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TRAPPING THE AFRICAN FIG FLY (DIPTERA: DROSOPHILIDAE) WITH COMBINATIONS OF VINEGAR AND WINE

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Abstract

The African fig fly, *Zaprionus indianus* Gupta (Diptera: Drosophilidae), is an invasive fruit pest that has spread rapidly through much of the eastern United States. Tests were conducted in southern Florida that recorded the response of *Z. indianus* to baits that included Merlot wine, rice vinegar, ethanol and acetic acid, alone and in combination. The flies were attracted to the wine but not to the vinegar or unbaited traps and were most strongly attracted to the combination of wine and vinegar. More flies were captured in traps baited with the combination of ethanol and acetic acid, the most abundant volatiles of wine and vinegar respectively, than in traps baited with either chemical alone or in unbaited traps. A subsequent test found that traps baited with wine plus acetic acid were as attractive as traps baited with wine plus vinegar. In this test, there was no difference in capture in unbaited traps or traps baited with ethanol plus acetic acid, and intermediate capture was obtained in traps baited with vinegar plus ethanol. These findings suggest that it may be possible to develop a synthetic chemical lure for *Z. indianus* that is based on volatiles from wine used in combination with acetic acid alone or in combination with other volatiles from vinegar.

Key Words: African fig fly, *Zaprionus indianus*, lure, bait, trap, kairomone

Resumen

La mosca Africana de la higuera, *Zaprionus indianus* Gupta (Diptera: Drosophilidae), es una plaga invasiva de frutas que se ha extendido rápidamente a través de gran parte del este de los Estados Unidos. Se realizaron pruebas en el sur de Florida, que registró la respuesta de *Z. indianus* a cebos que incluían vino Merlot, vinagre de arroz, etanol y ácido acético, solos y en combinación. Las moscas fueron atraídas por el vino, pero no por el vinagre o las trampas sin cebo y fueron más fuertemente atraídas a la combinación de vino y vinagre. Más moscas fueron capturadas en trampas cebadas con la combinación de etanol y ácido acético, los compuestos volátiles más abundantes de vino y el vinagre, respectivamente, que en trampas cebadas con cualquier de los dos químicos o en trampas sin cebo. Un examen subsiguiente reveló que las trampas cebadas con vino y etanol fueron tan atractivas como las trampas cebadas con vino y ácido acético. En esta prueba, no hubo una diferencia en la captura en trampas sin cebo o trampas cebadas con etanol y ácido acético, y se obtuvo una captura intermedia en trampas cebadas con vinagre y etanol. Estos hallazgos sugieren que puede ser posible desarrollar un señuelo de química sintética para *Z. indianus* que se basa en compuestos volátiles de vino utilizados en combinación con ácido acético solo o en combinación con otros compuestos volátiles de vinagre.

Palabras Clave: mosca Africana de la higuera, *Zaprionus indianus*, señuelo, cebo, trampa, kairomone

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The African fig fly, *Zaprionus indianus* Gupta, was first found in the U.S. in Florida in 2005 (Steck 2005). This drosophilid has spread rapidly and is now widely distributed in much of North America (van der Linde et al. 2006; Biddinger & Joshi 2012; Werle et al. 2013; van der Linde 2013). Fruits of a wide variety of plants are used as hosts (Lachaise & Tsacas 1983; van der Linde et al. 2006), making this species of great concern as a new pest of numerous tropical and temperate fruit crops. They have been reared primarily from ripe and damaged fruit collected from the ground and, although they have been reared from ripe fruit taken directly from the tree, there is some question as to whether they can act as primary pests in undamaged fruit while the fruit is on the tree (Steck 2005).

There is a need for lures, baits and traps to monitor the distribution and abundance of the African fig fly. However, there is little information available on trapping systems that might be useful for this drosophilid. Castrezana (2011) did not catch *Z. indianus* with banana baits, although small numbers of this insect were netted over bait made from mature bananas and yeast in surveys conducted in Brazil (Torres & Madi-Ravassi 2006). Fermentation volatiles are important in host and food finding behavior of drosophilid flies (Zhu et al. 2003; Stökl 2010; Cha et al. 2012), and fermentation products have been widely used to monitor and trap such flies (e.g., Kanzawa 1934; Hanna et al. 2010; Landolt et al. 2012a,b). The initial discovery of *Z. indianus* in Pennsylvania involved the capture of adults in survey traps baited with apple cider vinegar (Biddinger et al. 2012). It is possible then that *Z. indianus* is attracted to volatiles from fermenting fruit and fermented food products, such as the apple cider vinegar, consistent with food finding behavior of other pest drosophilids, such as *Drosophila suzukii* (Matsumura), the spotted wing drosophila (Kanzawa 1934; Landolt et al. 2012a,b).

Initial trapping of *Z. indianus* was obtained in field experiments that followed the results of Landolt et al. (2012a) with *D. suzukii*. For *D. suzukii*, ethanol and acetic acid, the most abundant volatiles of wine and vinegar, respectively, were more attractive as a mixture than as individual components in attracting *D. suzukii*, as was wine mixed with vinegar (Landolt et al. 2012a,b). Reported herein are the results of tests that were conducted in southern Florida that show field responses of *Z. indianus* adults to wine, vinegar, ethanol, and acetic acid; alone and in combinations.

Materials and Methods

Three experiments were conducted, all in plantings of carambola, *Averrhoa carambola* L., trees at the USDA/ARS, SHRS, Miami, Florida and at the University of Florida-Tropical Research and Education Center (UF-TREC), Homestead, Florida. Multilure traps (Better World Manufacturing Inc., Fresno, California, USA), which are plastic McPhail-type traps (17 cm diam at its widest point) with a yellow base (7 cm tall) and a clear top (11 cm tall), were used for all studies. All traps were placed ~ 1.5 m above ground in branches of trees with ripe fruit. Blocks consisted of either a single large tree, with all treatments in traps placed around the periphery, or in rows of trees, with one trap placed per tree. There were at least 5 m between traps within a single tree block and at least 10 m between traps placed in a row of trees. A randomized block design was used for all tests. Traps were sampled twice weekly, insects were removed, and baits were recycled (mid-week sample) or replaced (end of week sample). Traps were rotated one position after each sampling so that all treatments were tested in all positions within a test. The numbers of flies per treatment per block were summed across the sampling period, and divided by the total number of days to obtain number of flies per trap per day for subsequent analysis. Insect catch data were log (x+1) transformed to improve normality and homoscedasticity (Box et al. 1978). Data were analyzed by analysis of variance with block as a random factor and trap bait as a fixed factor (ANOVA, Proc GLM; SAS Institute 2010) followed by Tukey's HSD mean separation (P = 0.05).

All traps contained an aqueous drowning solution (200 ml) with a preservative (boric acid [1% w/v]) and a surfactant (unscented, dye-free soap [Seventh Generation Natural Dish Liquid] 0.0125% [v/v]) with or without an attractant. Attractants included vinegar (Nakano all natural rice vinegar [Mizkan Americas Inc, Mt. Prospect, Illinois, USA]), wine (Carlo Rossi Reserve Merlot wine [Carlo Rossi Vineyards, Fresno, California, USA]), ethanol (95%, Decon Laboratories, Inc., King of Prussia, Pennsylvania, USA) and acetic acid (> 96%, Fisher Scientific, Inc., Pittsburgh, Pennsylvania, USA). Concentrations of baits used in field tests were based on previous research that found that a 40:60 (v:v) mixture of vinegar and wine was equivalent to a solution of 2% acetic acid and 7.2% ethanol (Landolt et al. 2012a).

Experiment 1. Attractiveness of Vinegar and/or Wine

This experiment tested whether vinegar is attractive, wine is attractive, and a mixture of the 2 materials is attractive to *Z. indianus*. The 4 treatments were: (1) non-baited control, (2) aqueous vinegar solution (40% v/v), (3) aqueous wine solution (60% v/v), and (4) wine plus vinegar mixture (60% + 40% v/v). This experiment was placed in the field at 2 times (2 tests). The first test was conducted from 2 to 16 Mar 2012 at SHRS (14 day sampling period), and the second test was conducted from 4 to 17 Dec 2012 at UF-TREC (13 day sampling period). There were 5 replicate blocks for each of the 2 tests, providing 10 replicate blocks for this experiment.

Experiment 2. Attractiveness of Acetic Acid and/or Ethanol

This experiment tested whether acetic acid alone, ethanol alone, and a mixture of the 2 materials is attractive to *Z. indianus*. The 4 treatments were: (1) non-baited control, (2) aqueous acetic acid solution (2% v/v), (3) aqueous ethanol solution (7.2% v/v), and (4) aqueous acetic acid plus ethanol solution (2% + 7.2%). The 10 replicate blocks of this experiment were maintained in the field 4 to 17 Dec 2012 at UF-TREC (13-day sampling period).

Experiment 3. Role of Additional Wine and Vinegar Volatile

This experiment tested the hypotheses that chemicals in wine in addition to ethanol are involved in *Z. indianus* attraction to a bait possessing wine and that chemicals in vinegar in addition to acetic acid are involved in *Z. indianus* attraction to a bait possessing vinegar. The 5 treatments were: (1) non-baited control, (2) aqueous acetic acid plus ethanol solution (2% + 7.2%), (3) aqueous vinegar plus ethanol solution (40% + 7.2%), (4) aqueous wine plus acetic acid solution (60% + 2%), and (5) wine plus vinegar mixture (60% + 40%). Ten replicate blocks of this test were maintained from 22 Jan to 8 Feb, 2013 at UF-TREC (17-day sampling period).

Results

Experiment 1. Attractiveness of Vinegar and/or Wine

A total of 2,062 *Z. indianus* were captured in this experiment, and treatment affected capture ($F_{3, 27}$ = 37.03, P < 0.0001). No flies were captured in the control traps, and there was no difference between capture in control traps or traps baited with vinegar (Fig. 1a). The highest capture was in traps baited with the combination of wine and vinegar, with intermediate capture in traps baited with wine alone.

Experiment 2. Attractiveness of Acetic Acid and/or Ethanol

Capture was very low in this experiment, with only 99 *Z. indianus* captured, but treatment affected capture ($F_{3,27}$ = 15.50, P < 0.0001). The highest capture was in traps baited with acetic acid plus ethanol, which was the only treatment that captured more flies than the non-baited control (Fig. 1b).

Experiment 3. Role of Additional Wine and Vinegar Chemicals

A total of 3,114 *Z. indianus* were captured in test 3, and there was a significant treatment effect ($F_{4,36}$ = 226.78, P < 0.0001) (Fig. 1c). Again, the lowest capture was in the control traps, but in this test there was no significant difference between capture in the control or in traps baited with the combination of acetic acid and ethanol. The highest capture was in traps baited with wine plus

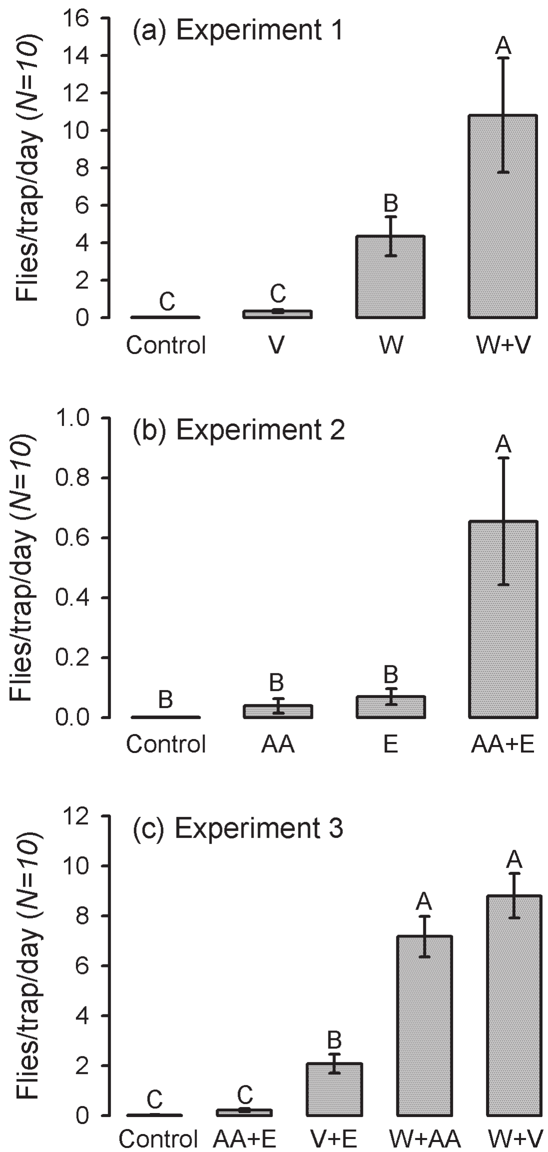

Fig. 1. Mean (± SE) numbers of *Zaprionus indianus* flies captured per trap per day in Multilure traps in field tests conducted in Homestead, Florida. Baits tested in separate studies were (a) Experiment 1: blank (control), vinegar (V), wine (W), or a mixture of wine and vinegar (W+V); (b) Experiment 2: blank (control), acetic acid (AA), ethanol (E), or a mixture of acetic acid and ethanol (AA+E); and (c) Experiment 3: blank (control), a mixture of acetic acid and ethanol (AA+E), a mixture of vinegar and ethanol (V+E), a mixture of wine and acetic acid (W+AA), or a mixture of wine and vinegar (W+V). For each graph, bars headed by the same letter are not significantly different (Tukey HSD test on log [x + 1] transformed data [P = 0.05], non-transformed means presented).

vinegar. Traps baited with wine plus vinegar and wine plus acetic acid captured similar numbers of flies, which were significantly greater than traps baited with vinegar plus ethanol. Traps baited with vinegar plus ethanol captured significantly more flies than traps baited with acetic acid plus ethanol.

Discussion

As was found in tests of *D. suzukii* response to wine and vinegar (Landolt et al. 2012a, b), results from our field trapping experiments demonstrate that the combination of wine and vinegar was a more effective bait for trapping *Z. indianus* than either substance alone. Both *D. suzukii* (Landolt et al. 2012a) and *Z. indianus* were attracted to wine alone. Unlike *D. suzukii* (Landolt et al. 2012a), *Z. indianus* was not attracted to vinegar alone, at least not to the rice vinegar used in our study. Apple cider vinegar was more attractive to *D. suzukii* than wine (Landolt et al. 2012a) and is widely used as bait for this species. *Z. indianus* has been captured in traps baited with apple cider vinegar, however, numbers captured in these traps were low relative compared to number of *D. suzukii* captured (Biddinger & Joshi 2012). It was not determined if this was due to differences in response or differences in populations levels of the 2 species. Landolt et al. (2012b) found that wine combined with rice vinegar was more attractive to *D. suzukii* than wine combined with apple cider vinegar. Studies of other types of vinegar are needed to further understand response of *Z. indianus* to vinegar.

Similarly to wine and vinegar, the combination of acetic acid and ethanol was more attractive to *Z. indianus* than ethanol or acetic acid alone, at least in the second experiment that tested only synthetics. The similar result was observed with *D. suzukii*, for which ethanol and acetic acid were shown to be key to their attraction to wine and vinegar, respectively (Landolt et al. 2012a,b). Interestingly, *Z. indianus* was not attracted to either acetic acid or ethanol alone, while *D. suzukii* was attracted to acetic acid alone, but not to ethanol alone. This may explain the stronger attraction by *D. suzukii* versus *Z. indianus* to vinegar. More *Z. indianus* were captured in traps baited with ethanol plus acetic acid than in unbaited control traps in experiment 2, but there was no difference between these treatments in experiment 3, which included more effective treatments. It is not known if differences were due to differences in population levels during these two studies or to presence of more effective attractants in experiment 3 that intercepted flies that would otherwise respond to the ethanol plus acetic acid baits.

Results of the third experiment indicate that there are volatile chemicals from wine, in addition to ethanol, that are attractive to *Z. indianus*, as found with *D. suzukii* response to wine and vinegar (Landolt et al. 2012b). That hypothesis would explain the increased catches of flies in traps baited with acetic acid plus wine compared to acetic acid plus ethanol (AA + W > AA + EtOH), and similarly the same hypothesis would explain the increased catches of flies in traps baited with vinegar plus wine compared to vinegar plus ethanol (V + W > V + E).

Although not as attractive as volatiles from wine, there is also support for the hypothesis that there are attractive volatiles from vinegar in addition to acetic acid. Unlike results with *D. suzukii* (Landolt et al. 2012b), however, the effect with *Z. indianus* was weak. More flies were captured with ethanol plus vinegar compared to ethanol plus acetic acid (EtOH + V > EtOH + AA). Numbers of flies captured with wine plus vinegar were numerically but not statistically greater than with wine plus acetic acid. Additional study would be needed to more clearly determine any role of vinegar chemicals, in addition to acetic acid, in *Z. indianus* attraction to the mixture of wine plus vinegar. In *D. suzukii*, all the vinegar volatiles that were detected by the fly antennae were also present in wine headspace (Cha et al. 2012), and there is potential for similar responses from *Z. indianus*. Together, these results indicate that the volatiles from these materials should be further investigated to determine a stronger blend of chemical attractants for *Z. indianus* that may be useful as a means of detection and monitoring. Such an approach was used to isolate and identify a 4-component synthetic attractant for *D. suzukii* from the same materials (Cha et al. 2012, 2014).

All together, these results with *Z. indianus* indicate that they can be captured in traps baited with wine or wine plus vinegar, and less effectively with vinegar only. Biddinger and Joshi (2012) indicated the capture of *Z. indianus* in traps baited with apple cider vinegar that were used to monitor *D. suzukii*. Our results, for *Z. indianus* response to vinegar in particular, suggest that *Z. indianus* and *D. suzukii*, although both drosophilids, may be sensitive to different sets of chemical cues from these and other fermented food baits, with the potential for different compositions of chemical attractants for *Z. indianus* versus *D. suzukii*.

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