Field survey of *Drosophila suzukii* (Matsumura) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) in Maui, Hawaii

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ABSTRACT

Drosophila suzukii (Matsumura) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) are notable agricultural pests of soft-skinned fruits. Efficient field surveying is vital in an integrated pest management program. A survey to identify *D. suzukii* populations was conducted in four localities in Maui County among seven host-plants. During the survey, adult *Z. indianus* specimens were collected at all four localities in traps positioned in six of the seven host plants, suggesting that this previously unreported exotic species may already be well-established. Though there are currently no species-specific attractants available for *D. suzukii* or *Z. indianus*, characterization of attractant specificity by species and understanding how attractant efficacy varies with time is needed to advance development. A modification of the deli-cup trap was used and five attractants (brown rice vinegar, apple cider vinegar, red wine, brown rice vinegar plus red wine, apple cider vinegar plus red wine) were deployed in cherimoya in Kula, Maui, Hawaii. This investigation includes the first reported use of brown rice vinegar as an olfactory attractant in the United States and the results suggest that it may have higher specificity in the field capture of *D. suzukii* than apple cider vinegar, red wine, and apple cider vinegar with red wine. No significant differences were observed in attractant specificity for the field capture of *Z. indianus*. To examine attractant efficacy over time with and without a preservative, traps were maintained daily in cherimoya. The results suggest that attractants up to seven days old had a significant effect on mean field captures of *D. suzukii* and non-target drosophilids. Inclusive of all attractants and field ages, The addition of 1% boric acid (w/v) to the attractant solution increased the total field captures of *D. suzukii* by 44%, but no effect was observed for non-target drosophilids. These investigations enhance our current understanding of attractant specificity, which is the first step towards identifying selective compounds for a species-specific attractant. Furthermore, the first report of *Z. indianus* in Hawaii highlights the importance of examining interspecies interactions between endemic and invasive drosophilids and the need for the establishment of economic thresholds for vinegar fly pests.

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ABBREVIATIONS

SWD	*Drosophila suzukii* (Matsumura), Spotted wing drosophila
AFF	*Zaprionus indianus* Gupta, African fig fly
ACV	Apple cider vinegar
BRV	Brown rice vinegar
RW	Red wine
ACV+RW	Apple cider vinegar plus red wine (60/40%)
BRV+RW	Brown rice vinegar plus red wine (60/40%)
SEM	Standard error of the mean

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AUTHOR CONTRIBUTIONS

For Chapter 1, the following attributions are acknowledged. First author Brittany Willbrand co-designed the experiment, conducted the field work, compiled the results, submitted voucher specimens (Hawaii Department of Agriculture, the Bishop Museum, and the Insect Museum at the University of Hawaii at Manoa), and authored the original manuscript draft. Co-author Dr. Douglas Pfeiffer co-designed the experiment, supervised the first author, submitted voucher specimens (Systemic Entomology Lab, USDA, ARS, and the Department of Entomology at Virginia Tech), enlisted Dr. Leblanc and Dr. Yassin with identification, and revised the original manuscript draft. Co-author Dr. Luc Leblanc provided taxonomic confirmation, and revised the original manuscript draft. Co-author Dr. Amir Yassin provided taxonomic confirmation, submitted voucher specimens (the Muséum d'Histoire Naturelle de Paris), and revised the original manuscript draft.

First Report of African Fig Fly, *Zaprionus indianus* Gupta (Diptera: Drosophilidae), on the Island of Maui, Hawaii, USA, in 2017 and Potential Impacts to the Hawaiian Entomofauna

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Abstract. This report confirms the first reported observation of *Zaprionus indianus* Gupta (Diptera: Drosophilidae), commonly known as African fig fly, on Maui (new island record). Adult specimens were collected in October and November 2017 while surveying for populations of *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae). Specimens were retrieved from four localities in Haiku and Kula among traps positioned at fruiting height in six host plant environments (orange, lemon, starfruit, banana, strawberry, and cherimoya). Historically, the earliest records of *Z. indianus* in the state were recorded on Oahu in 2013 (new state record, new island record), on Kauai in 2015 (new island record), and on the Big Island (Hawaii) in 2017 (new island record). Including this report, there are currently at least 33 introduced Drosophilidae species established in the state of Hawaii. Furthermore, it is the second member belonging to genus *Zaprionus* that has been identified on the Hawaiian Islands. Specimens were not only retrieved from farms and subdivisions but also within mountain ranges and state forest reserves, suggesting that further research is needed to evaluate potential impacts to endemic entomofauna.

Key words: Invasive drosophilids, *Zaprionus indianus,* African fig fly, new island record, Maui

The African fig fly, *Zaprionus indianus* Gupta (Diptera: Drosophilidae), is a red-brown vinegar fly with distinctive secondary coloring that is native to the Afrotropics (Gupta 1970). As illustrated in Figure 1, members of genus *Zaprionus* have longitudinal white stripes on the dorsal regions of the head and thorax (Yassin and David 2010). Genus *Zaprionus* contains two subgenera, *Anaprionus* and *Zaprionus*, which can be differentiated through examination of external morphology. While both groups have longitudinal white stripes, species belonging to subgenus *Anaprionus* have an odd number of white stripes whereas members of subgenus *Zaprionus*, including *Z. indianus*, have an even number of white stripes (van der Linde 2010). Furthermore, the black and white stripes on *Z. indianus* are of equal size with the stripe width maintained the full length of the head to the thorax (van der Linde 2010). The *vittiger* species group, of which *Z. indianus* is a member, is characterized by a row of composite spines fused with long bristles at the base of the forefemur (Yassin and David 2010). Once considered cryptic species,

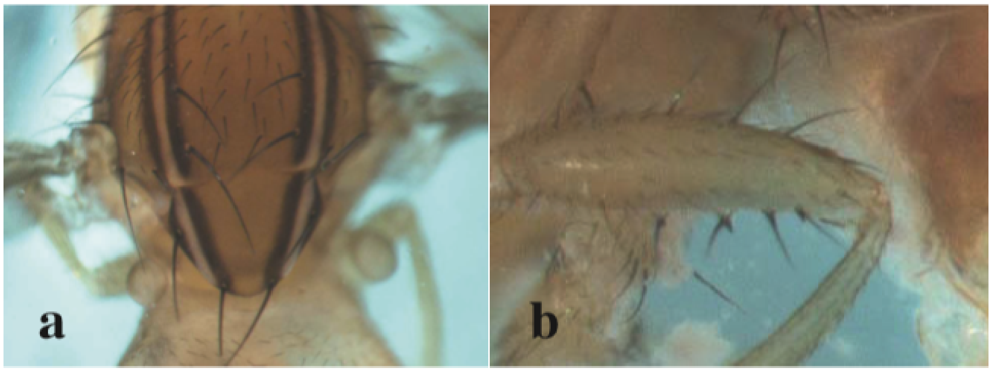

Figure 1. Thorax (a) and front tibia (b) of *Zaprionus indianus* collected on Maui. The lack of a white spot on the scutellum, and the presence of tibial spines, separates this species from *Zaprionus ghesquierei*.

members of the *indianus* species complex, including *Z. africanus*, *Z. gabonicus*, and *Z. indianus*, can be reliably distinguished by spermatheca shape with *Z. africanus* possessing a narrower spermatheca and an apically serrated aedeagal flap, *Z. gabonicus* with a basally smooth aedeagal flap and *Z. indianus* with a basally serrated aedeagal flap (Yassin and David 2010). Though morphologically similar, species belonging to the *indianus* species complex display a variety of ecological behaviors resulting in differences in pest status and invasive potential.

As a polyphagous drosophilid, *Z. indianus* uses a wide range of host plants for opportunistic feeding and breeding (Lavagnino et al. 2008). This results in damage to agricultural crops as well as making eradication of the drosophilid challenging once established. Even though *Z. indianus* is typically regarded as a secondary pest, it has demonstrated the potential to cause direct injury to select cultivars of both fig (Matavelli et al. 2015) and strawberry (Bernardi et al. 2017) fruits. Oviposition and subsequent larval feeding on agricultural crops can contribute to decreased yields and rejected product. On intact ripe strawberry fruit, adult females can oviposit on the fruit surface where larvae emerge, penetrate the epidermis, and consume pulp and yeast vital for development (Bernardi et al. 2017). Unlike *Drosophila simulans* Sturtevant and other drosophilids that oviposit on decaying figs, adult *Z. indianus* females can oviposit on ripening figs, increasing economic damage incurred by farmers (Matavelli et al. 2015). As a secondary pest, adult *Z. indianus* females can oviposit into fruits that have mechanical injury from other insects, including oviposition injury by *Drosophila suzukii* (Matsumura) (Bernardi et al. 2017) making the presence of both drosophilids particularly concerning to farmers of any soft-skinned fruit.

Aided by international trade and commerce, *Z. indianus* has been introduced to a wide variety of localities outside of its native range including North and South America, Europe, and Asia (Westphal et al. 2008, Hulme 2009). Invasive range expansion for *Z. indianus* has been reported on the Asian continent in India (Gupta 1970, Fartyal et al. 2014), Saudi Arabia (Amoudi et al. 1991), Egypt (Yassin and Abou-Youssef 2004), Iraq (Al T'Oma et al. 2010), and Jordan (Al-Jboory and Katbeh-Bader 2012). In Europe, specimens have been reported in France (Kremmer, et al. 2017) and Madeira archipelago (Rego et al. 2017). In South America, *Z. indianus* has invaded Brazil (Vilela 1999), Uruguay (Goñi et al. 2001), and Argentina (Soto et al. 2006). In Central America, *Z. indianus* was confirmed in Panama (van der Linde et al. 2006). In North America, reports of *Z. indianus* were published in Mexico (Lasa and Tadeo 2015), Canada (Renkema et al. 2013), and in the United States including Florida (van der Linde et al. 2006), Virginia (Pfeiffer et al. 2012), Michigan (Van Timmeren and Isaacs 2014), and Pennsylvania (Joshi et al. 2014). We report here an additional range expansion to the island of Maui, Hawaii, USA in 2017, discovered during a survey to identify whether populations of the invasive drosophilid *D. suzukii* were present within several localities and host plants.

Materials and Methods

Survey localities and host plants. Four locations on Maui were selected for the qualitative survey: a private estate in Haiku, HI (20.899859°N, –156.283533°W, elevation 302 m), Kula Agricultural Park (20.797167°N, –156.368971°W, 341 m), Kula Country Farms (20.747138°N, –156.337140°W, 889 m), and University of Hawaii at Manoa Kula Research Station (20.757534°N; –156.319755°W, 980 m). Seven host plants were selected to survey including fruiting trees (orange, lemon, starfruit, banana, cherimoya) and fruits and vegetables (strawberry, pumpkin). Orange, lemon, and starfruit were surveyed at the Haiku estate. Bananas were surveyed at Kula Agricultural Park. Strawberries, pumpkin, and lemon were surveyed at Kula Country Farms. Cherimoya was surveyed at Kula Research Station.

Trap assembly. The trapping device used in this experiment was a novel variation of the traditional deli cup design where a Mason jar, serving as a removable trap base, houses the drowning solution while a Solo cup permits entry and passive diffusion of the attractant within the headspace of the trap. Each trap was composed of a 236 ml Mason jar and a 473 ml red Solo cup and assembled with epoxy, sandpaper, an unfolded paperclip, and a soldering tool (Cold-Heat®). The first 2.5 cm of the Solo cup interior was scoured with sandpaper, then coated with a thin layer of epoxy. The silver band from the Mason jar lid was then firmly pressed down into the cup interior. The Solo cup was allowed to cure for 24 hours. An unfolded paperclip was pressed against the ceramic tip of the soldering tool to conduct heat. Then, the ceramic tip was used to pierce the solo cup in eight places (two on each side) to create 5 mm diameter entry holes. Nylon rope was strung through the top two holes of the Solo cup to enable trap installation (Figure 2).

Attractants. Attractants were selected based on drosophilids innate attraction to fermentation volatiles (Stensmyr et al. 2003, Stökyl et al. 2010, Faucher et al. 2013) and historical success in field capture using vinegars and wine as olfactory baits (Landoldt et al. 2012, Cha et al. 2015). The five attractants used in this survey were: apple cider vinegar (ACV, supplied by Marukan Vinegar Co. Ltd.), brown rice vinegar (BRV, supplied by Marukan Vinegar Co. Ltd.), red wine (RW, Oak Leaf Vineyards, Merlot), ACV+RW, and BRV+RW. The latter two attractant blends were prepared at a 60:40% concentration of vinegar to wine. Acetic acid (AcOH) concentrations were quantified by Dr. Naoki Akasaka with high-performance liquid chromatography (HPLC, Organic Acid Analysis System Prominence, Shimadzu) as previously described in Akasaka et al. (2017). The AcOH concentrations of undiluted BRV and ACV were 4.23% and 4.37% (% wt/vol), respectively. Once diluted with red wine, the AcOH concentrations of 60% BRV and 60% ACV were 2.54% and 2.62% (% wt/vol), respectively. Attractants were aliquoted (40 ml per trap) into Mason

Figure 2. Trap installation: example of trap positioning on a lemon tree

jars, a drop of unscented dish soap was added to each jar to break the surface tension, and the lids and bands were affixed for transport.

Trap installation and retrieval. Five traps were used (one of each attractant type) for each host plant at each location for a total of 40 traps. Once trap locations were delineated, the traps were installed at fruiting height, which varied by crop type. For fruiting trees (orange, lemon, banana, cherimoya, and starfruit), traps were hung at a ca. 1 m height. For fruit and vegetable crops (strawberry and pumpkin), traps were tied to a gardening stake that was secured in the ground at fruit height. After nylon rope was used to hang the trap to fruiting height, a Mason jar containing attractant solution was screwed on to the bottom of the Solo cup. On October 23rd 2017, 15 traps were deployed on orange, lemon, and starfruit trees at the Haiku estate. On 31 Oct 2017, 5 traps were deployed at the cherimoya grove at Kula Research Station. On 1 Nov 2017, 5 traps were deployed on the bananas at Kula Agricultural Park. On 2 Nov 2017, 15 traps were deployed on the strawberry, pumpkin, and lemon at Kula Country Farms. After one week, traps were removed and the specimens collected. Specimens were strained by pouring the attractant solution over a fine mesh filter (1 mm) and were stored in 70% isopropyl alcohol for three days prior to examination.

Identification and voucher specimens. Though the original objective of the survey was to identify whether *D. suzukii* populations were present (Yes/No) in the localities and host-plants surveyed, this objective was expanded to include *Z. indianus* after the first specimen was recognized. Specimens were identified as *D. suzukii*, *Z. indianus*, or non-target. Once *D. suzukii* and *Z. indianus* specimens were identified for a particular attractant type and host plant combination, no further quantification was performed. Identification as *Z. indianus* was confirmed through examination of external morphology by BW, using standard keys (van der Linde 2010, Yassin and David 2010). Voucher specimens were submitted to DP and LL, for imaging, identification, and collection submission. Taxonomic confirmation was provided by LL and AY. Specimens were deposited at the Systemic Entomology Lab, USDA ARS (Beltsville, MD), the Department of Entomology at Virginia Tech, the Muséum d'Histoire Naturelle de Paris, the Hawaii Department of Agriculture, the Bishop Museum, and the Insect Museum at the University of Hawaii at Manoa.

Results

Adult *Z. indianus* specimens were captured within traps installed in six of the seven host plant fruits surveyed (banana, cherimoya, lemon, orange, starfruit, and strawberry) and at all four localities surveyed. Survey results are presented (Table 1) simply to show that *Z. indianus* was attracted to the various baits and in different fruit systems, each with a different olfactory environment. At the private estate in Haiku, all three host plants surveyed including lemon, orange, and starfruit contained both *Z. indianus* and *D. suzukii* specimens. At Kula Agriculture Park, only *Z. indianus* specimens were retrieved from the traps placed in the banana grove. At Kula Country Farms, *D. suzukii* specimens were retrieved from all three host plants surveyed, while *Z. indianus* specimens were retrieved from the lemon and strawberry, but not the pumpkin traps. At Kula Research Station, the traps positioned in the Cherimoya grove contained both *D. suzukii* and *Z. indianus*.

Five attractants were used (ACV, BRV, RW, ACV+RW, BRV+RW) in the field capture of two exotic drosophilids (*Z. indianus*, *D. suzukii*) with results that varied by host plant and locality. At the private estate in Haiku, every attractant examined resulted in captures of *Z. indianus* and *D. suzukii*. At Kula Agricultural Park, every attractant was effective in capturing *Z. indianus* adults, but no *D. suzukii* specimens were retrieved. At Kula Country Farms, both *Z. indianus* and *D. suzukii* specimens were retrieved from all attractant types used in the lemon grove. In the strawberry fields, no target drosophilids were captured when RW was used as an attractant. However, BRV and ACV, alone and blended with RW, were effective in the field capture of both target drosophilids. For the pumpkin traps, *D. suzukii* specimens were captured using ACV+RW or BRV+RW attractants. No *Z. indianus* specimens were retrieved, regardless of attractant used. At Kula Research Station, every attractant used was effective in capturing *D. suzukii*, whereas *Z. indianus* specimens were retrieved only in RW, ACV+RW, and BRV+RW baited traps. It is noteworthy that BRV and BRV+RW were effective at field captures of both target drosophilids, as previous research has established BRV as an effective attractant to *D. suzukii* in laboratory trapping experiments (Akasaka et al. 2017). The attractants used in this survey and the resulting captures were reported simply to provide a detailed record of the circumstances from which target specimens were attracted. Whether an attractant resulted in captures in this survey, does not provide evidence that these results would be applicable to other surveys or experiments. Further research would be needed to discern any trends comparing attractant efficacy with confidence, as the lack of replicates and quantification limit the extrapolation of trends outside of this survey.

Discussion

This report established a new island record for *Z. indianus* on Maui, compiled collection data throughout the state (Table 2), and provided an update for introduced Drosophilidae established in Hawaii. Historically, the earliest *Z. indianus* specimens in the state were collected on Oahu in 2013 (new state record, new island

Table 1. Qualitative reporting of *Drosophila suzukii* (SWD) and *Zaprionus indianus* (AFF) and by attractant, survey locality, and host-plant. Attractant Abbreviations: ACV (Apple cider vinegar), BRV (Brown rice vinegar), RW (Red wine). Minus sign denotes zero captures; Plus sign denotes one or more captures.

Attractant:	**ACV**		**BRV**		**RW**		**ACV+RW**		**BRV+RW**	
Species:	**SWD**	**AFF**	**SWD**	**AFF**	**SWD**	**AFF**	**SWD**	**AFF**	**SWD**	**AFF**
Private Haiku Estate										
Lemon	+	+	+	+	+	+	+	+	+	+
Starfruit	+	+	+	+	+	+	+	+	+	+
Orange	+	+	+	+	+	+	+	+	+	+
Kula Agricultural Park										
Banana	–	+	–	+	–	+	–	+	–	+
Kula Research Station										
Cherimoya	+	–	+	–	+	+	+	+	+	+
Kula Country Farms										
Lemon	+	+	+	+	+	+	+	+	+	+
Strawberry	+	+	+	+	–	–	+	+	+	+
Pumpkin	-	–	–	–	–	–	+	–	+	–

Table 2. *Zaprionus indianus* records in the State of Hawaii. N.S. = new state record; N.I. = new island record. Collection abbreviations: HDOA (Hawaii Department of Agriculture), USDA-SEL (United States Department of Agriculture – Systemic Entomology Lab), BM (Bishop Museum), VT-ENT (Virginia Tech – Entomology Department), MNH-P (Muséum d'Histoire Naturelle de Paris), IM-UH (Insect Museum – University of Hawaii). [1](Evenhuis et al., 2017). [2](K. Magnacca, personal communication, 2018).

Year	Record	Locality	GPS N	GPS W	Elev. (m)	Habitat	Host plant environment	Collector	Collection
Oahu									
2013	N.S., N.I.	Pahole Gulch				Gulch	Unknown*	K. Magnacca	Personal[1]
2013		Central Kaluaa Gulch				Gulch	Unknown*	K. Magnacca	Personal
2013		Peahinaia Trail			610	Mountains	Unknown*	K. Magnacca	Personal
2014		Kaneohe				Residence	Jaboticaba	B. Azama	HDOA, USDA-SEL
2016		Lualualei	21.42582	–158.10302	1342	Mountains		C. Imada	BM
2017		Lualualei	21.42582	–158.10284	1356	Mountains		N. Evenhuis et. al.[2]	BM
2017		Lualualei	21.42475	–158.10360	1383	Mountains		N. Evenhuis et. al.	BM
2017		Lualualei	21.42457	–158.10357	1397	Mountains		N. Evenhuis et. al.	BM
2017		Lualualei	21.42480	–158.10370	1387	Mountains		N. Evenhuis et. al.	BM
Kauai									
2015	N.I.	Lihue				Residence	Litchi	L. Ishii	HDOA
2018		Kokee State Park	22.09820	–159.69015	905.3	Forest reserve		K. Adachi; Ruabora, A.	HDOA
Hawaii									
2017	N.I.	Kainaliu				Farm	Surinam cherry	R. Curtiss et. al.	HDOA
Maui									
2017	N.I.	Haiku	20.89986	–156.28353	302	Residence	Orange, lemon, starfruit	B. Willbrand et. al.	VT-ENT, USDA-SEL, BM
2017		Kula	20.79717	–156.33690	341	Farm	Banana	B. Willbrand et. al.	MNH-P
2017		Kula	20.74714	–156.33714	889	Farm	Strawberry, lemon	B. Willbrand et. al.	HDOA
2017		Kula	20.75753	–156.33714	980	Research station	Cherimoya	B. Willbrand et. al.	IM-UH

* captured with banana bait

record), on Kauai in 2015 (new island record), and on the Big Island in 2017 (new island record). Adding to the detailed list of introduced Drosophilidae compiled by Leblanc et al. (2009), there can now be considered at least 33 introduced Drosophilidae species established in the state of Hawaii.

This is the second member of genus *Zaprionus* that has been identified on the Hawaiian Islands. In 2005, *Z. ghesquierei* Collart was identified on the island of Hawaii and a year later specimens were identified throughout Kula, Maui (Leblanc et al. 2009). *Zaprionus ghesquierei* can be easily distinguished from *Z. indianus*, as specimens have a white spot on the tip of the scutellum of *Z. ghesquierei* (lacking in *Z. indianus* (Figure 1a) and the presence of spurs on the foretibiae of *Z. indianus* (Figure 1b) (Yassin and David 2010). Following this report of *Z. indianus*, earlier *Zaprionus* flies captured in Kula in 2006 were re-examined by L.L., and their identity as *Z. ghesquierei* was re-confirmed. Furthermore, four *Z. ghesquierei* vouchers at the University of Hawaii Insect Museum were re-identified by Camiel Doorenweerd to confirm the identification. No *Z. ghesquierei* specimens were identified in this survey.

The effectiveness of a given compound to elicit an attraction response is influenced by environmental cues (Faucher et al. 2013) and physiological adaptations (Matavelli et al. 2015, Nguyen et al. 2016). Thus, attractant efficacy is mediated not only by the species unique preferences, but also by environmental co-factors, such as olfactory and visual cues, that vary between host plant environments. In this survey, no *D. suzukii* specimens were retrieved from the banana grove at Kula Agricultural Park and no *Z. indianus* specimens were retrieved in the pumpkin field at Kula Country Farms. In both instances, it is possible that the attractants used were unsuitable for the given combination of target species and host plant environment. However, other factors such as climate, elevation, fruiting stage, and population dispersal may have contributed to the results. The absence of *Z. indianus* in the pumpkin fields of Kula Country Farms may be attributed to population dispersal to preferred hosts, such as the adjacent lemon grove. Even though *Z. indianus* specimens were retrieved from the majority of host plant environments surveyed (lemon, orange, starfruit, banana, cherimoya, strawberry), the presence of an insect is not sufficient evidence of economically significant damage. Further research is needed to determine economic thresholds by crop and to characterize ecological impacts to endemic species.

Unfortunately, the discovery of *Z. indianus* at four localities throughout Maui provokes more questions than answers—Is the range expansion limited to agricultural ecosystems, or are they also present in native forests? Throughout the state, specimens have not only been retrieved from farms and subdivisions but also within mountain ranges and state forest reserves, which suggests that the species is able to thrive in a wide variety of environments. Based on our results, it appears that further surveying on Maui is needed in regions producing soft-skinned fruit and berries, native forest reserves, and within diverse microclimates. Will the combined presence of *D. suzukii* and *Z. indianus* have deleterious impacts for Maui farmers? Considering that *Z. indianus* can cause direct injury to fig and strawberry (Matavelli et al. 2015, Bernardi et al. 2017), and that these crops are produced by Maui farmers, the pest potential of this introduced species should be examined and economic thresholds defined. Are there any adverse impacts to endemic drosophilids? *Zaprionus indianus* has led to declines of native drosophilids in other locations (Castro and Vilente 2001, Tidon et al. 2003, da Silva et al. 2005b, da Silva et al. 2005a) and the Hawaiian Islands are already struggling to cope with the appearance of many invasive species, including insects. On Hawaii and Maui, even though researchers found a strong association between exotic drosophilids with introduced host plant environments and endemic drosophilids with native plant species, invasive drosophilids were still encountered in native forests and endemic drosophilids in disturbed or nonnative environments (Leblanc et al. 2013).

This finding not only suggests ecological interactions between endemic and introduced species but also adaptations within a dynamic environment, both of which warrant characterization. Additional research is needed to quantify the economic and ecological significance of this finding and to examine whether eradication efforts are necessary and feasible. The first step would be to delineate the range of *Z. indianus* across Maui. Secondly, interspecies interactions need to be examined, not only between *Z. indianus* and other exotic drosophilids, but also between exotic and endemic drosophilids. Finally, economic thresholds for both species should be defined for use in survey protocols for integrated pest management programs. Nonetheless, this work has identified the first report of *Z. indianus* on Maui which requires further inquiries to identify the extent of the introduced species effect on the local ecology and agriculture economy.

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CHAPTER 2. Brown Rice Vinegar as an Olfactory Field Attractant for Drosophila suzukii (Matsumura) and Zaprionus indianus Gupta (Diptera: Drosophilidae) in Cherimoya in Maui, Hawaii, with Implications for Attractant Specificity between Species and Estimation of Relative Abundance

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AUTHOR CONTRIBUTIONS

For Chapter 2, the following attributions are acknowledged. First author Brittany Willbrand co-designed the experiment, conducted the field work, compiled the results, and authored the original manuscript draft. Co-author Dr. Douglas Pfeiffer co-designed the experiment, supervised the first author, and revised the original manuscript draft.

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Brown Rice Vinegar as an Olfactory Field Attractant for *Drosophila suzukii* (Matsumura) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) in Cherimoya in Maui, Hawaii, with Implications for Attractant Specificity between Species and Estimation of Relative Abundance

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Abstract: *Drosophila suzukii* (Matsumura) is an agricultural pest that has been observed co-infesting soft-skinned fruits with *Zaprionus indianus* Gupta. The characterization of olfactory preferences by species is a necessary step towards the development of species-specific attractants. Five olfactory attractants were used to survey the populations of two invasive drosophilids in cherimoya in Maui, Hawaii. The attractants used were apple cider vinegar (ACV), brown rice vinegar (BRV), red wine (RW), apple cider vinegar and red wine (ACV+RW; 60/40), and brown rice vinegar and red wine (BRV+RW; 60/40). For *D. suzukii*, BRV+RW resulted in more captures than BRV, ACV, and RW, while ACV+RW resulted in more captures than ACV. No differences were observed between BRV+RW and ACV+RW. BRV had greater specificity in attracting *D. suzukii* compared to ACV, ACV+RW, and RW. For *Z. indianus*, no significant differences were observed in either the mean captures or specificity for any attractant used. Collectively, these findings demonstrate that (1) BRV and BRV+RW are effective field attractants and (2) *D. suzukii* has unique olfactory preferences compared to non-target drosophilids, while (3) *Z. indianus*' preferences do not appear to vary from non-target drosophilids, and (4) the accuracy of relative abundance is impacted by the specificity of the attractants.

Keywords: drosophilids; invasive species; attractant effectiveness; field trapping; brown rice vinegar; apple cider vinegar; red wine

1. Introduction

International trade and travel has supported the dispersal of many insect species outside of their native range including *Drosophila suzukii* (Matsumura) and *Zaprionus indianus* Gupta. Native to Southeast Asia, *D. suzukii* is now commonly found throughout North America [1,2], South America [3], and Europe [4–6]. The first adult specimens in the United States were captured on Oahu and Kauai in 1983 [7], and records have since been published across the U.S. mainland [8]. Similarly, *Z. indianus*, native to the Afrotropics, is now reported throughout North America, South America, Europe, and Asia [9,10]. Like many dipterans, *D. suzukii* and *Z. indianus* play a vital ecological role in nutrient cycling through catalyzing decomposition processes. However, when introduced to a non-native range, temperate climates and the abundance of host plants paired with the lack of natural predators can contribute to unregulated population expansion [11]. Furthermore, these vinegar fly species possess high adaptability [12,13] and fecundity [14,15] that facilitate successful colonization. Not surprisingly, *D. suzukii* and *Z. indianus* are considered invasive agricultural pests for many soft-skinned fruits due to increased pest management costs, reduced yields and rejected product [16,17].

The pest potential of *D. suzukii* and *Z. indianus* can be attributed to their polyphagous nature [18], transmission of microorganisms to host plant tissues [19], and niche adaptations that enable larval development in ripening fruit [20]. As a polyphagous drosophilid, *D. suzukii* uses a wide range of host plants for feeding and breeding, including nectarines, peaches, plums, pluots, persimmons, figs, strawberry, blackberry, blueberry, raspberry, cherry, and grapes [18,21]. Unlike most vinegar flies that oviposit into overripe and decaying fruits, the evolution of a serrated ovipositor and novel behavioral adaptations enable *D. suzukii* to target undamaged ripening fruit [22]. Similarly, *Z. indianus* has been observed targeting ripening figs [23] and undamaged ripe strawberry [24] as oviposition substrates. Oviposition injury can damage fruit by inoculating the surface with microorganisms that promote decay [25,26] and through larval consumption of fruit pulp post-emergence [24,27]. Eventually, when farmers confirm *D. suzukii* or *Z. indianus* in the field, additional labor costs are warranted for surveying, pest management, and post-harvest handling [17,28].

Current field trapping strategies include the use of visual and olfactory attractants, neither of which exclusively attract *D. suzukii* [29] or *Z. indianus* [30]. Sticky cards can serve as visual attractants, particularly fluorescent red or yellow colors [31]. Vinegars and wines serve as olfactory attractants in the field [32,33] that take advantage of drosophilids' innate attraction to fermentation by-products [34–36]. Since there are no species-specific attractants for drosophilids available, farmers must learn to distinguish *D. suzukii* and *Z. indianus* from other insects that are captured in field survey traps. Unfortunately, this practice is not only laborious, but also results in the capture of non-target species, including beneficial parasitoids. Thus, there is crucial need for the development of species-specific attractants.

The concept of targeted attractants for drosophilid species is plausible as fermentation products are innately attractive to drosophilids [34–36], and drosophilids exhibit differences in olfactory preferences. These species-specific preferences to different yeasts [37] may be related to evolutionary adaptations for survival against competing drosophilids over finite resources [23,38], which may eventually result in speciation [39–41]. For *D. suzukii*, preferential attraction may be related to physiological adaptations that encourage oviposition in ripening fruit [38], while larval nutritional requirements may have changed over time to permit *Z. indianus* to oviposit in ripe figs [23]. This suggests that preferential attraction to different yeast communities may permit co-habitation within a given environment [37]. *Drosophila suzukii* is attracted not only to ripening fruit [42] and yeasts [37], but also to odors emitted from leaf tissue. After olfactory-mediated arrival to the plant, additional environmental cues may prompt subsequent behaviors such as feeding, breeding, or oviposition [42]. Furthermore, the odors emitted from leaf tissue and fruits will vary between plant species, enabling insect species to co-evolve with particular plants. Research has found that the ratio of protein to carbohydrates (P:C) in a substance affects adult fly behavior; for example, a low P:C ratio was associated with higher oviposition behaviors in *D. suzukii*, which is thought to be an evolutionary trait that enhances survivability in adult females [43]. These preferences, once fully characterized, can be used for targeted field surveillance, attract-and-sterilize, or attract-and-kill strategies.

The headspace from fermented solutions that are attractive to drosophilids, such as vinegars, wines, and yeast [30,44], can be analyzed to identify the volatile composition [45]. Individual volatiles that are suspected to be attractive can then be tested in laboratory choice assays or in the field to develop targeted bait. Some progress has already been made towards the development of synthetic drosophilid bait [46] for *D. suzukii* [47] and *Z. indianus* [30], but the identification of novel fermenting solutions that are attractive to particular drosophilids and the characterization of these species–attractant interactions can further advance the development of these species-specific attractants.

Since the volatile composition varies between vinegar products [48], examining the efficacy of novel vinegar products [30] and identifying species-specific preferences may lead to improvements in synthetic attractant development. Previous research has demonstrated that apple cider vinegar [49,50] and apple cider vinegar and red wine [51] are effective field attractants for *D. suzukii* and *Z. indianus*. Rice vinegar, with and without red wine, has also been characterized by several researchers [30,32,46]. Recently, Akasaka et al. [52] demonstrated that brown rice vinegar was more attractive to *D. suzukii* than apple cider vinegar in laboratory choice assays, substantiating the need for the characterization of attractant efficacy in field settings.

The primary objective of this experiment was to examine attractant efficacy and specificity by attractant type (apple cider vinegar, brown rice vinegar, red wine, apple cider vinegar blended with red wine, brown rice vinegar blended with red wine) and species (*Drosophila Suzukii* (Matsumura), *Zaprionus Indianus* Gupta, non-target drosophilids) in cherimoya (*Annona cherimola* Miller). The secondary objective was to examine whether attractant efficacy varied over time in the field capture of *D. suzukii*, *Z. indianus*, and non-target drosophilids. The tertiary objective was to characterize the relative abundance of two invasive drosophilids, *D. suzukii* and *Z. indianus*. This report provides the first published characterization of the relative abundance of *Z. indianus* in Maui, Hawaii, since the reported introduction in 2017 [53]. Furthermore, at the time of writing, this report provides the first published quantitative comparison of brown rice vinegar as an attractant solution in a field setting in the United States.

2. Materials and Methods

2.1. Trap Assembly

The traps used for the following three experiments were a novel modification of the traditional deli cup design (Figure 1). Assembled using a Mason jar (236 mL) and a red Solo cup (473 mL), the jar served to house the attractant-drowning solution, while the Solo cup provided the headspace that enabled passive diffusion of the attractant and fly entry into the trap. To fabricate the trap, the interior of the Solo cup was scoured with sandpaper, rinsed with 70% ethanol, and then coated with epoxy. The silver band from the Mason jar lid was secured inside the Solo cup, as deep as the diameter of the silver band permitted, and was then left to cure for 24 h. A soldering tool (Cold-Heat®) with a 5-mm diameter tip was used to gently puncture eight entry holes into the Solo cup—two on each of the four sides. Two of the holes were used in trap installation with nylon rope. The removable trap base permitted simple trap maintenance as the attractant solutions were aliquoted into clean jars weekly which then replaced the spent jars on-site. After the lids were affixed to the spent jars, they were transported off-site for specimen preservation in alcohol and quantification.

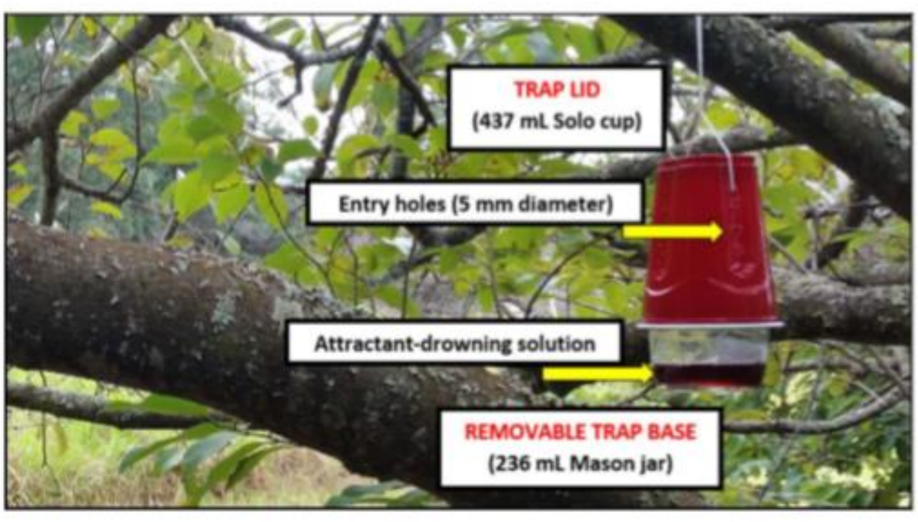

Figure 1. Assembled trap hanging in a cherimoya tree.

2.2. Attractant Solutions

Five attractants were used in this experiment: apple cider vinegar (ACV, supplied by Marukan Vinegar Co. Ltd., Kobe, Japan), brown rice vinegar (BRV, supplied by Marukan Vinegar Co. Ltd.), red wine (RW, Oak Leaf Vineyards, Merlot), ACV+RW (blended; 60/40%), and BRV+RW (blended; 60/40%). ACV, RW, and ACV+RW were chosen due to the historical use of vinegars and wine as olfactory baits in field traps [54]. BRV was selected due to recent evidence suggesting BRV is more attractive to *D. suzukii* than ACV in laboratory choice-assays [52]. BRV+RW was prepared to assess the dilution of BRV and synergy with red wine volatiles that had been previously observed with ACV+RW blends [30,32]. Attractants were aliquoted (40 mL per trap) into Mason jars on the morning of trap installation. A drop of unscented dishwashing liquid (Dawn® Ultra Free and Gentle, Procter & Gamble, Cincinnati, OH, USA) was added to each jar using a 3-mL transfer pipette (Karter Scientific, Lake Charles, LA, USA). The addition of a surfactant was necessary to break the surface tension, transforming the attractant into a drowning solution. After the surfactants were added, the Mason jar lids were affixed and ready for transport.

2.3. Trap Installation and Maintenance

The traps were installed at fruiting height and the attractant solution was replaced weekly. The trap height corresponded with low hanging fruit, as research in foraging ecology has shown higher *D. suzukii* capture rates in traps positioned on the lower canopy [55]. After trap installation, the trap bases were replaced weekly to collect captured specimens and to refresh the attractant solution. Once the specimens were collected, they were separated from the attractant solution using a 1-mm mesh filter and were stored in 70% isopropyl alcohol prior to identification.

2.4. Species Identification

The specimens were classified as *D. suzukii* (male or female), *Z. indianus* (male or female), non-target drosophilid, or other non-target captures. Standard keys were used in the examination of external morphology for *D. suzukii* [56] and *Z. indianus* [57,58]. Taxonomic confirmation for *D. suzukii* was confirmed by Dr. Douglas Pfeiffer while *Z. indianus* confirmation was provided by Dr. Luc Leblanc, University of Idaho, and Dr. Amir Yassin, Muséum d'Histoire Naturelle de Paris. The specimens were classified as non-target drosophilids if the individuals possessed all of the following attributes: (1) a body length of 3–4 mm, (2) a golden-yellow-brownish body color, (3) red eyes, (4) antennae with branched arista, and (5) wings that have three breaks in the costa vein [59]. The specimens that did not meet the criteria for *D. suzukii*, *Z. indianus*, or non-target drosophilid, were classified as non-target other captures. Note that this included the captures of any true fruit fly from Tephritidae, such as *Ceratitis capitata* (Wiedemann), the Mediterranean fruit fly, or *Bactrocera cucurbitae* (Coquillett), the melon fly.

2.5. Experimental Design

The survey location that was selected was a cherimoya grove at the University of Hawaii, at Mānoa: Kula Research Station (20.7° N; −156.3° W, 980 m) (Figure 2). There were 24 traps installed, which included four replicates of the six attractant treatments (five attractants and one control). Distilled water was used to control for a confounding variable—drosophilid attraction to visual stimuli [31], i.e., the red Solo cup. Once the traps were delineated and installed, attractant treatment was randomized. Each week, the captured specimens were collected, trap bases were replaced, and attractant treatment was re-randomized. Thus, each week corresponded to one run or replicate of the experiment. For each run, the four repeats of each attractant level were treated as individual data points and were not pooled or averaged for analysis, as the experimental unit was defined as an individual trap.

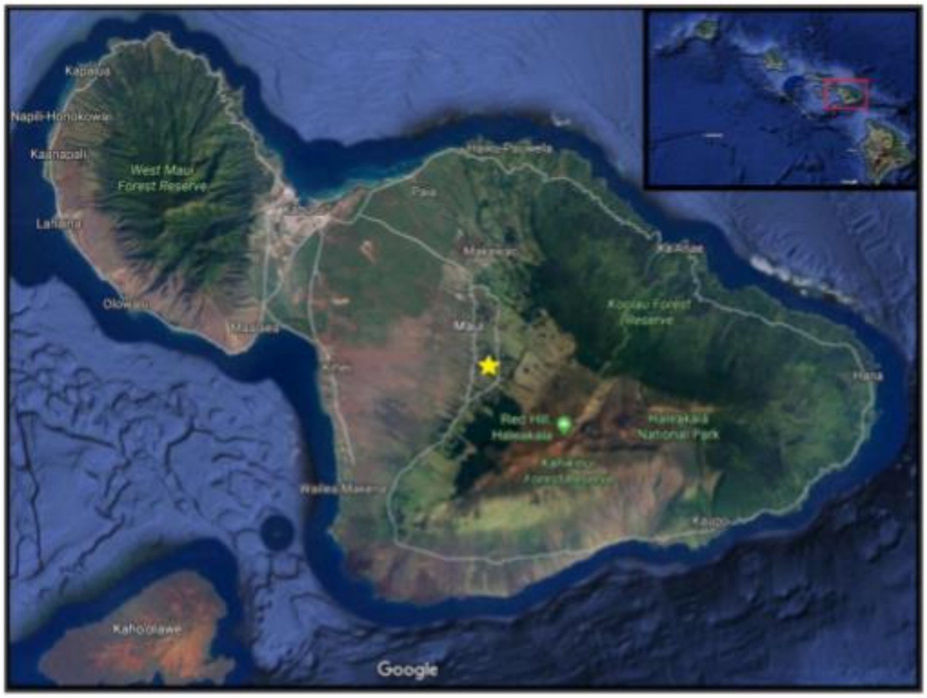

Figure 2. Satellite view of Maui with survey locality designated with yellow star.

The cherimoya grove at the University of Hawaii, at Mānoa: Kula Research Station, contained fruit that was ripening or ripe. The grove floor was shady with small amounts of plant debris in the early stages of decomposition and weeds that were routinely treated with herbicides. The perimeter of the grove contained approximately 2.5 m of well-managed turfgrass. On 13 November 2017, 24 traps were installed and were maintained weekly until 11 December 2017 (N = 16). Four rows of trees were selected, and six traps were installed within each row. The traps were placed in a single tree with at least 5 m between each trap.

2.6. Statistical Analysis

Statistical analysis was conducted with SAS® (SAS Institute Inc., Cary, NC, USA), with the statistical model as a generalized linear mixed model (GLMM) (PROC GLIMMIX procedure). A GLMM was selected as the data analyzed were non-normal and not transformed, and the model was subjected to both fixed and random effects. The significance threshold for mean separation was defined as $p < 0.05$ for post-hoc analysis. Tukey's Honestly Significant Difference (HSD) test was used for mean separation (within the LSMEANS statement). Several resources were utilized in the modeling of count data with a Poisson distribution [60–62]. In order to determine whether there were significant differences in the mean captures between the sexes, the total number of *D. suzukii* males was divided by the total number of male and female *D. suzukii* captures.

The sex specificity value results in a single response variable with a value between 0 and 1 for each datapoint (row of the spreadsheet). A value of 1.0 implies that only males were captured, while a value of 0.5 implies that there was equal male to female captures, and a value of 0.0 indicates that only females were captured.

To examine whether there were sex differences in mean capture between attractants, a generalized linear mixed model (GLMM) was used (PROC GLIMMIX) with the attractant as a fixed effect and with the week and replicate as random effects. The method was set as Laplace, and a beta distribution was specified as the sex specificity calculation was expressed as a decimal fraction. If there were no significant differences in sex specificity between either male and female *D. suzukii* or between male and female *Z. indianus*, further analysis was performed with the sex counts pooled.

To examine differences in mean capture between attractants, a GLMM was used (PROC GLIMMIX), with the attractant as a fixed effect and with the week and replicate as random effects. The method was set as Laplace, and a Poisson distribution was specified for the raw count measurements for each specimen category (*D. suzukii* female, *D. suzukii* male, *Z. indianus* female, *Z. indianus* male, non-target drosophilid, and other non-targets).

To examine the specificity between *D. suzukii* vs. drosophilid captures and *Z. indianus* vs. drosophilid captures, the raw data (capture counts) were manipulated to result in a single response variable (specificity ratios) on a per trap basis. For each trap, the attractant specificity for *D. suzukii* was calculated by dividing *D. suzukii* captures by drosophilid captures. Similarly, to calculate the attractant specificity for *Z. indianus*, *Z. indianus* captures were divided by drosophilid captures, resulting in a specificity value for each trap. The species specificity calculation results in a value between 0 and 1 for each datapoint (row of the spreadsheet) with the interpretation of values similar to as previously described for sex specificity. Using this calculation, mean specificity by attractant type could be evaluated.

To examine whether there were differences in the specificity of captures between attractants, a generalized linear mixed model (GLMM) was used (PROC GLIMMIX) with attractant as a fixed effect and with week and replicate as random effects. The method was set as Laplace, and a beta distribution was specified as the specificity results were expressed as decimal fractions. To support the secondary objective, a GLMM was used (PROC GLIMMIX) to examine whether the mean captures or specificity varied by week (fixed effects are attractant, week, and attractant*week; random effect is replicate). Similar to the previous analysis, the method was set as Laplace, and the distribution was set as Poisson and beta for count data and decimal fractions, respectively. To support the tertiary objective, relative abundance ratios were generated by dividing the total quantity of target specimens captured (*D. suzukii* or *Z. indianus*) by the total quantity of vinegar flies captured (*D. suzukii*, *Z. indianus*, and non-target drosophilid). Though similar to the calculation of specificity, the estimation of relative abundance produces a single value which includes all attractant solutions, while specificity is calculated on the level of each subject resulting in 80 values for statistical analysis.

3. Results

3.1. *Drosophila suzukii*: Mean Captures and Attractant Specificity

Since there were no significant differences between male and female *D. suzukii* captures by attractant ($f = 0.73$, $df = 4, 68$, $p = 0.5761$), the captures were pooled for both sexes for analysis. There were significant differences in *D. suzukii* captures by attractant type ($f = 5.86$, $df = 4, 75$, $p < 0.001$) (Figure 3). Mean separation using Tukey's HSD test indicated that BRV+RW (29.0 ± 4.4) resulted in more captures than BRV (16.5 ± 3.0), RW (12.8 ± 1.6), and ACV (9.3 ± 2.3). There were no significant differences between BRV+RW (29.0 ± 4.4) and ACV+RW (17.6 ± 3.0). There were no significant differences between ACV+RW (17.6 ± 3.0), BRV (16.5 ± 3.0), and RW (12.8 ± 1.6). The use of ACV+RW (17.6 ± 3.0) or BRV (16.5 ± 3.0) resulted in higher captures than ACV alone (9.3 ± 2.3).

There were significant differences observed in the mean captures of *D. suzukii* by week surveyed ($f = 25.93$, $df = 3, 60$, $p < 0.0001$) (Figure 4). The greatest mean captures of *D. suzukii* occurred in weeks 2 (24.5 ± 2.8) and 3 (22.0 ± 3.4), followed by week 1 (14.3 ± 2.7), with the lowest captures observed in week 4 (7.5 ± 1.1).

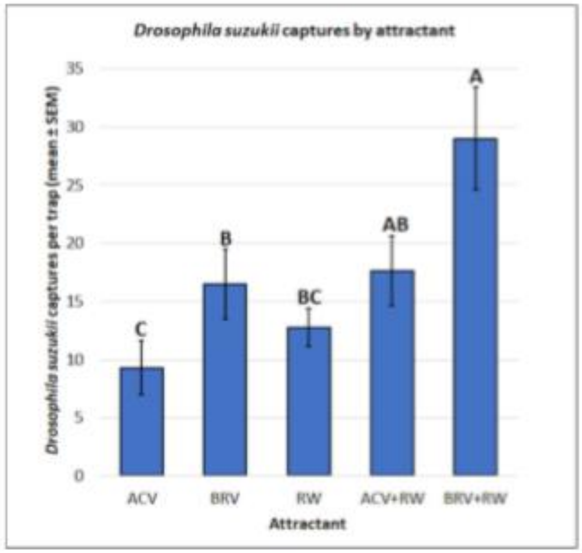

Figure 3. Mean (± SEM) *Drosophila suzukii* captures by attractant type. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

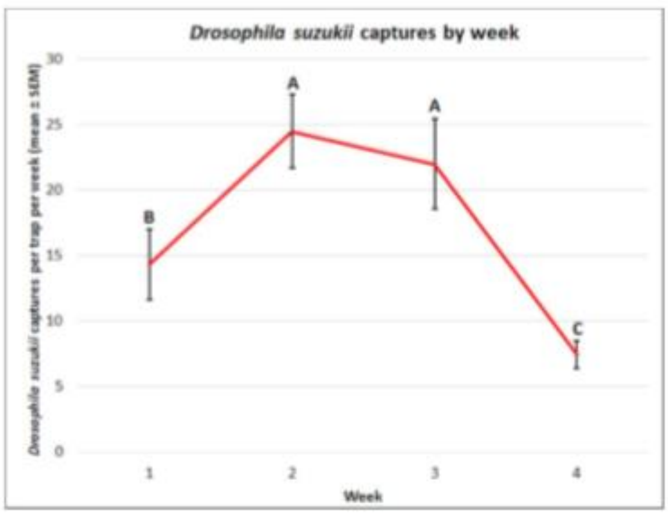

Figure 4. Mean (± SEM) *Drosophila suzukii* captures by week. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

Since the interaction between week and attractant was significant (f = 3.43, df = 12, 60, $p < 0.001$), attractant preferences were examined separately for each week. Significant differences were observed in the mean captures by attractant in week 1 (f = 6.13, df = 4, 15, $p < 0.01$), week 2 (f = 6.73, df = 4, 15, $p < 0.01$), week 3 (f = 4.64, df = 4, 15, $p < 0.05$), and week 4 (f = 5.76, df = 4, 15, $p < 0.01$) (Figure 5). For week 1, the use of BRV+RW (33.5 ± 4.3) resulted in more captures than BRV (11.5 ± 5.7), RW (10.0 ± 3.0), ACV+RW (9.5 ± 2.9), and ACV (6.75 ± 1.8). For week 2, the use of BRV+RW (32.0 ± 4.5), ACV+RW (31.8 ± 7.3), BRV (30.0 ± 5.6) or RW (19.3 ± 2.5), resulted in more captures than ACV (9.5 ± 3.5). Week 3 was similar to week 1—the use of BRV+RW (43.8 ± 10.1) resulted in more captures than ACV (19.3 ± 5.7), ACV+RW (16.8 ± 4.5), BRV (16.5 ± 3.2), and RW (13.5 ± 2.5). In contrast, for week 4, ACV+RW (12.5 ± 1.3) resulted in more captures than BRV+RW (6.75 ± 1.6) and ACV (1.75 ± 0.5). The use of RW (9.25 ± 1.9), BRV (8.0 ± 2.7), or BRV+RW (6.75 ± 1.6) resulted in more captures than ACV (1.75 ± 0.5). There were no significant differences between ACV+RW (12.5 ± 1.3), RW (9.25 ± 1.9), and BRV (8.0 ± 2.7). There were no significant differences between RW (9.25 ± 1.9), BRV (8.0 ± 2.7), and BRV+RW (6.75 ± 1.6).

There were significant differences observed in the specificity of *D. suzukii* captures per trap by attractant ($f = 4.39$, $df = 4, 74$, $p > 0.01$) (Figure 6). The use of BRV alone (0.47 ± 0.05) resulted in higher specificity than ACV (0.38 ± 0.06), ACV+RW (0.32 ± 0.04), and RW (0.26 ± 0.03). There was no significant difference in specificity for *D. suzukii* between BRV (0.47 ± 0.05) and BRV+RW (0.41 ± 0.04). There were no significant differences in specificity for *D. suzukii* between BRV+RW (0.41 ± 0.04) and ACV+RW (0.32 ± 0.04).

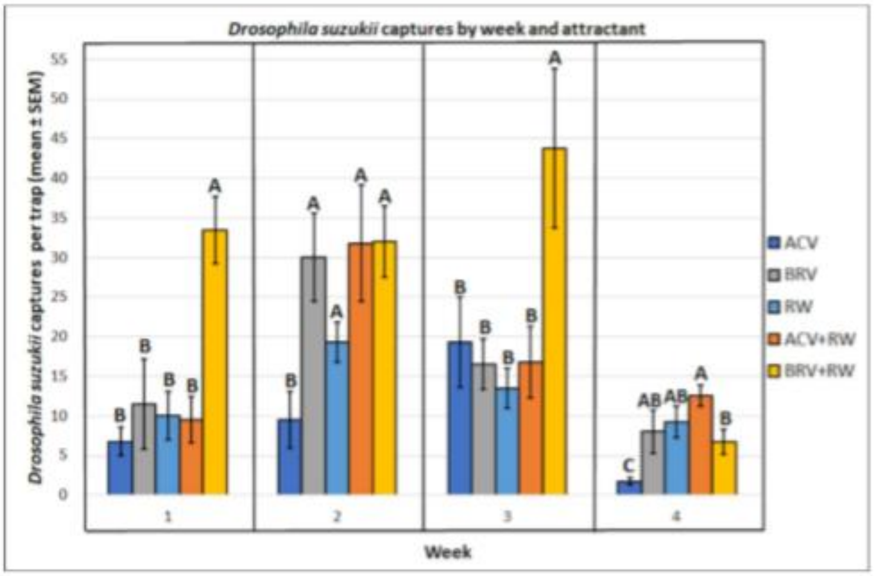

Figure 5. Mean (± SEM) *Drosophila suzukii* captures per trap by week and attractant. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

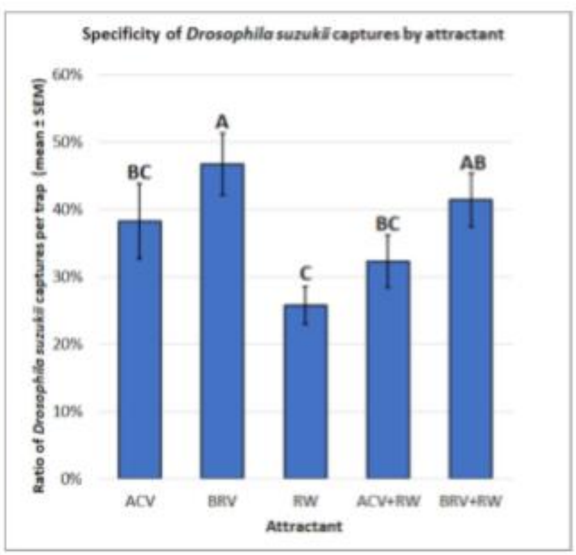

Figure 6. Specificity of *Drosophila suzukii* captures by attractant. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

3.2. *Zaprionus indianus*: Mean Captures and Attractant Specificity

Since there were no significant differences between male and female *Zaprionus indianus* captures by attractant (f = 1.43, df = 4, 14, $p = 0.2762$), the captures were pooled for both sexes for analysis. No significant differences were observed for *Zaprionus indianus* captures by attractant type (f = 1.41, df = 4, 75, $p = 0.2376$) (Figure 7).

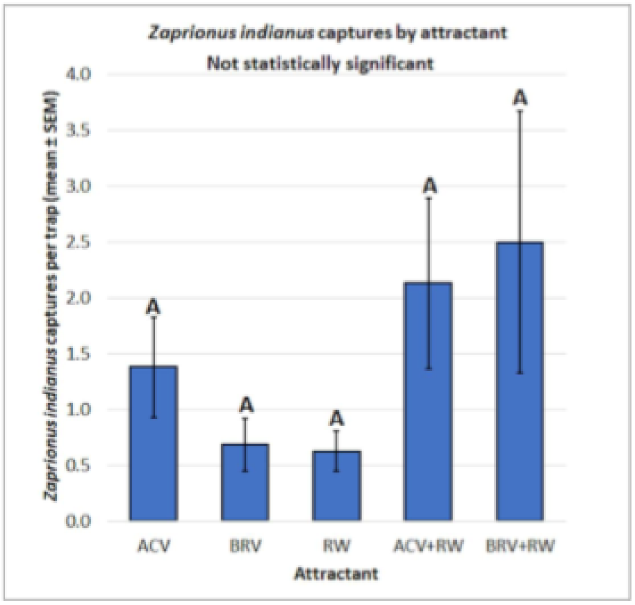

Figure 7. Mean (± SEM) *Zaprionus indianus* captures by attractant type. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

There were no significant differences observed in the mean captures of *Z. indianus* by week (f = 2.74, df = 3, 60, $p = 0.0511$); the interaction between attractant and week was also non-significant (f = 1.81, df = 12, 60, $p = 0.0671$) (Figure 8).

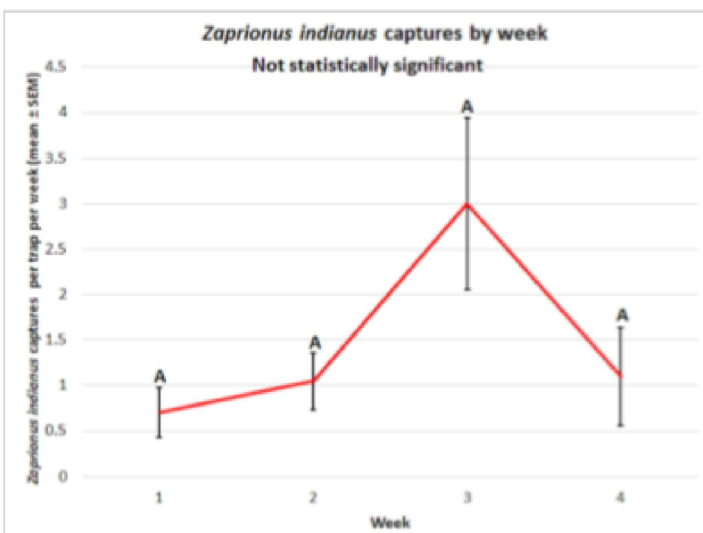

Figure 8. Mean (± SEM) *Zaprionus indianus* captures by week. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

There were no significant differences observed in the specificity of *Z. indianus* captures per trap by attractant (f = 1.82, df = 4, 37, $p = 0.1450$) (Figure 9).

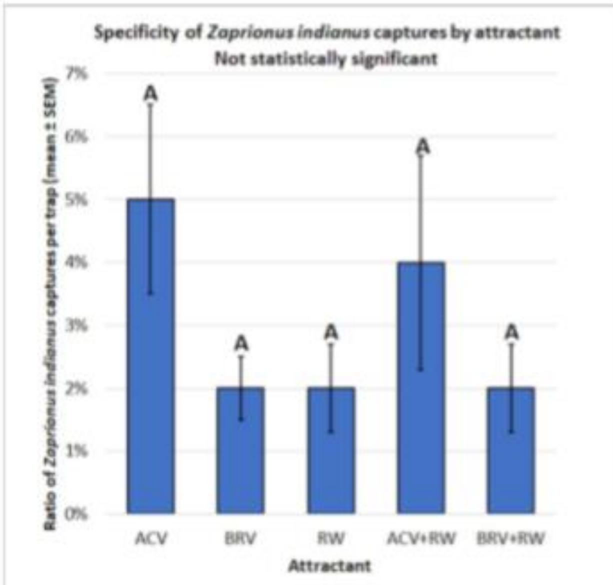

Figure 9. Specificity of *Zaprionus indianus* captures by attractant. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

3.3. Non-Target Drosophilid: Mean Captures and Attractant Specificity

There were significant differences in the mean captures of non-target drosophilids by attractant (f = 8.63, df = 4, 75, $p < 0.0001$) (Figure 10). The use of RW (43.8 ± 6.7), BRV+RW (41.7 ± 7.5), and ACV+RW (39.1 ± 5.8) resulted in more captures than BRV (18.4 ± 3.6) and ACV (13.1 ± 2.0) alone.

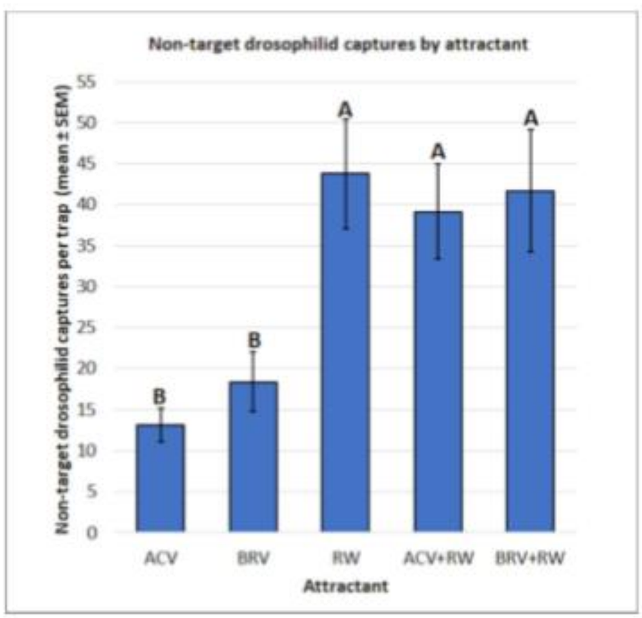

Figure 10. Mean (± SEM) non-target drosophilid captures by attractant. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

There were significant differences observed in the mean captures by week surveyed (f = 37.25, df = 3, 60, $p < 0.0001$) (Figure 11). The greatest mean captures of non-target drosophilids occurred in week 3 (52.8 ± 6.8), followed by week 2 (36.4 ± 4.4), week 1 (23.0 ± 4.6), and the lowest were observed in week 4 (12.7 ± 1.7). The interaction between week and attractant was not significant (f = 1.44, df = 12, 60, $p = 0.1748$).

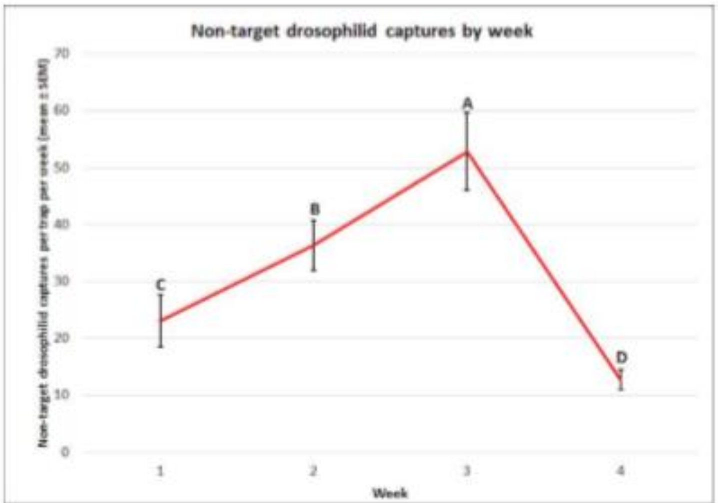

Figure 11. Mean (± SEM) non-target drosophilid captures by week. Different letters represent statistically significant differences ($p < 0.05$, Tukey's HSD test) between attractant solutions.

3.4. Estimated Relative Abundance of D. suzukii, Z. indianus, and Non-Target Drosophilids in Cherimoya

To characterize the relative abundance of exotic drosophilids, total captures by species and attractant type were reported in a contingency table (Table 1), then the relative abundance of *D. suzukii*, *Z. indianus*, and non-target drosophilids was calculated (Figure 12). All experimental data, including raw counts and calculated specificity ratios, can be found in the supplementary workbook (S1: Data).

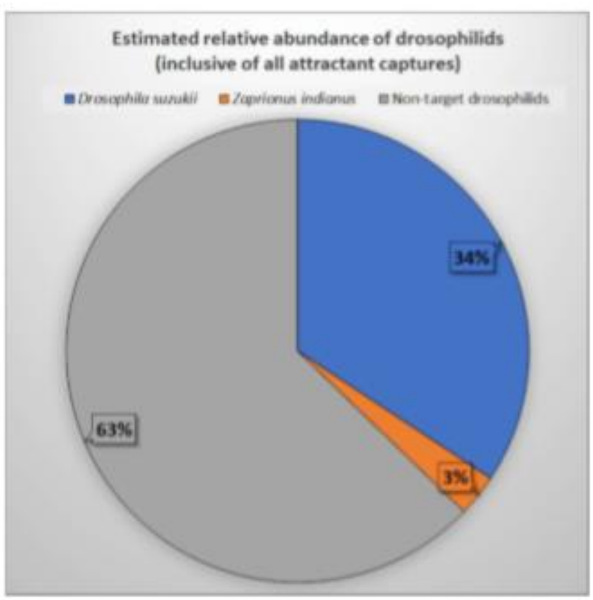

Figure 12. Estimated relative abundance of drosophilids.

Table 1. Contingency table listing all field captures by attractant type and specimen category.

Attractant Type	*Drosophila suzukii* Male	*Drosophila suzukii* Female	*Zaprionus indianus* Male	*Zaprionus indianus* Female	Non-Target Drosophilids	Non-Target Other	Total Captures
Apple cider vinegar	99	50	14	8	210	11	392
Brown rice vinegar	173	91	8	3	294	21	590
Red wine	123	81	5	5	700	76	990
Apple cider vinegar + red wine	190	92	12	22	626	59	1001
Brown rice vinegar + red wine	300	164	21	19	667	51	1222
Distilled water	1	2	0	0	5	9	17
Total	886	480	60	57	2502	227	4212

4. Discussion

The results of this experiment for the field capture of drosophilids in cherimoya in Maui suggest that (1) BRV and BRV+RW are effective attractants for *D. suzukii* and *Z. indianus*, (2) *D. suzukii* has unique olfactory preferences compared to all non-target drosophilids, while (3) there are no differences in olfactory preferences between *Z. indianus* and non-target drosophilids, (4) attractant preferences vary over time for *D. suzukii* but not for *Z. indianus*, and (5) the accuracy of relative abundance is impacted by the specificity of the attractants used. At the time of writing, this is the first reported use of BRV and BRV+RW as olfactory attractants in a quantitative field trapping study in the United States. Furthermore, this report provides the first characterization of the relative abundance of *Z. indianus* since its reported introduction in Maui [53]. Since there were relatively few captures in the control treatment (distilled water), which controlled for drosophilid attraction towards visual stimuli (to the red solo cup) [31], it can be concluded that the captures in this experiment were due to olfactory attraction or the combination of olfactory and visual stimuli—not to visual stimuli alone. The limitations of this experiment include limited duration (4 weeks) and relatively low repeats ($N = 16$), suggesting that further research is needed for greater confidence in these trends. Nonetheless, this experiment provides insight into attractant efficacy by species and emphasizes the need to assess the synergy between attractant solutions and other compounds within the host plant environment, such as the outgassing of volatiles emitted from plants and fruit, which has been a topic of recent investigation [63,64]. Furthermore, since the experiment was limited to one host plant on one island, additional experiments in other host plants and localities are needed to have confidence in extrapolating these trends across multiple environments.

Five attractants were used (ACV, BRV, RW, ACV+RW, and BRV+RW) to evaluate attractant effectiveness for *D. suzukii* and *Z. indianus* in cherimoya. There were significant differences in the mean captures of *D. suzukii* by attractant type, but no significant differences were observed for *Z. indianus*. The use of BRV+RW resulted in greater *D. suzukii* captures than BRV, RW, and ACV. There were no significant differences between BRV+RW and ACV+RW, suggesting that BRV+RW may be equivalent to ACV+RW as an olfactory attractant for *D. suzukii*. Similar to the results of previous experiments, the use of ACV+RW resulted in greater *D. suzukii* captures than ACV alone [32,46]. Researchers conducting field experiments in berry crops found that wine-vinegar blends were more attractive to *D. suzukii* than wine and vinegar alone [46] or aqueous red wine [32]. Cha et al. [46], reported that blended RW and rice vinegar (60/40%) was more attractive to *D. suzukii* than rice vinegar or red wine alone, while Landolt et al. [32] found that RW blended with rice vinegar (60/40%) was more attractive to *D. suzukii* than RW with ACV (60/40%) or RW with white wine vinegar (60/40%) or aqueous RW (60%). In contrast to the previous experiments described, there was no significant difference between ACV+RW and RW, which could be attributed to other factors present between the different host plant environments examined—cherimoya in this experiment vs. blackberry [32] and berry crops [46]. For *Z. indianus*, no significant differences were observed in the mean captures for any attractant used, which suggests that, in practice, any of the attractants assessed could be used to identify whether *Z. indianus* adults are present in a field. For non-target drosophilids, greater captures were observed with RW, ACV+RW, and BRV+RW than with ACV or BRV alone. This suggests that for non-target drosophilids and *Z. indianus*, wine volatiles alone performed similarly to the synergy of wine and vinegar volatiles, while the latter was a stronger preference for *D. suzukii*.

There were significant differences in the mean captures by week for *D. suzukii* and non-target drosophilids, but not for *Z. indianus*. After analysis of *D. suzukii* attractant preferences by week, there appeared to be slight differences in the distribution of the captures. For non-target drosophilids, even though the captures varied by week, attractant preferences were similar throughout the duration of the experiment. Inclusive of all attractant types examined, 65% of total *D. suzukii* captures were male and 35% were female. Since there were no significant differences in the proportion of male to female captures by attractant type, this suggests that the trend was consistent regardless of which attractant was used with 60–67% male captures to 33–40% female captures. Though it is not possible to know the actual sex ratio for the population and since the visitor rate was not assessed (e.g., pesticide treatment of traps coupled with a bucket), it is not possible to determine whether there were more males present in the population or simply more male captures.

In order to develop species-specific attractants, the characterization of attractant specificity by species is needed. BRV had greater specificity in attracting *D. suzukii* compared to ACV, ACV+RW, or RW. On average, ~50% of the drosophilid captures in the BRV traps were *D. suzukii*, which was reduced to ~40% with BRV+RW and ACV, ~30% with ACV+RW, and ~25% with RW. The results of this field experiment support previous research where, in a laboratory trapping assay, BRV was found to be significantly more attractive to *D. suzukii* than ACV [52]. Though there were no significant differences in specificity observed between BRV and BRV+RW, there were also no differences between BRV+RW and ACV+RW, suggesting that the volatile compound present in BRV, once diluted with red wine, becomes less specific to attracting *D. suzukii*. Since the results suggest that BRV has greater *D. suzukii* specificity, it follows that using BRV as a field attractant for surveying could reduce non-target impacts and the time necessary to identify specimens. However, further research in additional host plants and localities is needed to better understand this trend and identify any potential ecological and economic impacts. The specificity reported in this experiment was calculated from observed captures and did not take into account the true population, as this was an open-field experiment. Future research could examine attractant specificity between *D. suzukii* and *Z. indianus* in a controlled environment, which may better isolate the variables in question and provide valuable data towards the development of a species-specific attractant. The preferential attraction observed in this experiment is likely associated with evolutionary adaptations that enable co-habitation of *D. suzukii* and *Z. indianus* with other drosophilids over finite resources in a given environment [23,41,42]. These unique olfactory preferences have implications for both farmers and researchers. In order to mitigate revenue loss, farmers must detect the presence of pests as early in the season as possible to start implementing pest reduction strategies. These findings suggest that for field surveying drosophilid pests, multiple attractants should be used to cover a range of attractive compounds.

The results of this experiment beg the question—if specificity varies depending on attractant, how can an accurate representation of relative abundance be depicted from the field captures of wild flies? In this experiment, the relative abundance of *D. suzukii* and *Z. indianus* ranged from 22.3–46.4% and 1.1–5.8%, respectively, depending on the attractant used. The low relative abundance of *Z. indianus* observed could be the result of low attractant efficacy in the host plant environment, or that cherimoya was not a preferred host—both of which may have underestimated the actual population. Alternatively, the low relative abundance could correspond with the recent introduction of this species to the region [53] coupled with interspecies competition for resources that limited population expansion. In central Brazil, Tidon et al. [65] reported that *Z. indianus* had an initially low relative abundance followed by a population explosion the following year when the populations were re-surveyed. Since the actual relative abundance cannot be known in wild populations, and there is attractant bias, the interpretation of field data on relative abundance is challenging and may overestimate or underestimate the actual population. This experiment emphasizes the challenges and limitations associated with the interpretation of relative abundance calculations from field-generated data.

Previous research has demonstrated the potential for co-infestation of *D. suzukii* and *Z. indianus* in a variety of crops including strawberry [24], guava [2] and grape [66]. As a secondary pest with opportunistic oviposition tendencies, *Z. indianus* can increase damage to soft-skinned fruits with oviposition injury from *D. suzukii* [66] or mechanical damage from other insects [24]. In Veracruz, Mexico, *D. suzukii* and *Z. indianus* co-infested guava orchards comprising more than 80% of the total drosophilids captured (55.0 and 26.3% relative abundance, respectively) [2], which suggests that there may be deleterious interspecies competition between endemic and invasive drosophilids. There is also evidence of interspecies competition between *D. suzukii* and *Z. indianus*. For example, larval competition between *Z. indianus* and *D. suzukii* increased development time and mortality for *D. suzukii* in Virginia [66]. Even though co-infestation was observed in the cherimoya grove, no attempt was made to examine interspecies competition or to quantify the losses from direct and indirect injury. Furthermore, research is needed regarding *D. suzukii* and *Z. indianus* co-infestation to identify economic thresholds and provide practical pest management strategies for farmers.

Several limitations were identified in this experiment including the short duration of the study. The short-duration of the experiment (4 weeks) limited the characterization of olfactory preferences by fruiting stage over time. Over the four-week experiment, there were few total captures for traps treated with distilled water—three *D. suzukii* specimens were captured (one male, two female), zero *Z. indianus* specimens were captured, five non-target drosophilids were captured, and nine non-target other specimens were captured. Due to the low quantity of the control captures, there is confidence that the dataset analyzed contained values that were the result of olfactory attraction and not confounded by visual stimuli. Additional research is necessary to assess whether an attractant is more or less attractive in different visual environments. Further characterization of attractant specificity by species is needed as fruit ripeness, population dispersal, and interspecies competition alter drosophilid species composition throughout the growing season. Future research could assess attractant efficacy by host plant fruiting stage by collecting fruit samples weekly and identifying the outgassing fruit volatiles. If novel compounds or the synergistic effects of multiple compounds were identified, this would aid in the development of a species-specific attractant. In the meantime, this may also provide farmers with the most effective attractant to use while surveying depending on host plant fruiting stage.

5. Conclusions

The results of this study support the notion that *D. suzukii* displays unique olfactory preferences compared to non-target drosophilids, whereas no attractant preferences were observed between *Z. indianus* and non-target drosophilids. The results suggest that BRV, or BRV+RW, is an effective alternative to ACV or ACV+RW for the field survey of drosophilid pests. Furthermore, BRV appears to have high specificity for *D. suzukii* as compared to non-target drosophilids, which warrants further research towards the development of a species-specific attractant. This study provides an initial characterization of the relative abundance of *Z. indianus* (inclusive of all attractants, 2.9%) in a cherimoya grove in Kula, Maui, Hawaii, since the reported introduction in 2017. However, the estimation of relative abundance varied by attractant type (between 1.1–5.8%) and may have been underestimated as none of the attractants used in the experiment had high specificity for *Z. indianus* field captures. Alternatively, the low relative abundance observed could be indicative of a low population level. The actual drosophilid distribution can only be estimated, as the true population is unknown.

Supplementary Materials: The following are available online at http://www.mdpi.com/2075-4450/10/3/80/s1, Workbook S1: Data.

Author Contributions: Conceptualization, B.N.W.; methodology, B.N.W. and D.G.P.; formal analysis, B.N.W.; investigation, B.N.W.; resources, B.N.W.; data curation, B.N.W.; writing—original draft preparation, B.N.W.; writing—review and editing, D.G.P.; visualization, B.N.W.; supervision, D.G.P.

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Conflicts of Interest: The authors declare no conflict of interest.

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CHAPTER 3. Attractant efficacy and boric acid supplementation over time for the field capture of *Drosophila suzukii* (Matsumura) and *Zaprionus indianus* Gupta (Diptera: Drosophilidae) in cherimoya

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Abstract

Drosophila suzukii (Matsumura) and *Zaprionus indianus* Gupta are agricultural pests of many soft-skinned fruits and berries, which are often surveyed with vinegars and wines. These attractants may continue to ferment in the field changing the composition of the chemicals outgassed, and thus attractant efficacy. Furthermore, drosophilids vector yeast and once exposed to the drowning solution, these microorganisms may be transmitted to the attractant. Boric acid is a preservative commonly used to suppress microbial activity in field traps. Little research has been done to assess the effects of fermentation or boric acid supplementation and any potential effects to attractant efficacy over time. To examine these effects, daily captures were recorded in traps installed in cherimoya for seven days. There were significant differences in mean captures by day for *D. suzukii* and non-target drosophilids. Regardless of attractant used, there were greater *D. suzukii* captures on day 1 than days 4 or 5. For non-target drosophilids, there were greater captures on days 1 and 3 than days 4, 5, or 6. Due to the low capture rate for *Z. indianus* (many zero captures), the model was unable to analyze field captures for this species. Boric acid (1% w/v) was associated with a 44% increase in *D. suzukii* captures while no significant differences were observed for non-target drosophilid captures, which suggests that inhibiting active fermentation may increase the capture rate of *D. suzukii*. For *D. suzukii*, BRV+RW resulted in higher mean captures of than ACV, ACV+RW, and RW with no significant differences between BRV+RW and BRV. While for non-target drosophilids, BRV+RW resulted in higher captures than ACV with no significant differences observed between BRV+RW and ACV+RW, RW, or BRV or between ACV and ACV+RW, BRV, or RW. Because this experiment did not directly assess the effectiveness of 1% boric acid in inhibiting microbial activity or the suitability of each attractant as a substrate for microbial growth, further research is needed for confidence in these results.

Keywords: *invasive drosophilids*, Hawaii, attractant preservatives, apple cider vinegar, brown rice vinegar

Introduction

Drosophilids vector microorganisms including yeast (Becher et al., 2012; Stamps et al., 2012) and bacteria (Ioriatti et al., 2018), transmitting microorganisms to host-plant tissues through contact, feeding, oviposition, and defecation (Bakula, 1969; Basset et al 2003; Hamby et al., 2012). The inoculation not only enriches larval development (Stamps et al., 2012) and enhances nutrient recycling, but also may affect the efficacy of attractants used in field surveying. Active fermentation of the attractant (Epsky and Gill, 2017) and inoculation of microorganisms from insects captured in the drowning solution may change the efficacy of the attractant over time. To prevent changes to the attractant over time and delay decomposition of captured specimens, researchers have added preservatives, such as boric acid.

Drosophilids vector yeast (Becher et al., 2012) and subsequently modify yeast density and colony diversity on oviposition substrates (Stamps et al., 2012). Higher yeast abundance was observed in cherry and raspberry fruits that had been infested by *D. suzukii* than non-infested fruits collected from the field (Hamby et al., 2012), which suggests that either yeasts were transmitted from the insect to the fruit or that oviposition sites were selected based on the presence of established yeast colonies. In a laboratory experiment, Becher et al. (2012) observed that a yeast-free strain of *D. melanogaster*, after being fed a diet containing yeast for one day, was able to vector yeast to sterile grapes. Similarly, Stamps et al. (2012) demonstrated that bananas exposed to *D. melanogaster* adults or larvae had higher yeast densities and less diversity than unexposed fruit, validating the theory that drosophilids not only vector yeast, but also modify the environment. Therefore, it is plausible that, when drosophilids are exposed to the drowning solution present in field traps, microorganisms could be transmitted to the attractant. Furthermore, this exposure could change the volatile composition of the attractant solution over time, due to secondary fermentation, thus altering attractant efficacy.

Many attractants continue to ferment in the field becoming more or less effective over time, and these changes in attractiveness vary by drosophilid species (Epsky et al., 2015). As aqueous grape juice aged, lower field captures were observed for *Anastrepha suspensa* (Loew) and higher captures for *Z. indianus* (Epsky et al., 2015), suggesting that the continued fermentation of the attractant may have altered attractant volatilization and thus behavioral responses. Research has demonstrated that *D. suzukii* and *D. melanogaster* exhibit preferential attraction to particular yeast strains (Scheidler et al., 2015). Understanding how attractant exposure to yeast affects field captures, particularly as the attractant ages over time, provides vital information not only regarding bait longevity (Epsky and Gill, 2017) but also for the development of a species-specific attractant.

Any temporal changes to the attractant solution may adversely affect the interpretation of field surveys. Because drosophilids are gregarious creatures (Durisko et al., 2014; Battesti et al., 2015) that vector yeast (Becher et al., 2012) and have a high sensitivity to low concentrations of fermentation compounds that elicit attraction (Stensmyr et al., 2003), it would not be surprising if attractant exposure to insect microbiome would affect attractant efficacy. Higher densities of captured drosophilids in the attractant solution may potentially increase the rate of change. If microbiome exposure alters attractant efficacy and the effects are observed within days of inoculation, then the interpretation of captures by attractant type could be affected. If the capture rate increased, the data could mislead researchers into thinking the attractant volatiles were responsible for differences in capture rates when, in fact, introduction of yeast to the attractant was influencing attractiveness. Therefore, it is necessary to distinguish the effect of volatile composition and microbiome inoculant on the attractiveness of lures.

A preservative can suppress proliferation of microorganisms and therefore minimize any potential changes to attractant volatile composition. The addition of borax (1%, w/v) to an attractant solution eliminated specimen decomposition and discoloration of the attractant (Lopez and Becerril, 1967), suggesting that microbial activity was suppressed. However, there could be species-specific responses to this decrease in microbial activity. The addition of borax (1%, w/v) to laboratory-aged grape juice increased field captures of *A. suspensa* while decreasing *Z. indianus* captures, suggesting that the preservative reduced active fermentation, and caused species-specific effects on attractant efficacy (Epsky et al., 2015). Boric acid (1%, w/v) is another preservative commonly added to attractant solutions (Landolt et al., 2012; Cha et al., 2014), but the effects of this addition to attractant efficacy on field captures is not well understood.

Standard attractants for drosophilid field surveying include red wine and apple cider vinegar, alone or in combination (Lee et al., 2013; Wang et al., 2016; Kremmer et al., 2017). It has also been demonstrated that brown rice vinegar was effective in a laboratory choice-assay in

D. suzukii captures (Akasaka et al., 2017). Because gas chromatography-mass spectrometry has demonstrated that different vinegars contain different compounds (Chinnici et al., 2009), there is reason to suspect that the efficacy of the attractant over time may vary between apple cider vinegar and brown rice vinegar. The objective of this experiment is to examine the effects of attractant type and boric acid treatment on captures over time (repeated measures, days 1-7) for *D. suzukii*, *Z. indianus*, and non-target drosophilids in cherimoya.

Materials and Methods

Survey locality and host-plant. The cherimoya grove at the University of Hawaii at Mānoa: Kula Research Station (20.7 ˚N; -156.3 ˚W, 980 m) was selected for this experiment. The cherimoya was ripe and some fruits were observed on the ground. The grove floor was shady with substantial plant debris. Weeds present within the grove were routinely treated with herbicides. The grove perimeter contained the approximately eight feet of well-managed turfgrass.

Attractants and boric acid. Five attractants were used in this experiment: apple cider vinegar (ACV, supplied by Marukan Vinegar Co. Ltd.), brown rice vinegar (BRV, supplied by Marukan Vinegar Co. Ltd.), red wine (RW, Oak Leaf Vineyards, Merlot), ACV+RW (mixed; 60/40%), and BRV+RW (mixed; 60/40%). In addition to the five attractants, distilled water was used to control for drosophilid attraction to the red solo cup. ACV, RW, and ACV+RW were chosen due to historical use of vinegars and wine as olfactory baits in field traps (Cha et al., 2015). BRV was selected due to recent evidence suggesting BRV as more attractive than ACV in laboratory choice-assays (Akasaka et al., 2017). BRV+RW was prepared to assess the dilution of BRV and synergy with red wine volatiles that had been previously observed with ACV+RW blends (Landolt et al., 2012; Epsky et al., 2014). Attractants were aliquoted (40 mL per trap) into Mason jars the morning of trap installation. Using a 3mL transfer pipette (Karter Scientific), a drop of unscented dishwashing liquid (Dawn® Ultra Free and Gentle) was added to each jar to break the surface tension of the attractant solution. To examine the effects of preservative treatment on mean captures, two levels of boric acid were examined. For the 1% w/v treatment group, 0.4 g of boric acid was added to each jar, while no boric acid was added for the 0% treatment group. Because there were six attractants and two boric acid levels there were a total of 12 treatment groups.

Trap maintenance and specimen collection. Twenty-four traps were installed in the cherimoya grove. Each trap was installed in a single tree at a 1 m height. There were four rows of six traps with at least 5 m between rows and 5 m between traps within rows. Specimens were collected daily for seven days and the attractant solution, exposed to captured insects, was reused for the duration of the run. During collection, the attractant solution was poured over a fine-mesh filter, forceps were used to transfer captured specimens to labeled vials containing hand sanitizer with 70% ethanol, and the trap base was re-installed to the trap. All of the tools used including the extra trap base, forceps, the fine mesh filter, were sanitized with 70% isopropyl alcohol before and after each use.

Specimen preservation and identification. Specimens were stored in hand sanitizer containing 70% ethanol prior to identification. Specimens were then classified as *D. suzukii* (male or female), *Z. indianus* (male or female), non-target drosophilid, or other non-target captures. Standard keys were used in the examination of external morphology for *D. suzukii* (Vlach, 2013) and *Z. indianus* (van der Linde, 2010; Yassin and David, 2010). Specimens were classified as drosophilids if individuals possessed all of the following attributes: 1) having a body length of 3-4 mm, 2) a golden-yellow-brownish body color, 3) red eyes, 4) antennae with branched arista, 5) and the presence of three breaks in the costa vein (Oregon State University, 2013). Specimens that did not meet the criteria for *D. suzukii*, *Z. indianus*, or non-target drosophilid, were classified as non-target other captures. For example, field captures belonging to family Tephritidae, such as the Mediterranean fruit fly or the melon fly, were categorized as non-target other captures.

Research design and statistical analysis. Statistical analysis was performed using SAS® Studio (SAS Institute Inc.). A generalized linear mixed model was used to assess the repeated effect of time (random effect; subject=trap) on the fixed effects of attractant (ACV, BRV, RW, ACV+RW, BRV+RW) and boric acid (1% w/v, 0% w/v) for the capture of *D. suzukii*, *Z. indianus,* and non-target drosophilids. Tukey's Honestly Significant Difference Test was used to evaluate significant differences in mean capture rate within factor levels with a significance threshold of $p<0.05$. The installation of 24 traps permitted two repeats (N=2) for each attractant level (ACV, BRV, RW, ACV+RW, BRV+RW, distilled water) and preservative (boric acid, no boric acid) combination. Each run (daily captures for seven days) had two repeats and the experiment was performed in two runs (two-weeks) for a total of four replicates (N=4). Due to the temporal correlation between both runs, the four replicates are not entirely independent. . For the first run, traps were maintained daily from 12 December 2017 to 19 December 2017. The trap bases were removed at the end of the week, the attractant/preservative treatment was re-randomized, and fresh attractant was added. The second run occurred from 3 January 2018 to 10 January 2018.

Results

The effect of boric acid treatment on mean captures. There were significant differences in mean captures by boric acid treatment for *D. suzukii* (df=1, 34, f=7.10, $p<0.05$), but not for non-target drosophilids (df=1, 34, f=0.24, p=0.6302)**.** For *D. suzukii***,** there were greater captures observed for attractants treated with 1% (w/v) boric acid (1.35 ± 0.14) than no boric acid (0.94 ± 0.13) (Figure 1).

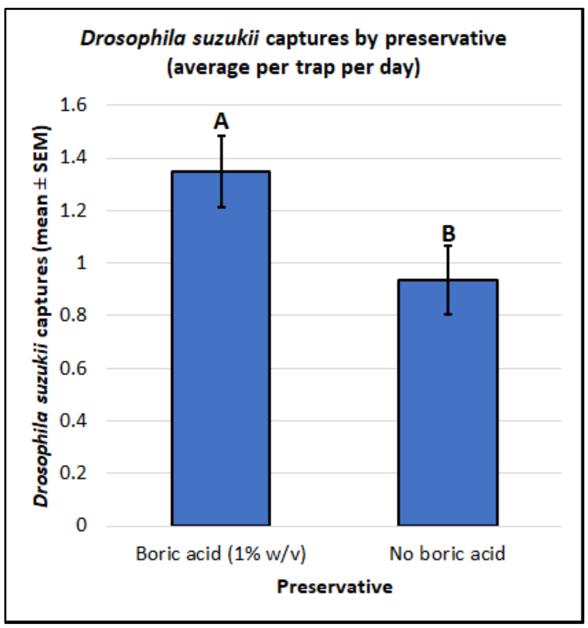

Figure 1. *Drosophila suzukii* captures (mean ± SEM) by preservative.

The effect of attractant type on mean captures (inclusive of all days and boric acid levels). There were significant differences in mean captures observed by attractant for *D. suzukii* (df=4, 34, f=5.22, $p<0.01$) and non-target drosophilids (df=4, 34, f=3.68, $p<0.05$). For *D. suzukii* greater mean captures were observed with BRV+RW (2.02 ± 0.31) than ACV+RW (0.91 ± 0.14), RW (0.89 ± 0.19), and ACV (0.84 ± 0.17), and with no significant differences between

BRV+RW (2.02 ± 0.31) and BRV (1.05 ± 0.17) or between BRV (1.05 ± 0.17), ACV+RW (0.91 ± 0.14), RW (0.89 ± 0.19), and ACV (0.84 ± 0.17) (Figure 2).

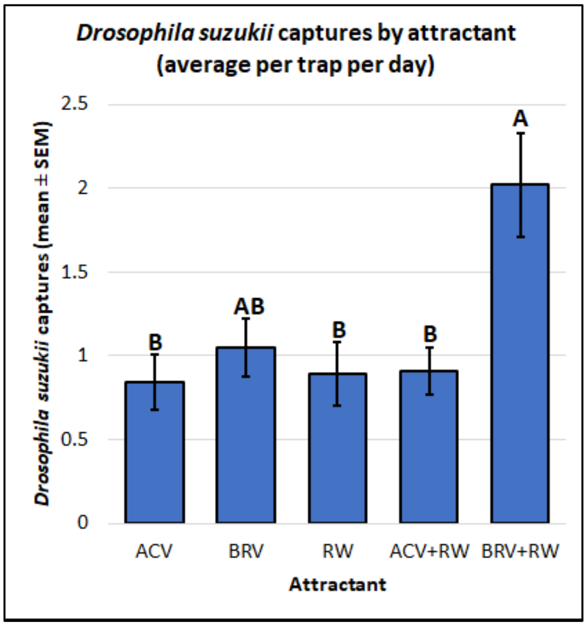

Figure 2. *Drosophila suzukii* captures (mean ± SEM) by attractant.

For non-target drosophilids greater mean captures were observed with BRV+RW (10.7 ± 1.4) and ACV (1.77 ± 0.38) or with no significant differences between BRV+RW (10.7 ± 1.4), ACV+RW (7.91 ± 1.02), RW (5.21 ± 0.55) and BRV (3.91± 0.65) (Figure 3).

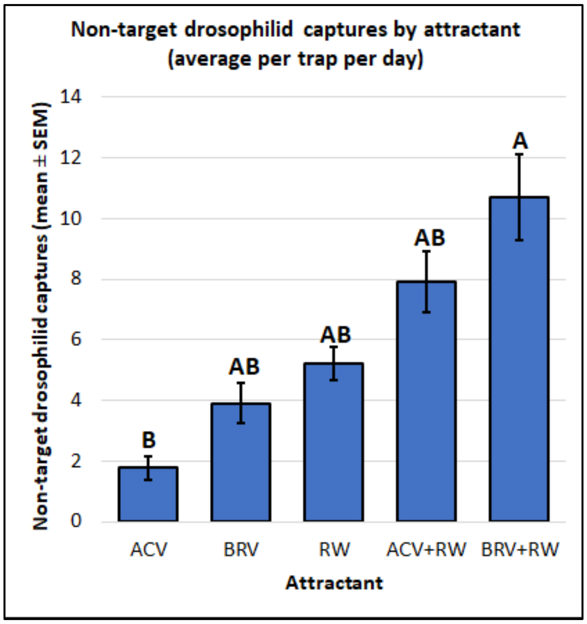

Figure 3. Non-target drosophilid captures (mean ± SEM) by attractant.

The effect of day on mean captures (inclusive of all attractant and boric acid levels). There were significant differences in mean captures by day for both *D. suzukii* (df=6,228, f=3.03, $p<0.01$) and non-target drosophilids (df=6,228, f=5.63, $p<0.001$). There were greater *D. suzukii* captures on day 1 (1.68 ± 0.39) than day 4 (0.50 ± 0.10) or 5 (0.70 ± 0.22) with no significant differences between days 1 (1.68 ± 0.39), 2 (1.23 ± 0.22), 3 (1.23 ± 0.24), 6 (1.30 ± 0.23), and 7 (1.38 ± 0.24) (Figure 4).

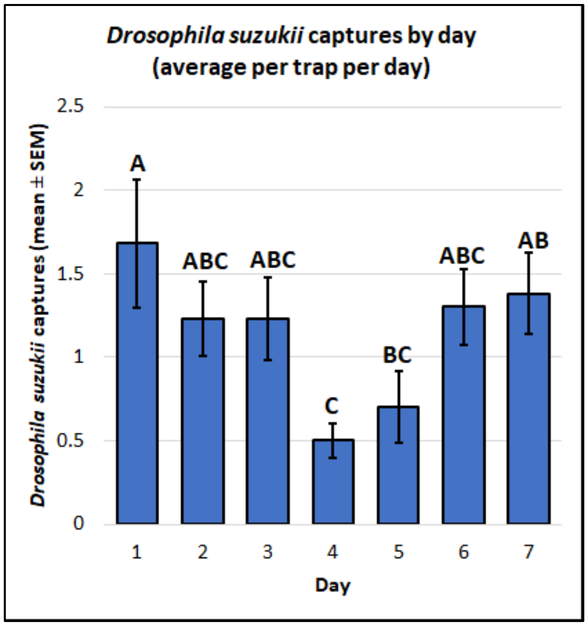

Figure 4. *Drosophila suzukii* captures (mean ± SEM) by day inclusive of all attractant and boric acid levels.

For non-target drosophilids, there were greater captures on days 1 (9.15 ± 1.39) and 3 (7.1 ± 1.11) than days 4 (4.98 ± 1.07), 5 (4.25 ± 1.11), or 6 (3.83 ± 0.80) with no significant differences between days 1 (9.15 ± 1.39), 2 (6.10 ± 1.20), 3 (7.1 ± 1.11), and 7 (5.93 ± 1.13) (Figure 5).

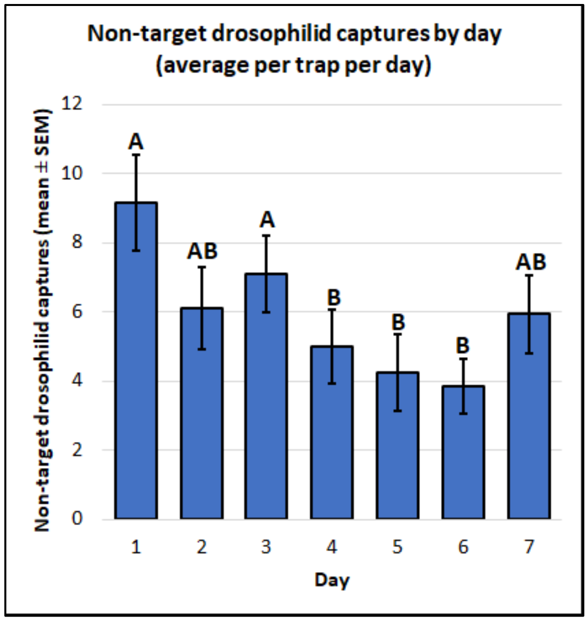

Figure 5. Non-target drosophilid captures (mean ± SEM) by day inclusive of all attractant and boric acid levels.

Because of the temporal correlation between the four replicates, mean captures were qualitatively assessed by week for *D. suzukii* (Figure 6) and non-target drosophilids (Figure 7). For *D. suzukii*, there appeared to be lower captures near day 4 for most attractant types with the exception of RW in week 2. For non-target drosophilids, there appeared to be considerable differences in mean captures by day and attractant depending on the week surveyed. For example, for BRV+RW in week 1 peak captures appear around days 1, 3, and 7 while in week 2 peak captures appear around days 1, 5, and 7. For ACV the trend appears similar in both weeks with more captures on day 1 and a decline throughout the rest of the week.

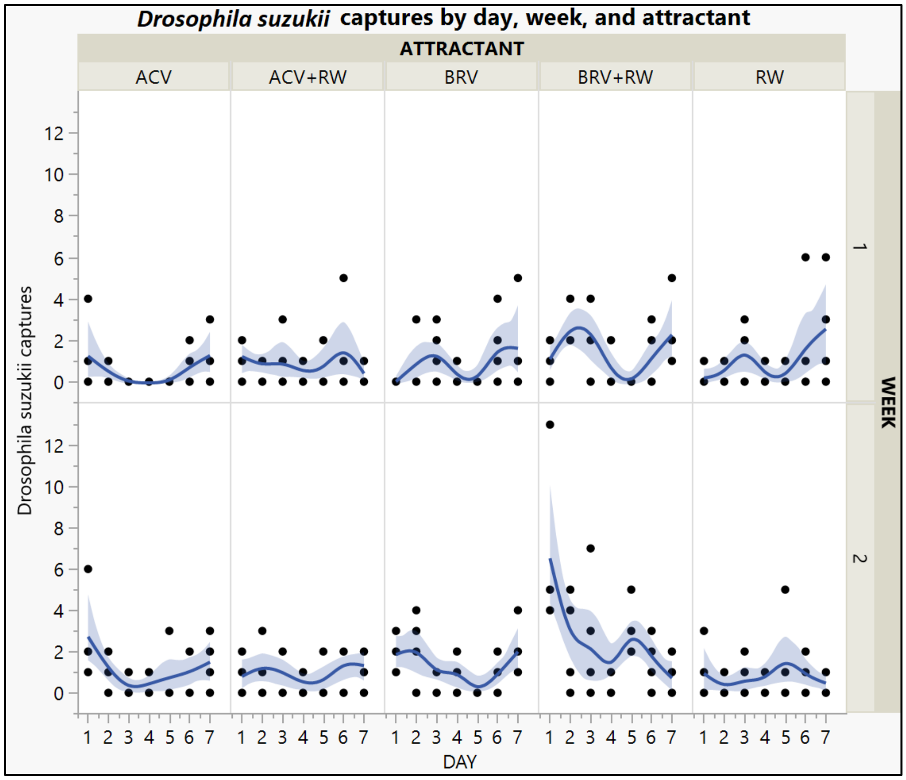

Figure 6. *Drosophila suzukii* captures by day, week, and attractant.

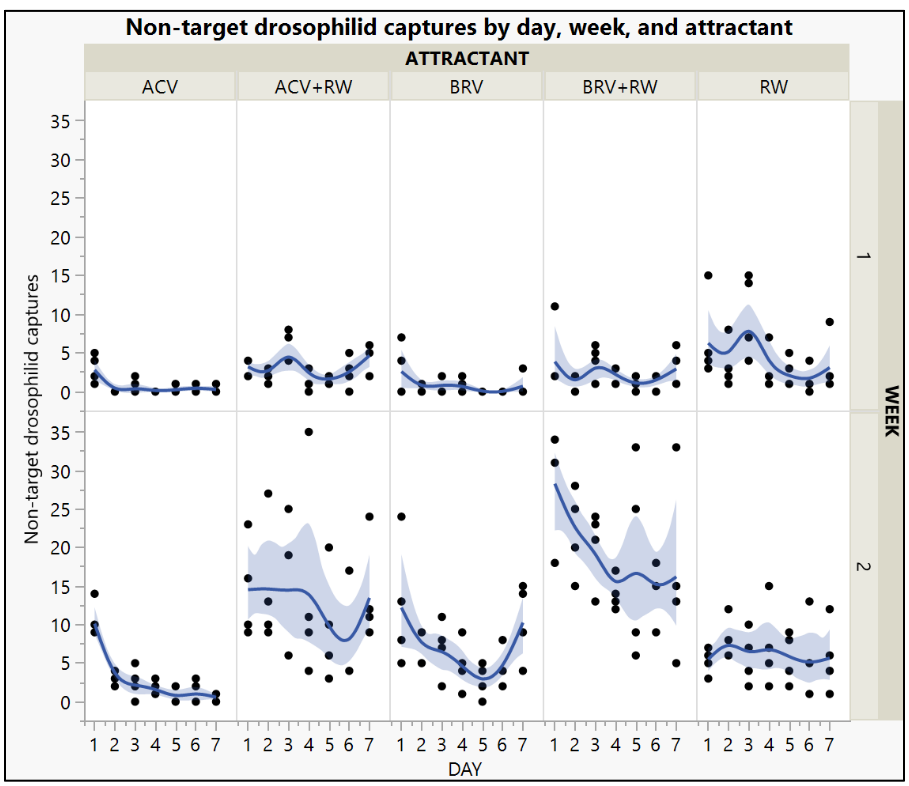

Figure 7. Non-target drosophilid captures by day, week, and attractant.

The interaction effect of time and boric acid treatment. There were no significant differences in mean captures by the interaction of day and boric acid for either *D. suzukii* (df=6, 228, f=1.51, p=0.1754) or non-target drosophilids (df=6, 228, f=0.52, p=0.7961). Due to the low capture rate for *Z. indianus* (many zero captures), the model was unable to analyze field captures for this species.

The interaction effect of time and attractant type. There were no significant differences in mean captures by the interaction of day and attractant for either *D. suzukii* (df=24, 210, f=1.16, p=0.2866) or non-target drosophilids (df=24, 210, f=1.06, p=0.3912). Due to the low capture rate for *Z. indianus* (many zero captures), the model was unable to analyze field captures for this species.

Discussion

Daily field captures were assessed to examine the effects of time on attractant type and preservation with boric acid for invasive drosophilid captures in cherimoya. There were significant differences in mean captures by day for *D. suzukii* and non-target drosophilids. For *D. suzukii*, greater captures were observed on day 1 than day 4 with no significant differences between days 1, 2, 3, and 7 or between days 2, 3, 4, 5, and 6. While for non-target drosophilids, there were greater captures on days 1 and 3 than days 4, 5, and 6 with no significant differences between days 1, 2, 3, and 7 or between days 2, 4, 5, 6, and 7. The results suggest that either 1) microbiome exposure from captured drosophilids affected the field efficacy of attractants or 2) there was another variable responsible for differences in captures with time. Landolt et al. (2012) observed no significant differences in mean captures of *D. suzukii* from ACV+RW (+ 1% boric acid) that had been aged in the laboratory for 0, 1, 3, 5, or 7 days before field deployment, suggesting that either fermentation was no longer active in ACV+RW or that boric acid suppressed any further fermentation. Further research is necessary to better elucidate these findings, as the repeated measures design in this experiment did not account for extraneous variables between days. The differences between days sampled, such as humidity, wind speed, precipitation and temperature are may have interfered with the rate of capture. Therefore, it is hard to isolate the effect of attractant age from other factors.

There were significant differences observed in mean captures by attractant type for *D. suzukii* and non-target drosophilids, but there were no significant differences in the interaction between day and attractant type. For *D. suzukii*, BRV+RW resulted in higher captures than ACV+RW, ACV or RW with no significant differences observed between either BRV+RW and BRV or BRV and ACV, ACV+RW, or RW. Similar to the results reported in chapter 2, BRV+RW resulted in higher captures than ACV or RW. In contrast, BRV+RW was not statistically different from ACV+RW. Collectively, the results suggest that there are differences in attractant preferences by week surveyed. One possible explanation is that attractant preferences vary with changes in fruit ripeness. In the present experiment, , BRV+RW resulted in higher non-target drosophilids captures than ACV with no significant differences observed between BRV+RW and ACV+RW, RW, or BRV or between ACV and ACV+RW, BRV, or RW. Similar to the results of the experiment in chapter 2, there were no differences between BRV+RW, ACV+RW, and RW. In contrast, BRV+RW was more attractive than BRV and ACV+RW was more attractive than ACV. There were no significant differences observed in mean captures of *D. suzukii* or non-target drosophilids for the interaction effect of day and attractant. For *D. suzukii*, there were higher captures towards the beginning of the week, lower captures towards the middle of the week, followed by an increase of captures at the end of the week. The non-significant interaction effect between day and attractant indicates that this v-shaped capture trend for *D. suzukii* was similar across all attractant types used. The results suggest that there may have been a third, unexamined factor that was responsible for the trend observed and the effect was misattributed to time, which cannot be determined from the data collected in this experiment.

There were significant differences in mean captures by boric acid treatment for *D. suzukii*, but not for non-target drosophilids and there was no interaction effect between day and boric acid treatment. For *D. suzukii* there were greater captures in attractant solutions preserved with boric acid than no preservative treatment. Boric acid (1% w/v) was associated with a 44% increase in *D. suzukii* captures compared to no boric acid treatment with 189 and 131 total field captures, respectively. In contrast, there was no significant difference by boric acid treatment for non-target drosophilids. Epsky et al. (2015) found no significant difference in field capture rate of *Z. indianus* by borax treatment for red wine. In the present experiment, the abundance of zero capture data prevented analysis for *Z. indianus*. For the Mexican fruit fly, *Anastrepha ludens*, Lopez and Becerril (1967) reported that borax (1%, w/v) added to the attractant reduced total field captures by 12%. Though not statistically significant, there was a

15% decrease in non-target drosophilid captures in this experiment when boric acid (1% w/v) was added to the attractant. A 33% decrease in *Z. indianus* captures was observed when boric acid (1% w/v) was added to attractant solutions, but since the analysis was not performed it is unknown whether this value is statistically significant. The results suggest that adding boric acid (1% w/v) to attractant solution may not only affect field capture rates, but also have species-specific effects. Future research is needed with higher replication in order to examine potential effects of boric acid on captures by species. There were no significant differences observed in mean captures of *D. suzukii* or non-target drosophilids for the interaction effect of day and boric acid treatment. Therefore, it can be concluded that the differences in mean captures by day are consistent regardless of whether boric acid was used. For *D. suzukii*, there was a v-shaped capture trend with higher captures in the beginning and end of the week with decreased captures mid-week. The non-significant interaction effect between day and boric acid indicates that this v-shaped capture trend was similar across all attractant types used. The results suggest that the effects of boric acid (1%) on attractant effectiveness was consistent throughout the 7 day experiment, whether this is due to changes in pH or inhibition of microbial activity is unknown.

There were several limitations in the experimental design that effect the interpretation of the results including the use of repeated measures and the lack of controls. Because all attractant age levels were not deployed simultaneously and no attempt was made to correlate variables such as temperature, wind speed, precipitation and humidity, it cannot be ruled out that the differences observed in mean captures by day were influenced by a third factor. The experiment was performed in one location among a single population per species so the accuracy of generalizing the results to other environments remains unknown. Furthermore, this experiment did not directly assess the suitability of each attractant type as a substrate for microbial growth or confirm that 1% (w/v) boric acid would inhibit microbial growth. Because vinegar and red wine has already been fermented, there may be a limited amount of sugar left for microbial activity, i.e., the differences in volatile outgassing could be attributed to a diffusion curve over time rather than microbial activity. Even if the attractant solution was exposed to insect skin microbiome, proliferation rates for vectored yeast in the attractant solutions are unknown. The attractant solution itself could have suppressed microbial activity through limited food sources and there would be no need for an additional preservative. Microbial activity was not directly assessed, so conclusions cannot be made regarding the suitability of each attractant as a growth medium for the yeast strains vectored by drosophilids. Boric acid may have altered other attractant properties, such as pH, that were not assessed in this experiment. Lopez and Becerril (1967) reported that adding borax (1%, w/v) to the attractant solution increased pH to 8.8, while without borax, the attractant pH gradually increased from 5.5 to 8.1 over a seven-day period. Additionally, there were no controls in place to assess whether 1% (w/v) boric acid solution is effective and whether it would remain effective outdoors over a seven-day period. There may have been significantly higher *D. suzukii* captures in attractants with boric acid, but the underlying reason, whether it is due to microbial suppression, increased pH, or another factor can only be speculated.

Future research should further examine how microbiome exposure from the attractant solution affects attractant efficacy over time. The suitability of various attractant solutions as a potential growth medium for drosophilid vectored yeasts would reveal if a preservative is necessary to suppress fermentation. The suitability of an attractant to act as a substrate is expected to vary significantly by attractant type, as fresh fruit puree baits (such as mashed banana) will have readily available carbohydrate sources, while for vinegars, this would likely be a limiting factor for microbial growth. If an attractant was found to be a suitable substrate, then the percentage of boric acid needed to inhibit growth would need to be optimized. Once boric acid concentration is optimized, the next step would be to examine the length of time that boric acid would remain effective in outdoor settings and whether temperature or humidity would affect efficacy. To better elucidate attractant age from other factors, an alternative experimental design would be to inoculate the attractants with yeast strains vectored by drosophilids, age the attractant in a laboratory and then, deploy all attractant ages simultaneously.

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CHAPTER 4. Conclusions

Brittany N. Willbrand

Drosophila suzukii (Matsumura) and *Zaprionus indianus* (Gupta), commonly known as spotted wing drosophila (SWD) and the African fig fly (AFF), respectively, are notable pests of soft-skinned fruits and berries. This study was first reported field experiment in the United States that characterized the efficacy of BRV and BRV+RW for drosophilid field surveying. Results suggest that BRV+RW may be a suitable alternative to ACV+RW for SWD and AFF surveying and that BRV may be uniquely attractive to SWD. Chapter 1 documented the invasive range expansion of AFF to Maui, Hawaii, which was previously unreported. Chapter 2 demonstrated the potential of using BRV as an olfactory attractant for SWD and AFF field capture. Chapter 3 examined the relationship between attractant efficacy, field age, and preservation with boric acid. This chapter will briefly summarize the findings of the preceding chapters, interpret the results as a collective, and identify gaps in the literature for future research.

During field surveys for SWD on Maui, suspect AFF specimens were collected, taxonomic verification was provided by experts, and the resulting confirmation of an invasive range expansion was published in the *Proceedings of the Hawaiian Entomological Society* (PHES) (Chapter 1). The variety of localities (Haiku, lower Kula, and upper Kula), host-plants (orange, lemon, starfruit, cherimoya, banana, strawberry, and lemon), and elevations (302, 341, 889, and 980 m above sea level) surveyed suggest that the AFF may already be well-established on Maui island. Now that this new record is published, funding could potentially be allocated towards assessment of economic and ecological effects and to determine whether eradication efforts are warranted and feasible.

In order to advance the development of a species-specific attractant, BRV was evaluated as a field attractant for SWD and AFF with the results published in the journal *Insects* (Chapter 2). Whereas BRV+RW as an olfactory attractant resulted in greater mean captures of SWD relative to other attractants, BRV had greater specificity. On average, ~50% of the drosophilids captured in traps containing BRV were SWD compared to ~40% for BRV+RW, ~40% with ACV, ~30% with ACV+RW, and ~25% with RW. The results suggest that using BRV for SWD surveying may limit non-target captures and reduce the time needed to identify pest drosophilids. Diluting BRV with RW appeared to reduce specificity for SWD, but it cannot be concluded whether that was due to the dilution of an attractive compound or the addition of or synergy with a less attractive or repulsive compound present in RW. Future research could include a serial dilution experiment with BRV to examine this trend. Although the initial relative abundance of *Z. indianus* appeared to be low, other researchers have reported dramatic population expansion within a few years of invasion. Interspecies interactions should also be examined, not only between *Z. indianus*, *D. suzukii* and other invasive drosophilids, but between invasive and endemic drosophilids. The economic effects should be estimated and economic thresholds should be defined to give farmers the appropriate support for field surveying and integrated pest management.

This study examined whether field age and preservation with boric acid (1% w/v) affected attractant efficacy (Chapter 3). Because there were differences in daily captures of SWD and non-target drosophilids, it was concluded that attractant field age up to seven-days affects attractant efficacy, regardless of the attractant type used. The addition of boric acid appeared to increase SWD field captures, regardless of the attractant used, which was not observed for non-target drosophilids. It was hypothesized that when the attractant solution was exposed to the skin microbiome of drosophilids the composition of the volatiles outgassing would change. Therefore, attractant efficacy would change over time. However, since this experiment did not directly assess whether each attractant could act as a substrate for microbial growth, it did not confirm that exposure to the microbiome inoculated the attractant or changed volatile outgassing. Furthermore, even if the attractants were confirmed as suitable substrates, there were no experiments that confirmed that the concentration examined (1% w/v) was optimal to inhibit microbial growth. Future research should directly assess the effects of microbiome exposure from captured drosophilids to the attractant solution. These secondary fermentation processes may alter attractant efficacy over time. There is a gap in the literature regarding characterization of attractant solutions as potential growth media for drosophilid vectored yeasts. These data would determine whether preservatives are necessary for field surveying and if so, the extent to which vectored yeasts could proliferate and alter the attractant solution. If there are significant differences in volatilization between attractants exposed to captured drosophilids and attractants without exposure, then that confounding variable would have considerable effects towards the interpretation of field survey research with regards to attractant efficacy. There is also a gap in the literature regarding the effectiveness of boric acid over time and how it effects attractant solution properties and attractant efficacy.

Field pest surveys are an essential component of integrated pest management programs. Rapid identification of pest drosophilids throughout the growing season are necessary in determining the timing of pesticide applications. Characterizing the capture specificity rates for novel attractant solutions are needed for the development of synthetic species-specific attractants. During an initial survey of SWD on Maui in 2017, an invasive range expansion of AFF was documented. This study contained the first published experiment on drosophilid field surveying with BRV and BRV+RW in the United States with promising results that demonstrate the potential of BRV as an attractant for SWD in the field. Because this study took place in a geographically isolated region within one host-plant environment, further research is needed to test these findings.