels to find their hosts. However, this chemical landscape may be altered by the invasion of exotic insect species. The spotted-wing drosophila (SWD), *Drosophila suzukii* (Diptera: Drosophilidae), is a highly polyphagous fruit pest native to eastern Asia and recently arrived in South America. Our study aimed to characterize the effect of SWD attack on the volatile organic compounds (VOCs) of blueberries, a common host fruit, and to correlate these odor changes with the olfactory-mediated behavioral response of resident populations of *Trichopria anastrephae* parasitoids, here reported for the first time in Uruguay. Using fruit VOC chemical characterization followed by multivariate analyses of the odor blends of blueberries attacked by SWD, we showed that the development of SWD immature stages inside the fruit generates a different odor profile to that from control fruits (physically damaged and free of damage). These differences can be explained by the diversity, frequency, and amounts of fruit VOCs. The behavioral response of *T. anastrephae* in Y-tube bioassays showed that female wasps were significantly attracted to volatiles from SWD-attacked blueberries when tested against both clean air and undamaged blueberries. Therefore, *T. anastrephae* females can use chemical cues from SWD-infested fruits, which may lead to a successful location of their insect host. Since resident parasitoids are able to locate this novel potential host, biological control programs using local populations may be plausible as a strategy for control of SWD.

Keywords Spotted wing drosophila · Local parasitoids · VOC blends, biocontrol agents

Introduction

Global trade is one of the main causes of the expansion of the range of pest species. The flow of agricultural products between countries facilitates the movement of species to novel environments (Anderson et al. 2004; Lantschner et al. 2019), threatening not only the production of goods but also the local biodiversity (Harvey and Fortuna 2012). When exotic insect species arrive in local natural communities, the native food webs can be altered, as is the entire ecosystem by cascading chemical, physiological and ecological changes across trophic levels (Chabaane et al. 2015). The presence or absence of antagonists (i.e., predators or parasitoids) is a key component in the success or failure of non-native species in a novel range, involving both top–down and bottom–up biotic influences among multiple trophic levels (Schultz et al. 2019). In this context, understanding how non-native pest species establish and interact with the native community is critical to forecast their success and to devise pest management strategies.

Chemical cues and signals are essential mediators in the ecological interactions of insects. Trophic interactions within native communities often rely on these cues and are therefore susceptible to chemical landscape alterations that may result from the invasion of exotic insect species (Chabaane et al. 2015; Mair and Ruther 2019; Rombaut et al. 2017). A common third trophic level involved in plant defense involves parasitic wasps, hymenopteran insects whose immature life stages occur in or on other arthropods, mostly other insects (Cusumano et al. 2020; Schultz et al. 2019). To find adequate habitats for potential mates

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✉ G. J. de la Vega
delavega.gerardo@gmail.com

✉ A. González
agonzal@fq.edu.uy

[1] Laboratorio de Ecología Química, Facultad de Química, Universidad de La República, Montevideo, Uruguay

[2] Grupo de Ecología de Poblaciones de Insectos, IFAB CONICET-INTA EEA Bariloche, Bariloche, Argentina

or hosts, parasitic wasps use long-range volatiles and host-associated cues from lower trophic levels (Desurmont et al. 2020; Mumm and Hilker 2005). While searching for hosts, parasitic wasps need to integrate specific chemical cues with context background odors, which may be classified as irrelevant odors (no role in foraging behavior), masking odors (interfere and neutralize an attractive odor source) and enhancing odors (increase the attractiveness of an odor source) (Schröder and Hilker 2008). In this sense, mixtures of host-associated volatiles and background odors may be important mediators in tritrophic interactions involved in parasitoid host location (Desurmont et al. 2020; Schröder and Hilker 2008). In agricultural systems, these synergistic effects may be necessary to enhance the behavioral response of parasitic wasps to find their hosts efficiently (Liu et al. 2019).

Even though invasion ecology is a growing field, studies seldom focus on the effect of new exotic insects on multitrophic interactions in native communities (Carrasco et al. 2017; Chabaane et al. 2015,). Our study system involved three trophic levels: a local population of the parasitoid wasp *Trichopria anastrephae* Lima (Hymenoptera: Diapriidae), a potential new insect host that has recently arrived in South America, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae), and one of its soft-skin fruit hosts, *Vaccinium corymbosum* (blueberries). *D. suzukii*, known as the spotted wing drosophila (SWD), is a highly invasive insect native to eastern Asia (Walsh et al. 2011). In the last decade, it became a risk for soft-skin fruits affecting a variety of cherry and berry crops (i.e. strawberries, blueberries) (Bolda et al. 2010; Walsh et al. 2011). SWD invasions were initially detected in Europe and North America in 2008 (Cini et al. 2012) and between 2012 and 2015 arrived in South America spreading from Brazil to the Patagonia region in southern Argentina (de la Vega and Corley 2019, de la Vega et al. 2020). The fly presents an important difference with most *Drosophila* species, which oviposit in decaying or overripe fruit, in that SWD females have a serrated ovipositor to pierce fruit skin, allowing them to lay eggs inside undamaged fresh and ripening fruit (Atallah et al. 2014). Before *D. suzukii* invaded agroecosystems, wounds on fruits such as grapes were due to climatic factors (i.e., hail, heat shock, heavy rain) or physical damages by birds or wasps (Rombaut et al. 2017). Consequently, the attack of SWD facilitates *D. melanogaster* infestation. As a consequence, rather than competing with closely related species, the invader makes available otherwise non-accessible resources, hence opening a new ecological niche for native, fructivorous insects (Rombaut et al. 2017).

Soft-skin fruits such as blueberries change their profile of volatile organic compounds (VOCs) as they ripen or senesce (Farneti et al. 2017). Therefore, fruit VOCs may potentially be used by *D. melanogaster* and *D. suzukii* to find different fruit stages (Karageorgi et al. 2017; Keesay et al. 2015). A host preference shift from rotten to fresh fruit has been proposed for SWD (Keesay et al. 2015), an ecological shift that may have had an impact on higher trophic levels as well. In this scenario, understanding multitrophic chemical ecology aspects related to the invasion of *D. suzukii* to new environments represents an opportunity to understand its effect on established populations of natural enemies. In turn, this understanding may result in improvements for integrated pest management programs.

There is growing interest in the development of environmentally friendly pest management methods to reduce the application of harmful pesticides (Kruitwagen et al. 2018). Thus, both larval and pupal parasitic wasps have been tested as biological control agents for SWD. The former group includes species of the genus *Asobara* (Hymenoptera: Braconidae), *Ganaspis* (Hymenoptera: Figitidae), and *Leptopilina* (Hymenoptera: Figitidae). Pupal parasitoids include *Pachycrepoideus vindemiae* Rondani (Hymenoptera: Pteromalidae), *Spalangia erythromera* Förster (Hymenoptera: Pteromalidae), *Trichopria drosophilae* (Perkins), and *T. anastrephae* Lima (Hymenoptera: Diapriidae). These were all able to parasitize *D. suzukii* under laboratory conditions (Ibouh et al. 2019; Vieira et al. 2019). While potential biological control agents may be identified in SWD's native range (Lee et al. 2019), complex international regulations and biodiversity risks associated with the introduction of exotic natural enemies underline the need for improving the efficacy of resident species of natural enemies (Kruitwagen et al. 2018). In this sense, the presence of *T. anastrephae* populations in Latin America has been reported since 2001 (summarized in Vieira 2019), although biological control studies against *D. suzukii* are still only starting in the region (i.e., Wollmann et al. 2016; Vieira et al. 2019), with no studies focusing on the chemical ecology of these interactions. In this particular system, understanding if parasitoids have an innate ability to find and exploit fruit infested by SWD by using volatile cues bears potential implications for the biological control of this fruit pest. More specifically, if the parasitoid shows plasticity in exploiting volatile cues from various infested fruits, it is then capable of switching its preference patterns toward non-native host fruits or laboratory artificial substrates, which becomes also important for the development of rearing strategies for pest management programs (Biondi et al. 2017).

Using a chemical ecology approach, we here explored two ecological questions in our tritrophic study system. First, we investigated the effects of SWD infestation on the VOCs of ripening blueberries. Second, we addressed the behavioral response of female parasitoid wasps of a local population of *T. anastrephae* to fruits infested with this novel insect host.

Methods and Materials

Fruits

Organic grown blueberry fruits (*Vaccinium corymbosum* var. O'Neil and Blue Jay) were used to analyze the effect of SWD attack on VOC profiles. The fruits were harvested weekly from multiple plants between December 2019 and January 2020 in a local organic farm (La Micaela, Canelones, Uruguay, http://lamicaelaorganico.com/). They were harvested before the fully ripe stage, still on the red stage as described by Gilbert et al. (2013) and Farneti et al. (2017) (see Supplementary Data Fig. S1). The fruits were harvested in the morning (0900–1200 h) and VOC sampling was performed in the laboratory the same afternoon.

Insect Rearing

Drosophila suzukii adults came from a laboratory colony established from flies collected locally in April 2019. The rearing was maintained on common cornmeal diet (504 ml distilled water, 66 g sucrose, 6 g bread yeast, 2.3 g agar, 52 g corn-flour, 1.3 ml propionic acid, 0.8 g nipagin), in vials (12 cm high, 2.5 cm diam.) placed in an incubator under controlled conditions (21.5 ± 1 °C, 65 ± 5% relative humidity, 12:12 h photoperiod).

The parasitoid, *T. anastrephae*, also came from a laboratory rearing established at the same time (April 2019) from field-collected insects. It should be noted that, while *T. anastrephae* is well known at the regional level, these field collections represent the first report of the presence of this parasitoid in Uruguay. To work with wasps naïve with respect to SWD, the parasitoid rearing was maintained continuously on *D. melanogaster*, under the same conditions as described above for SWD.

Collection of Fruit Volatiles

Blueberries free from external damages or irregularities (25 g) were placed in glass collecting chambers (20 cm length, 8 cm diam.) for dynamic headspace volatile collection. VOCs were collected by passing air pushed from an air compressor at a flow rate of 0.5 l min^{-1}. The air was previously dehumidified with silica gel and filtered through activated carbon. VOCs were adsorbed in glass tubes filled with 50 mg of HaySep Q (Alltech, USA) for 24 h. VOC collections were made at a temperature of 25 ± 2 °C, 70 ± 5% relative humidity, and a photoperiod of 12:12 h (L:D). Adsorbed volatiles were eluted with 1 ml hexane, concentrated to 150 µl under a gentle flow of nitrogen, and stored in 250 µl vial inserts at – 20 °C until GC–MS analysis. An internal standard solution (100 µl) was added prior to concentrating the sample (tridecane in hexane, 1 µg/ml).

Effect of Attack by SWD Females on Blueberries

After the initial 24-h VOC collection the blueberry samples were assigned to the different treatments. To obtain SWD-attacked blueberries, the fruits were enclosed for 24 h with ten SWD couples ranging 2–7 days old. After 24 h the flies were anesthetized with CO2 and removed, and oviposition was confirmed under a stereo microscope by the presence of egg breathing tubes (9 ± 6 egg in each blueberry). The fruits were stored in clean glass containers covered with a fine mesh, under the same conditions as described for the insect rearing. Ten days later, a period that correlates with egg to pupae development of SWD (Tochen et al. 2014), fruit VOCs were collected under the same conditions as described above (SWD-attacked samples). Two controls were performed to differentiate the effect of SWD attack on fruit VOCs from the effect of physical damage and the natural ripening of the fruit. Physical damage (Physically damage samples) was mimicked by gently punching all fruits in the 25 g cluster with a 5 µm diam. microcapillary tube (three holes per fruit). The fruits were punctured ten days before VOC collection to match the maturation time of SWD-attacked fruit. Natural ripening was allowed under the same conditions with no treatment as an additional control treatment (undamaged control samples).

Chemical Analyses

Blueberry VOCs were analyzed by gas chromatography coupled with mass spectrometry (GC–MS) using a Shimadzu QP 2010 PLUS (Shimadzu Corp., Tokyo, Japan) equipped with a Rtx®-5MS column (30 m, 0.25 mm i.d, 0.25 µm film thickness; Alltech, USA). Samples (1 µl) were injected in the splitless mode with He as carrier gas at a flow rate of 1 ml/min (49.7 kPa). The oven temperature was programmed from 40 °C for 4 min, then increased to 150 °C at 5 °C/min and to 250 °C at 10 °C/min and held for 10 min. Injector and MS transfer line temperatures were both set at 250 °C.

Volatile compounds were identified and quantified using the GCMS Solution software (Shimadzu GCMS Solution V 4.45SP1). The chromatograms were analyzed first by comparison with a system blank (without blueberries) for background volatiles, then by comparison among the fruit VOC samples under the three treatments (SWD-attacked, physical damage, undamaged control). VOCs were identified from their mass spectra and retention indices, using the NIST08 and Adams' MS databases (Adams 2007). Amounts of Individual compounds present were calculated relative to the internal standard by peak area comparison and are hence expressed as µg/25 g/24 h.

Olfactometer Bioassays

The behavioral responses of *T. anastrephae* females to volatiles from blueberries were evaluated using a glass Y-tube olfactometer consisting of two arms (6 cm long by 0.6 cm internal diameter) connected to chambers (9 cm long by 5 cm internal diameter) for the volatile stimuli. The chambers were located so that no visual contact was possible from the Y-tube. Humidified, charcoal-filtered air was pushed through the stimuli and olfactometer with a pump at a rate of 0.5 l/min. The olfactometer was laid horizontally on a glass surface homogeneously trans-illuminated with cold white LED lights (3600 lumens). To further eliminate visual cues, the olfactometer was fully enclosed in a box made from white corrugated plastic with a hole on top to allow video recording. All bioassays were conducted at 22 ± 2 °C, 70 ± 5% RH, and performed between 09:00 and 18:00 h. After each trial, the olfactometer arms were rotated to avoid position bias. At the end of the day the olfactometer was washed with distilled water and neutral soap, rinsed with ethanol and acetone, and oven-dried at 100 °C for 24 h.

Trichopria anastrephae females were used 2–5 days after hatching from *D. melanogaster* pupae. Upon hatching, females and males were placed in glass containers with access to honey-water (50:50) absorbed in cotton balls, until their use in the experiments. To conduct the bioassay, each female wasp was individually placed at the base of the common section of the Y-tube, and a 5-min period was video recorded. From this video, we measured the time spent in each arm, the first arm selected, and the position at the end of the recording period. For the three variables, the assignment of the wasp position was defined relative to a perpendicular line at the base of each arm.

Three olfactometer experiments were performed: one bioassay tested undamaged blueberries versus clean air, the second tested SWD-attacked blueberries versus clean air, and the third compared SWD-attacked blueberries versus undamaged blueberries. Physically-damaged blueberries used as a control in VOC chemical analyses were not used in the behavioral bioassays because the VOC profiles from undamaged and physically-damaged blueberries were not different (see "Results"). For the experiments with SWD-damaged fruit, 25 g of blueberries were exposed to oviposition by SWD under the same conditions as described for the VOC collections, and similarly kept for 10 days prior to the bioassays. Undamaged blueberries were stored under identical conditions. Blueberries for olfactometer bioassays were of commercial origin (*Vaccinium corymbosum*, Frusan, Frutera San Fernando, Chile); they were used fresh upon purchase and gently washed with distilled water.

Statistical Analyses

All statistical analyses were performed using R (Version 3.6–2) (R Core Development Team 2019).

To visualize VOC profiles in the different treatments a nonmetric multidimensional scaling (NMDS) was performed on the amount of VOCs matrix by using a Bray–Curtis distance matrix (Vegan package V2.4-6 in R). The data include many zero values, so it was fourth-root transformed (Hervé et al. 2018). To test for significant differences in the chemical composition of VOCs produced among the treatments (SWD-attacked, physical damage, and control) a permutational multivariate analysis of variance (perMANOVA) was also carried out on the distance matrix based on 9999 permutations. The analysis was performed using the *adonis* function in R (Vegan package V2.4-6).

The behavioral response of parasitoid wasps was analyzed by a paired *t*-test for the time spent in each arm, and an exact binomial test both for the first arm chosen and the position at the end of the bioassay. The exact binomial test performs an exact test of a simple null hypothesis about the probability that the number of wasps for first choice of either olfactometer arm had a 50:50 distribution. Also, to test the position at the end of the bioassay we performed the same test with a probability of 0.33 for the number of wasps present in either olfactometer arm or the common section. Females that did not respond were excluded from the analyses.

Results

Effects of Attack by SWD on Blueberry VOCs

Analyses of collection of volatiles from blueberries before initiation of the treatments and visualization by NMDS confirmed that the batches assigned to treatments were homogenous (Supplementary Data Fig. S2). We then measured VOCs from 15 samples of SWD-attacked blueberries, 10 samples of physically damaged fruit, and 15 samples of undamaged control fruits. Twenty-four compounds were identified in the VOC extracts from blueberries (Table 1, Fig. 1, Supplementary Data Table S1). Of these, nine compounds were exclusively present in the VOCs from blueberries attacked by *D. suzukii* (SWD-attacked) (Table 1). Taking into account the frequency in which it was found and the relative amount, isoamyl acetate was one of the main compounds that separates VOCs of SWD-attacked blueberries from VOCs of the fruit treatments (Table 1). Overall, volatiles produced in the highest amounts were short-chain aliphatic esters (Table 2).

Multivariate analyses also showed that the VOC profiles of SWD-attacked blueberries differed from the VOC profiles of both control treatments (perMANOVA:

Table 1 Amounts of volatile organic compounds (VOCs) found in collections from blueberries subjected to the different treatments: attacked by *Drosophila suzukii* (SWD-attacked); physical damage and natural fruit ripening (undamaged control)

Compound	Amount (μg/25 g/24 h; mean ± standard deviation)					
	SWD-attacked	*N*	Physical damage	*N*	Undamaged control	*N*
Short-chain aliphatic esters						
1. Ethyl isobutyrate	0.025 ± 0.03	3	0.021	1	0.005	1
2. Methyl 3-methylbutanoate	0.503 ± 1.15	8	0.341 ± 0.09	2	0.125 ± 0.10	4
3. Ethyl butanoate	0.257	1	0.149	1		
4. Methyl 3-methyl-2-butenoate	0.073 ± 0.03	4				
5. Ethyl 2-methylbutanoate	0.167 ± 0.15	15	0.097 ± 0.07	10	0.143 ± 0.10	15
6. Ethyl 3-methylbutanoate	2.559 ± 3.45	15	0.973 ± 1.31	10	1.549 ± 1.54	15
8. 3-Methylbutyl acetate (isoamyl acetate)	0.144 ± 0.09	7				
9. isoPropyl 3-methylbutanoate	0.020 ± 0.01	5	0.034 ± 0.03	2	0.037 ± 0.02	2
10. Ethyl 3-methyl-2-butenoate	0.112 ± 0.08	2	0.045	1	0.036 ± 0.00	2
15. 3-Methylbutyl 2-methylbutanoate	0.03	1				
16. 3-Methylbutyl 3-methylbutanoate	0.293 ± 0.10	2				
Sesquiterpenes						
19. delta-Elemene	0.182 ± 0.12	12	0.269 ± 0.15	9	0.246 ± 0.21	13
20. β-Caryophyllene	0.003 ± 0.003	4	0.008 ± 0.01	2	0.014 ± 0.02	4
21. cis-Thujopsene	0.015 ± 0.01	6	0.002 ± 0.001	2	0.006	1
23. 4,5-di-epi-Aristolochene	0.037 ± 0.02	2				
24. trans-beta-Guaiene	0.007 ± 0.005	4	0.009 ± 0.01	4	0.014 ± 0.01	6
Monoterpenes						
11. Anhydrolinalool oxide	0.090 ± 0.04	3	0.049	1	0.043 ± 0.01	3
12. Limonene	0.093 ± 0.15	11	0.087 ± 0.08	4	0.133 ± 0.23	9
13. Cineole	0.032 ± 0.01	3				
14. Terpinolene	0.023 ± 0.01	3	0.072	1	0.053 ± 0.05	4
Aromatics						
17. 2-Phenylethanol	0.178 ± 0.03	3				
18. Ethyl benzoate	0.059 ± 0.02	3				
Others						
7. 1-Hexanol	0.055 ± 0.03	2				
22. 12-Methyl-oxacyclododec-9-en-2-one	0.069 ± 0.04	2			0.039	1

VOC components are classified in groups. Compounds are numbered as in Fig. 1 and Table S1 *N* indicates the number of samples in which a compound was found. See Table S1 for details of compound identification

$F_{2,37} = 1.91$ $P = 0.04$, permutation = 9999). The NMDS ordination of the VOC composition partially separated VOCs of SWD-attacked fruits from physically damaged and control VOCs (Fig. 2). Further, the multivariate pairwise comparison showed significant differences between the VOCs from SWD-attacked blueberries and those from physically attacked controls (perMANOVA: $F_{1,23} = 2.63$, $P = 0.03$, permutation = 9999), as well from SWD-attacked blueberries and undamaged control fruit VOCs (perMANOVA: $F_{1,28} = 2.26$, $P = 0.04$, permutation = 9999). Finally, the NMDS ordination did not graphically-show differences between VOCs from physically damaged and undamaged blueberries, nor did the multivariate analyses (perMANOVA: $F_{1,23} = 0.34$, $P = 0.83$, permutation = 9999).

Olfactory Responses of Trichopria anastrephae to Blueberry Volatiles

In the Y-tube bioassay, females of *T. anastrephae* reared on *D. melanogaster* responded preferentially to volatiles from blueberries attacked by *D. suzukii*, in comparison to undamaged blueberries or clean air. In both bioassays involving SWD-attacked fruits (vs. air-control and vs. undamaged blueberries), about 60% of females made a

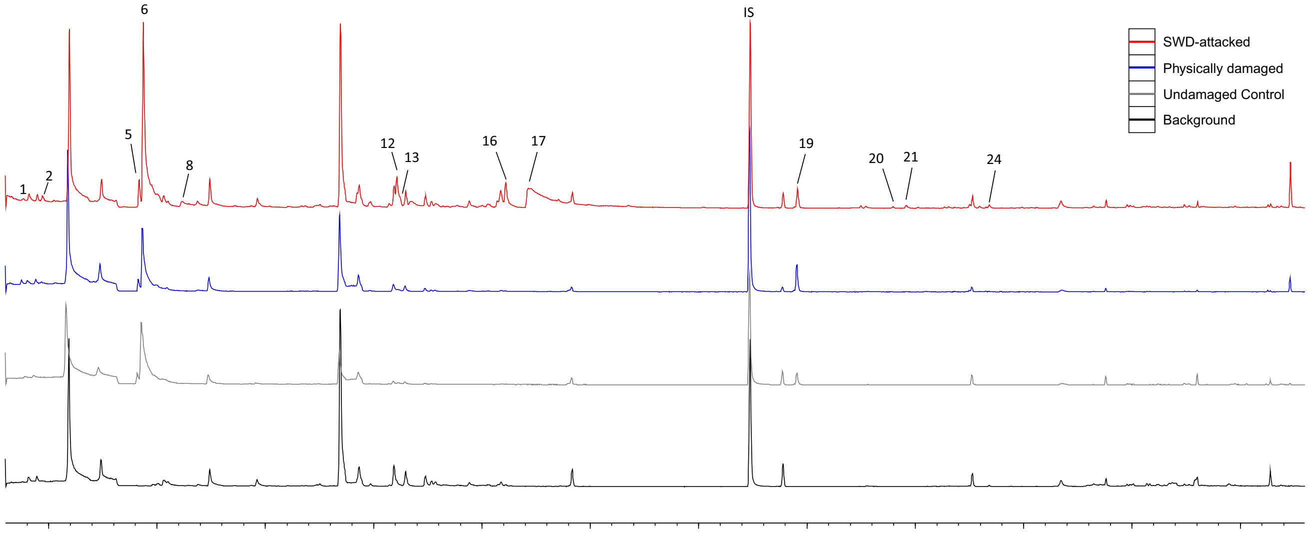

Fig. 1 Typical GC–MS chromatogram (TIC) of VOC collections from blueberries attacked by SWD (red), physically damaged control (blue), undamaged control (grey) and ambient background volatiles (black). Numbers indicate compounds as described in Tables 1 and S1. Non-numbered peaks correspond to background volatiles. Missing numbers are blueberry VOCs not found in the samples chosen for the Figure (IS internal standard 100 ng tridecane)

choice during the 5-min test period (72 out of 109 and 53 out 92, respectively). In contrast, in the bioassay comparing undamaged control blueberries vs. clean air only around 30% of the females made a choice (28 out of 103). In general, the behavioral responses of *T. anastrephae* females showed a tendency to prefer the blueberry-treated arm. Parasitoid wasps were significantly attracted to SWD-attacked blueberries when compared to clean air in all three variables measured (first choice binomial test $P = 0.01$; final position binomial test $P = 0.0003$ and time in each arm $t_{52} = 2.52$, $P = 0.01$) (Figs. 3, 4). Also, *T. anastrephae* females were significantly attracted to the volatiles from SWD-infested blueberries relative to undamaged blueberries when considering their final position (binomial test $P = 0.002$), but not in the first arm choice (binomial test $P = 0.1$) or the time spent in each olfactometer arm ($t_{71} = 1.28$, $P = 0.20$). In the case of undamaged blueberries compared to clean air, there were no significant differences in any of the three variables measured (first choice binomial test $P = 0.28$; final position binomial test $P = 0.33$ and time in each arm $t_{27} = 1.31$, $P = 0.19$).

Discussion

The aim of our study was to characterize the effect of SWD attack on the VOCs of ripening blueberries, and to correlate these odor changes with the olfactory-mediated behavioral responses of local populations of *T. anastrephae* female parasitoids. Our study is the first to use a chemo-ecological approach to test the capacity of *T. anastrephae* to find *D. suzukii*-infested fruit.

Multivariate analyses of the odor blends produced by SWD-attacked blueberries showed that the development of SWD immature stages inside the fruit generates a different odor profile in comparison with physically damaged and natural fruit ripening (undamaged control).

Table 2 Amounts of blueberry volatile organic compounds (VOCs) clustered by chemical group, according to fruit treatments: attacked by *Drosophila suzukii* (SWD-attacked); physical damage control and natural fruit ripening (undamaged control). *N* indicates the number of samples in which compounds of the chemical group were found

Compound group	Amounts (μg/25 g/24 h; mean ± standard deviation)					
	SWD-attacked	*N*	Physical damage	*N*	Undamaged control	*N*
Short-chain aliphatic esters	3.17 ± 4.63	15	1.17 ± 1.52	10	1.74 ± 1.61	15
Sesquiterpenes	0.17 ± 0.13	14	0.28 ± 0.16	9	0.24 ± 0.22	14
Monoterpenes	0.12 ± 0.15	12	0.12 ± 0.14	4	0.17 ± 0.29	9
Aromatics	0.14 ± 0.09	5				
Others	0.06 ± 0.03	4			0.04	1

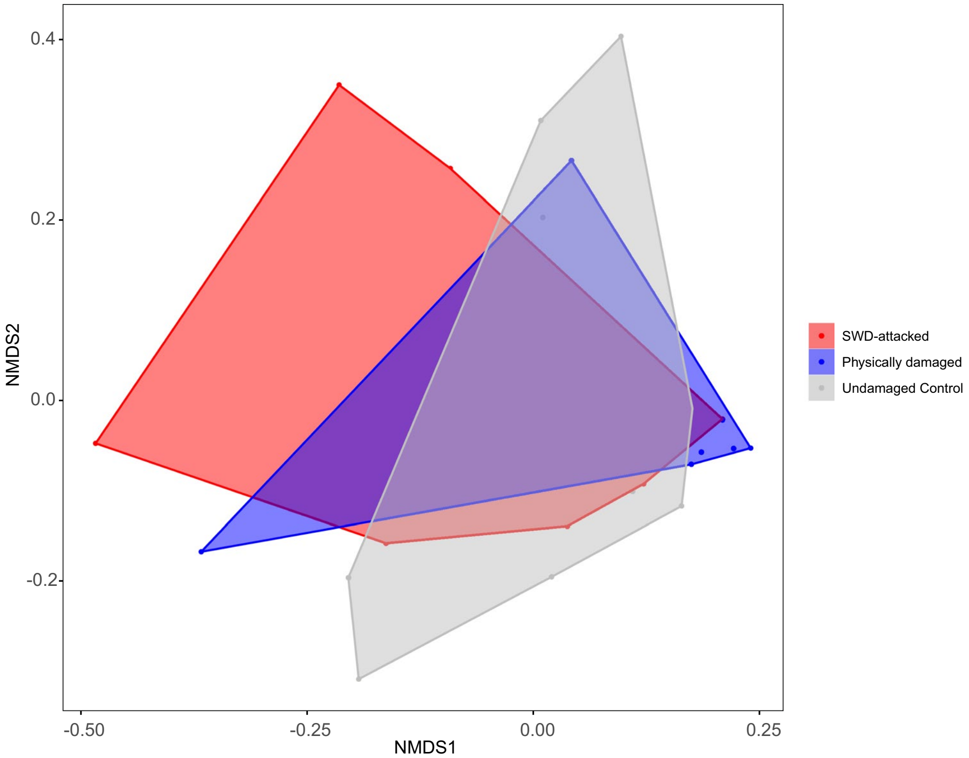

Fig. 2 Non-metric multidimensional scaling (NMDS) ordination based on Bray–Curtis dissimilarities of the volatile organic compounds (VOCs) from blueberries attacked by *Drosophila suzukii* (SWD-attacked, $N = 15$); VOCs from physically-damaged blueberries ($N = 10$) and from undamaged fruit (Control, $N = 15$). Stress value = 0.1615

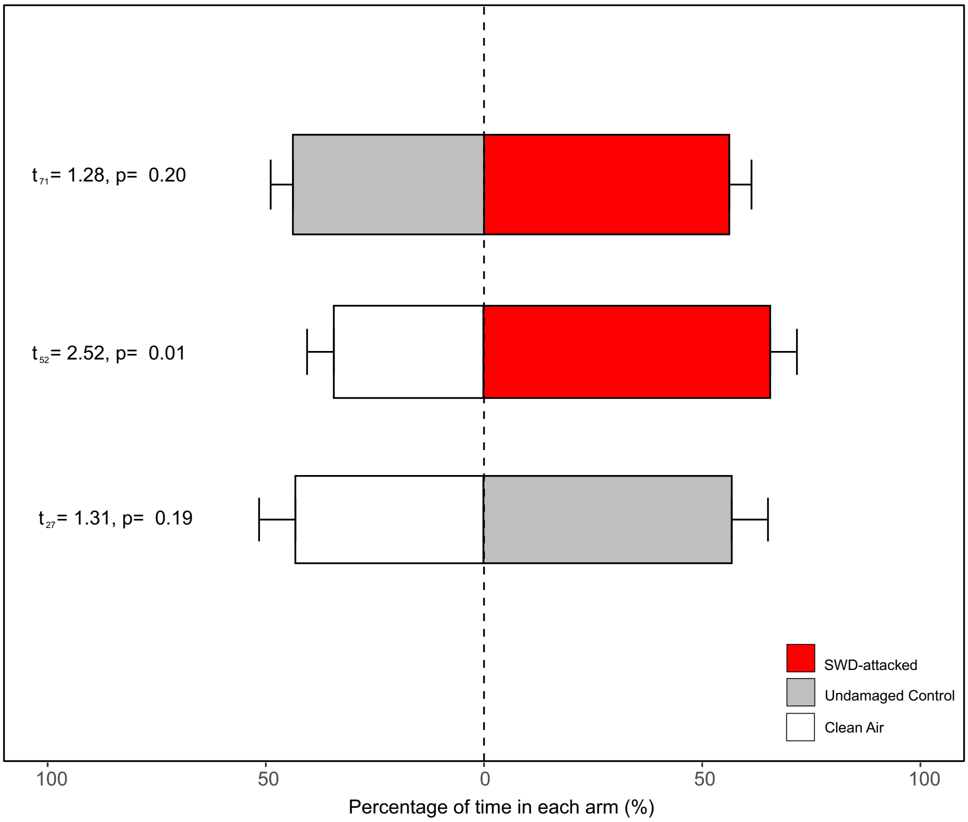

Fig. 3 Percent time spent by *Trichopria anastrephae* females in each arm of an olfactometer tube in choice bioassays testing volatile cues produced by blueberries attacked by *Drosophila suzukii* (SWD-attacked), control blueberries (undamaged) or clean air. Paired *t*-test are reported and error bars show Standard Error

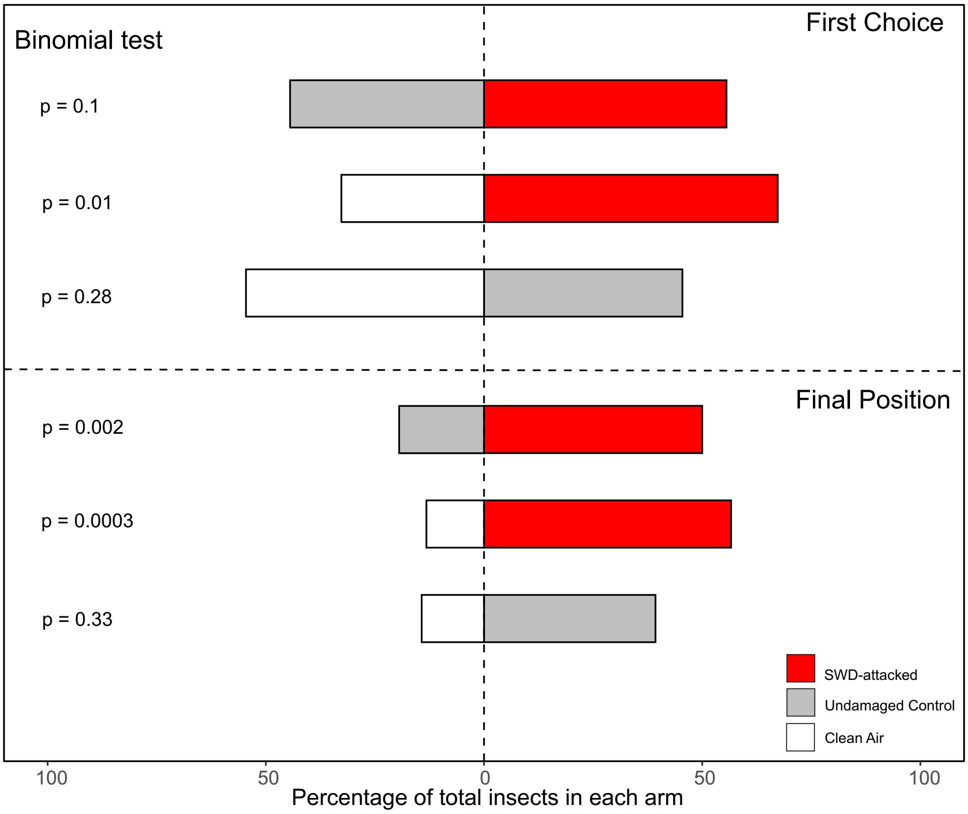

Fig. 4 Percent *Trichopria anastrephae* females in each arm of an olfactometer tube in choice bioassays with volatile cues produced by blueberries attacked by *Drosophila suzukii* (SWD-attacked), undamaged control or clean air. The upper and lower panels show the first arm choice and the final position at the end of a 5-min test period, respectively. *P* values for the binomial test are reported

undamaged control fruits. Ten days after SWD infestation, a period that correlates with SWD larval development (Tochen et al. 2014), the odor differences among SWD-attacked and control blueberries (physically damaged and undamaged) were significant and observable in the diversity, frequency, and amounts of volatile compounds. These differences in the blueberry VOC profiles may be the result of various factors associated with SWD infestation, such as fruit tissue collapse due to larval feeding, larval metabolic wastes and their associated microorganisms, and opportunistic microorganisms associated with oviposition wounds (Hamby and Becher 2016; Rombaut et al. 2017).

Short-chain aliphatic esters, particularly ethyl 3-methylbutanoate, constituted the most abundant group of chemicals in all three treatments. Along with the esters, the blueberries emitted sesqui- and monoterpenes as relatively minor components. Among the sesquiterpenes, delta-elemene was the most abundant and frequent, while limonene was the most abundant monoterpene in the control treatments. In SWD-attacked blueberries, however, the amount of limonene was similar to that of anhydrolinalool oxide, a probable fungal biotransformation metabolite. VOCs from SWD-attacked fruits also contained 2-phenylethanol and ethyl benzoate, volatiles commonly found in flowers and fruits and also typical from fermentation processes in the case of the former. These aromatic volatiles were not found in either of the control treatments. Finally, SWD-attacked blueberries emitted seven additional compounds that were not found in the VOC collections from both control treatments. Among these, the most abundant were 3-methylbutyl 3-methylbutanoate (isoamyl isovalerate) and isoamyl acetate. The latter was also the most consistently found among compounds exclusive to SWD-attacked fruits.

Compounds such as hexanol, (*Z*)-linalool oxide and linalool are probably produced throughout ripening and preserved during the last maturation phases, reaching high amounts in the overripe stage (Farneti et al. 2017; Horvat et al. 1996). Other compounds seem to be emitted by unripe fruits and drastically reduced during ripening (e.g. caryophyllene), while compounds such as δ-elemene are stable during all ripening phases (Farneti et al. 2017). In contrast, esters such as ethyl 3-methylbutanoate (ethyl isovalerate), ethyl acetate, and methyl 2-methylbutanoate are exclusively produced in the last phase of ripening, increasing as the blueberries overripe (Farneti et al. 2017). In this scenario, our results suggest that SWD attack results in volatile emissions that resemble overripe fruit.

Fruit volatiles are important in the chemical ecology of drosophilids and may play a role in niche differentiation among sympatric species. Even though cosmopolitan *Drosophila* species are host generalists, different species may separate along resource-based niche dimensions such as fruit maturation time (Nunney 1996). SWD may be attracted to leaf volatile cues to mate-finding and also fresh unripe fruits odors to locate areas for oviposition (Cloonan et al. 2018). As fruits ripen, other drosophilids such as *Drosophila simulans*, *D. melanogaster*, and *Drosophila immigrans* may further colonize this rotten fruit, following a preference order for increasing maturation stages (Atallah et al. 2014; Nunney 1996; Rombaut et al. 2017). Fruit volatile esters may provide cues for ripening stages and may encode enough information to enable drosophilid flies to detect and discriminate their niches (Scheidler et al. 2015). For instance, isoamyl acetate, a "fruity" odor often present in ripening, ripe, and early fermenting fruits, is known to attract many drosophilids (Stökl et al. 2010). This compound, along with isobutyl acetate and ethyl hexanoate, were present in headspace VOC samples of fruit-associated yeasts and caused antennal responses in *D. melanogaster* and *D. suzukii* (Scheidler et al. 2015). Moreover, isoamyl acetate was one of the EAD-active compounds from wild blueberries, attractive to *D. suzukii* both individually and as part of a blend (Urbaneja-Bernat et al. 2021). However, tested in formulated blends for trapping SWD, isoamyl acetate showed no attraction and even a decrease in SWD adult captures (Cha et al. 2012). In this case, it is possible that concentration modulates SWD response to volatiles such as isoamyl acetate, since high concentrations may signal an overripe fruit that is not a preferred oviposition site for SWD females (Revadi et al. 2015).

The dynamics of fruit volatile blends associated with the temporal separation of *Drosophila* species may provide host-finding cues to the next trophic level (Vet and Dicke 1992). Using behavioral bioassays, we demonstrated that resident populations of *T. anastrephae*, a pupal parasitoid reared in the laboratory on *D. melanogaster*, responded differentially to volatiles emitted by blueberries infested by SWD. We measured three variables to characterize female wasp responses to the volatiles of blueberries: the first choice, the time spent in each arm, and the final position after five minutes of bioassay. Females of *T. anastrephae* showed a preference in all three variables when SWD-attacked fruits were tested against clean air. When the bioassay compared SWD-attacked and undamaged blueberries, a preference was only found in the position of the wasp at the end of the tested period, a variable that may correlate with active seeking behavior of the preferred source. These results are in line with those obtained from a related parasitoid, *T. drosophilae*. Using an olfactometer arena with four chambers and testing seven wild non-crop fruits as stimuli, Wolf et al. (2020) showed that female *T. drosophilae* spent more time walking over chambers with SWD-infested fruits compared to clean air. When comparing SWD-infested fruits with non-infested fruits, significant preferences were not so consistent, indicating that fruit odors alone may not be sufficient for host location in these fruits (Wolf et al. 2020). The preference showed by *T. anastrephae* females towards volatiles of SWD-infested blueberries appear to be innate, since they have had no access to SWD or SWD-infested fruit prior to the tests. SWD infestation of blueberries resulted in an increase of typical volatile compounds that other *Drosophila* species use for locating food, mating, and oviposition sites. It is then likely that *T. anastrephae* females use these same general odorant cues to locate their established drosophilid hosts. The foraging females may switch hosts if other alternatives are more abundant (Jaworski et al. 2013) or if they find earlier infested fruits that are more likely to contain pupae. In support of this, the generalist parasitoid *T. drosophilae* showed no differential preference for *D. melanogaster* or *D. suzukii* pupae (Wang et al. 2016). Similarly, a recent study by Biondi et al. (2021) showed that the larval parasitoids, *Asobara japonica* Belokobylskij (Hymenoptera: Braconidae), *Leptopilina japonica* Novković & Kimura, and *Ganaspis brasiliensis* (Ihering) (Hymenoptera: Figitidae), respond to fruit volatile cues associated with the presence of either *D. suzukii* or *D. melanogaster* (Biondi et al. 2021).

From an applied perspective, our results represent a relevant contribution to the development of a biological control program for SWD, since it deepens our understanding of how SWD impact blueberry VOCs, and how a parasitoid responds to the presence of its potential pest host (Biondi et al. 2017). Our study highlights the potential use of established populations of *T. anastrephae* since they are naturally able to cue on VOCs from SWD-attacked fruit and use SWD as a viable host (Vieria et al. 2020). Although most SWD pupae are found in the soil (Wolz et al. 2017), by using fruit VOCs as a long-range cue, parasitoids could target the right ecological niche to then refine their search. Understanding the behavior of this parasitoid in challenging environments is important to obtain higher efficiency in biological control programs against SWD (Krüger et al. 2019), stressing the need for more studies on the biology of *T. anastrephae* in the region. In line with this, the absence of management in adjacent crops or wild fruits could be reservoir of SWD where resident populations of parasitoids could have a greater importance. Our finding of local parasitoid populations suggests that the wasp is well adapted to local environmental conditions, which further underlines its potential as a biological control agent also in unmanaged adjacent crops or nearby wild fruits, which could serve as reservoirs for SWD (Krüger et al. 2019). In a broader sense, studies on these local parasitoid populations at the regional level may offer opportunities to manage SWD in Latin America without the new introduction of exotic species. Hence, a non-native organism such as SWD in Latin America may become controlled by a regulating mechanism in the introduced range, which limits its density and expansion (Schulz et al. 2019).

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Availability of Data and Material Data is available from the corresponding authors on request.

Code availability.

Code is available from the corresponding authors on request.

Code Availability Code is available from the corresponding authors on request.

Declarations

Conflict of interest All authors declare that they have no conflict of interest.

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