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Sources of protein as food baits for *Anastrepha obliqua* (Diptera: Tephritidae): tests in a wind tunnel and the field

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Abstract

The West Indian fruit fly, *Anastrepha obliqua* Macquart (Diptera: Tephritidae), is an economically important fruit pest in the Americas. Food attractants are used as bait in traps for monitoring the population of flies in orchards, but their effectiveness differs with location, fruit fly variety, and the type of trap deployed. In this work, we tested the effectiveness of a hydrolyzed protein, BioAnastrepha®, and a yeast extract, Bionis YE MF®, under field conditions and in a laboratory bioassay and identified the main volatile compounds emitted from such mixtures. Hydrolyzed protein and yeast extract were attractive in a wind tunnel, but in the field, hydrolyzed protein was not attractive and only pure yeast extract and yeast extract with sugar were attractive for *A. obliqua*. Sugar alone was not attractive to the flies in either experiment. Yeast extract itself is a good attractant for *A. obliqua*. The addition of sugar, however, will stimulate feeding, which could be useful in insecticide-bait sprays.

Key Words: Food attractant; fruit fly; hydrolyzed protein; volatile organic compounds; yeast extract

Resumo

A mosca das frutas *Anastrepha obliqua* Macquart (Diptera: Tephritidae) é uma importante praga de frutas com valor econômico nas Américas. Atrativos alimentares são utilizados como isca em armadilhas para monitoramento da população de moscas em pomares, porém suas eficácias divergem conforme local, espécie de mosca da fruta e tipo de armadilha utilizada. Neste trabalho testamos a eficácia alimentar de uma proteína hidrolisada, BioAnastrepha®, e um extrato de levedura, Bionis YE MF®, em condições de campo e em bioensaio laboratorial, bem como identificamos os principais compostos voláteis exalados destas misturas. A proteína hidrolisada e o extrato de levedura foram considerados atrativos nos testes em túnel de vento, inclusive quando se adicionou açúcar no extrato de levedura. Nos testes a campo, apenas o extrato de levedura puro e o extrato de levedura com açúcar foram atrativos para *A. obliqua*. O açúcar sozinho não foi atrativo para as moscas em nenhum experimento. Nosso estudo indica que o extrato de levedura é um bom atrativo alimentar para *A. obliqua*, sendo que a adição de açúcar pode melhorar a resposta atrativa.

Palavras Chave: Atrativo alimentar; mosca das frutas; proteína hidrolisada; compostos orgânicos voláteis; extrato de levedura

The West Indian fruit fly, *Anastrepha obliqua* Macquart (Diptera: Tephritidae), is found throughout the tropics and subtropics of the Americas where it is an economically important pest of many fruit crops (Hernández-Ortiz & Aluja 1993). The species shows a strong preference for mango (*Mangifera indica* Bl. [Anacardiaceae]) and other fruits of Anacardiaceae, causing great damage to production (Carvalho et al. 1998; Cruz-López et al. 2006; Jenkins et al. 2011). Its presence in mango orchards triggers a strict quarantine for export, mainly because of the risk of spreading the pest to climatically favorable areas, such as the southern United States, sub-Saharan Africa, Southeast Asia, and northeast Australia (Fu et al. 2014).

Monitoring of populations to detect increases early allows for the timely use of control measures and is essential in integrated pest management (Kogan 1998). One option for monitoring fruit fly populations in the field is the use of food baits in traps, based on the need females have for proteins to complete ovarian development and egg maturation (Drew & Yuval 2000). The most widely used system for monitoring *Anastrepha* species consists of glass or plastic versions of the McPhail trap containing a protein source (Aluja 1994). However, difficulties often are reported relating to the required handling times, low trap capture efficiency for *Anastrepha* flies, and the capture of large numbers of nontarget organisms of such traps (Epsky et al. 1993; Thomas et al. 2001).

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Some of the commonly used attractants to detect and monitor *Anastrepha* flies are (i) Nulure®, which is a hydrolyzed corn protein bait (Miller Chemical & Fertilizer, Hanover, Pennsylvania, USA); (ii) BioLure®, a combination of ammonium acetate and putrescine (BioLure, Suterra LLC, Bend, Oregon, USA), and (iii) Torula®, hydrolyzed yeast extract (Epsky et al. 2011; Jenkins et al. 2011; Arredondo et al. 2014). Baits containing alternative protein sources have shown some degree of attractiveness, such as poultry feces (Robacker et al. 2000), bacterial compounds (Nigg et al. 1994), and human urine (Aluja & Piñero 2004).

This study sought to compare the efficacy of 2 protein sources in attracting *A. obliqua*: (i) a hydrolyzed protein produced in Brazil (BioAnastrepha®) used in traps for monitoring several species of fruit flies, and (ii) a yeast extract (*Saccharomyces cerevisiae* Meyen [Saccharomycetaceae]) (Bionis YE MF®) produced in Brazil and marketed originally as a food supplement in human and animal diets. This yeast extract has been used successfully in the development of low-cost diets for mass rearing of *Ceratitis capitata* Weidemann and *A. fraterculus* Weidemann (both Diptera: Tephritidae) (Morelli et al. 2012; Silva-Neto et al. 2012). In addition to comparing the attractiveness of these baits, we also identified the main chemical substances in these materials as the possible basis for their attractiveness.

Materials and Methods

INSECTS

The *A. obliqua* flies used in laboratory bioassays were obtained from a mass-reared colony maintained at the Fruit-flies Laboratory of Centro Tecnológico da Agropecuária da Bahia-CETAB, Bahia State, Brazil. In the larval stages, these flies were fed fruits (mango or guava) and adults were fed an artificial diet composed of protein sources derived from a yeast extract (Silva-Neto et al. 2012). Rearing took place at 25 ± 1 °C, 40 to 70% RH, and a 12:12 h L:D photoperiod. After emergence, adults were placed in cages covered with voile netting, containing water and the same diet used for rearing, ad libitum, for 3 days. On the fourth d adults were separated by sex and starved for 22 h until being used in the study. Water, but not food, was provided in the containers. By starving the flies, we hoped to increase their attraction response to the analyzed treatments (Cruz-López et al. 2006).

SAMPLE PREPARATION

Four treatments and 1 negative control (distilled water) were tested: (1) hydrolyzed corn protein diluted to 5% (w/v) - BioAnastrepha® (hydrolyzed protein); (2) yeast extract protein diluted to 5% (w/v) - Bionis MF® Ye (yeast extract); (3) yeast extract protein with sugar diluted to 5% (w/v), 3.33%(w/v), and 1.67% (w/v), respectively (yeast extract with sugar); (4) pure sugar diluted to 3.33% (w/v) (sugar). Dilution and mixing of the components was done using a magnetic stirrer at a temperature of 80 °C, followed by cooling to room temperature for a period of 15 min. Three treatments (yeast extract, yeast extract with sugar, and sugar alone) contained 5% sodium tetraborate (Borax®) as a stabilizer.

BIOASSAYS IN WIND TUNNEL

A wind tunnel was used to assess bait attractiveness. The wind tunnel was constructed of polycarbonate, and was 150 cm long × 60 cm high × 60 cm wide. The distance between the odor source and the fly release box was 120 cm with an air flow of 40 cm per s. Illumination was supplied by 1,000 LED lamps, placed 5 cm above the wind tunnel, providing a light intensity of 1,300 lux. Insects were evaluated in groups of 5 individuals (all male or all female), which were placed inside acrylic release boxes (6.5 cm × 6.5 cm × 6.5 cm) and held for at least 20 min under room conditions in the wind tunnel to acclimatize (25 ± 1 °C and 60 ± 10% relative humidity). Two hundred µL of each test substance was applied to filter paper (4 cm × 4 cm) for each bioassay.

In each test, insects were observed for 10 min. The behavior of the insect leaving the release box and flying towards the odor source was called "activation." Flies were used only once during the experiments. Ten replicates were done for each sex for each of the 5 treatments. At the end of each treatment, the wind tunnel was cleaned with absolute alcohol.

Preliminary tests indicated that both males and females were more responsive in the morning and so tests were conducted from 8:00 AM to 11:00 AM.

ATTRACTIVENESS OF FIELD INSECTS

The field attractiveness of the different treatments was monitored in a mango orchard at the Active Germplasm Mango Bank in the Embrapa Mandioca e Fruticultura, Cruz das Almas, Bahia, Brazil (12.6700°S, 39.1019°W). The mango orchard is at an altitude of 200 masl and has a climate classified as hot humid tropical, containing 26 mango varieties spaced 5 m × 5 m, with a total of 144 trees. The tests took place between Sep and Oct 2014, the off-season for mango in this region. The experimental design was conducted over a total area of 30,000 m^2. Traps were arranged in a randomized block design, with 1 sampling block per wk, making a total of 4 blocks over 4 consecutive wk. In each sample block, 35 McPhail traps were deployed, grouped into 7 groups of 5 traps. Each of these 7 groups contained 1 trap of each treatment. The distance between groups and between traps within groups was 20 m and 10 m, respectively. Traps were placed on randomly selected trees of the various cultivars in the orchard about 2 m above the ground and their position within each block was rotated sequentially every week.

Each trap contained 300 mL of bait, for a total of 2.1 L of bait per treatment. All baits were changed and the flies collected weekly, for 4 consecutive wk. Flies were removed from the trap and stored in 70% alcohol until being identified to species. The taxonomic identification of the captured fruit flies was done by examination of the everted ovipositor (Zucchi 2000).

EXTRACTION OF CHEMICAL COMPOUNDS

To collect volatile compounds emitted by the different treatment materials, we used a solid-phase micro-extraction technique in headspace mode (HS-SPME) with a manual sampler. Ten mL of the headspace sample was put in a sealed 20 mL glass vial, and the extraction was performed by placing the vial into an aluminum heating block (4 cm height × 14 cm diam) on a temperature-controlled heating plate at 60 °C. The extraction of the volatile organic compounds (VOCs) was done with 75 µm of the fiber Carboxen/PDMS (Supelco, Bellefonte, Pennsylvania, USA) previously conditioned according to the manufacturer's instructions. After an 18 min extraction period, the fiber was inserted into the gas chromatograph injector for 3 min at 250 °C for desorption of the VOCs. The extraction procedure was performed in triplicate for each of the evaluated treatments.

ANALYSIS AND IDENTIFICATION OF COMPOUNDS

The volatile compounds present in the samples were detected using a gas chromatograph coupled to a mass spectrometer (GCMSQP2010 Plus model, Shimadzu, Japan) equipped with a split/splitless injector in the splitless mode and at 250 °C during the chromatographic run. VOCs were separated and detected under the following conditions: (1) HP-1

capillary column 30 m × MS 0.25 mm id × 0.25 uM (Agilent, Palo Alto, California, USA) and 0.69 mL min^{-1} carrier gas flow (He) and, (2) temperature programming of 40 °C for 1 min, 4 °C min^{-1} to 140 °C, 140 °C for 3 min, 8 °C min^{-1} to 240 °C and 240 °C for 3.5 min (total time of 45 min). The mass detector conditions were a transfer line temperature of 250 °C, ion source temperature of 250 °C, and ionization mode with electron impact at 70 eV.

Identification of VOCs was achieved by (i) comparing the GC retention times and mass spectra with those of the pure standard compounds, when available; (ii) all mass spectra were also compared with the data system library (NIST 147 Database); and (iii) Kovats retention index (KI) values were determined using a homologous series of n-alkanes C_8–C_{40} and the values compared with values reported in the literature for similar chromatographic columns. The percentage of individual peaks was achieved by peak area normalization measured without correction factors.

STATISTICAL ANALYSIS

Chi square tests were used to compare the proportions of males and females responding to each treatment. Initially, the test was conducted for all 5 groups simultaneously. Thereafter, if a significant difference was verified, each of the 2 groups was compared separately, and P values < 0.05 were considered significant.

For the data field, analysis of variance was used to identify possible differences between treatments and between capture periods. The capture data were transformed into the $\sqrt{y + 0.5}$; to meet the data normality assumptions and homogeneity of variance of treatments. After a transformation of the data, these were analyzed by Kolmogorov-Smirnov test, with a Lilliefors correction.

To determine significance of differences of means among treatments, Tukey's test ($P < 0.05$) was applied using the system for statistical analysis, SAEG version 9.1, Foundation Arthur Bernardes-UFV, Viçosa, Brazil, 2007.

Results

BIOASSAYS IN WIND TUNNEL

In the wind tunnel experiment, the greatest response of fruit flies was to hydrolyzed protein and to the yeast extract with sugar, both of which differed significantly from the response to the negative control (p = 0.027 and 0.0455, respectively) (Fig. 1). Meanwhile, the response to the pure yeast extract did not differ significantly from that for the hydrolyzed protein or from the yeast extract with sugar treatment (p = 0.1601 and 0.2348, respectively), nor did it differ from that for the negative control (p = 0.4008). Sugar alone was not attractive. There was no difference in the attraction of males versus females across treatments (Fig. 1).

ATTRACTIVENESS IN THE FIELD

A total of 217 fruit flies were captured from all traps in the field experiment, including 120 females and 96 males of *Anastrepha* spp. and 1 female of *C. capitata*. The following species of *Anastrepha* were captured: *A. obliqua* (57%), *A. pickeli* Lima (18%), *A. barnesi* Aldrich (11%), *A. sororcula* Zucchi (4%), *A. fraterculus* Weidemann (4%), *A. montei* Lima (2%), *A. zenildae* Zucchi (2%), *A. amita* Zucchi (1%).

The treatment with yeast extract captured the largest number of females and showed more attractive potential for all species captured. In the case of *A. obliqua*, yeast extract with sugar captured a similar number to using pure yeast extract (30 and 37, respectively).

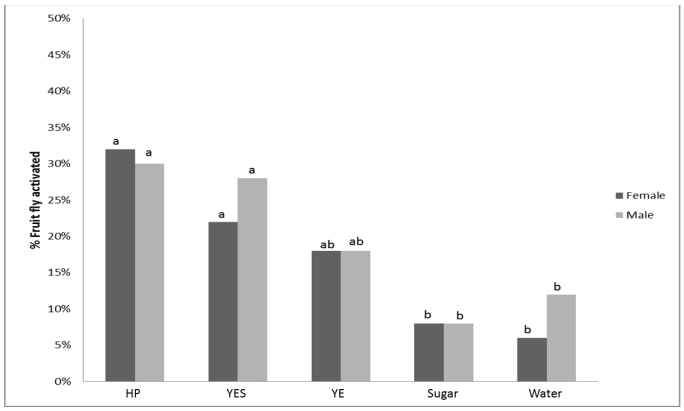

Fig. 1. Activation response of male and female *Anastrepha obliqua* to different food baits tested in the wind tunnel bioassay. HP - hydrolyzed protein, YE - yeast extract, YES - yeast extract with sugar, pure sugar diluted in water and negative control (water). Bar heights refer to total percentage responding over all replicates, in each sex separately. Bars headed by the same letter are not significantly different by X^2 test ($P < 0.05$).

Comparing the average total number of females captured for each treatment, there was a significant difference between treatment with yeast extract and the others, although the yeast extract with sugar treatment and the hydrolyzed protein treatment did not differ (Table 1).

CHEMICAL IDENTIFICATION

Considering the evaluated treatments, it was possible to identify 10 different VOCs (Table 2). The compounds benzaldehyde, phenylacetaldehyde and ethylester L-isoleucine were present in hydrolyzed protein and treatments containing yeast extract, suggesting a possible relationship of these compounds with the attractiveness of the treatments.

Discussion

The effectiveness of hydrolyzed protein and yeast extracts against different species of economically important flies in the field has been demonstrated by a number of studies (Kendra et al. 2010; Leblanc et al. 2010; Epsky et al. 2011; Mangan & Thomas 2014). Specifically, in the case of *A. obliqua*, a synthetic mixture of ammonium acetate and putrescine, known as BioLure®, was more attractive than the protein hydrolyzate known as Nulure® in field tests at carambola orchards in Puerto Rico (Jenkins et al. 2011) and mango orchards in Mexico (Díaz-Fleischer et al. 2009). However, Nulure® was more attractive to *A.*

Table 1. Number of female fruit flies caught in each treatment with McPhail traps in the Active Germplasm Mango Bank, in Cruz das Almas, Brazil, 2014.

Treatments	Female		Mean[1]	Transformed mean[2]
	N	%		
YE	79	65	2.96	1.68 A
YES	35	29	1.29	1.18 B
HP	6	5	0.25	0.83 BC
Sugar	1	1	0.04	0.72 C
Water	0	0	—	—

[1]Means followed by the same letter within a column are not significantly different by Tukey test ($P < 0.05$); [2]transformed data in y = ; HP - hydrolyzed protein, YE - yeast extract, YES - yeast extract with sugar, pure sugar diluted in water and negative control (water).

Table 2. Volatile compounds identified in each treatment.

Compounds	IK_{exp}	IK_{lit}	HP	YE	YES	Sugar
2-methylpyrazine	—	—	—	+	+	—
2-furanmethanol	859	858	+	—	—	—
methional	869	865	+	—	—	—
benzaldehyde	1,028	1,003	+	+	+	—
phenylacetaldehyde	1,105	1,100	+	+	+	—
2-ethyl-1-hexanol	1,116	1,115	—	+	+	+
nonanal	1,183	1,102	—	+	+	+
ethylester L-isoleucine	1,191	1,102	+	+	+	—
camphor	1,216	1,200	+	—	—	—
decanal	1,284	1,284	—	+	+	—

HP - hydrolyzed protein, YE - yeast extract, YES - yeast extract with sugar and pure sugar diluted in water.

obliqua than BioLure® in mango cultivars in the Dominican Republic (Thomas et al. 2008) and Mexico (Arredondo et al. 2014), while López-Guillén et al. (2010) found no difference in the attractiveness of these protein sources for *A. obliqua* in mango orchards in Mexico. The differences in the attractiveness of these protein sources to *A. obliqua* has already been discussed by some authors, and it has been suggested that the type of bait used, as well as the model of the trap employed, should be chosen specifically for each locality, which increases the importance of studies in different regions of the world. In addition, there is general consensus that the volatiles from baits are affected by local environmental conditions and how traps are deployed.

The hydrolyzed protein, BioAnastrepha®, which already had been shown to be attractive to other species of fruit flies in field tests (Scoz et al. 2006), was attractive to *A. obliqua* only in laboratory tests in this study and did not differ from the negative control in field tests. The loss of attractiveness of this compound in field tests is likely due to environmental weathering in the orchards, where varying pH, temperature, microbial flora, and other features may interfere with the release of volatile organic compounds. In this work, 5% borax was added to the yeast extract treatments, which may be an important factor in its continued attractive ability to *A. obliqua* under field conditions. Despite the fact that the hydrolyzed protein BioAnastrepha® had borax in its formulation, its concentration is not provided by the manufacturer and may not have been sufficient to buffer it from weather changes in the field.

The literature regarding the performance of borax in maintaining the attractiveness of lures for different fruit fly species is controversial (Heath et al. 1994; Duyck et al. 2004). In this work, the addition of borax did not affect the attractiveness of yeast extract.

Studies in semi-field conditions have shown that both endogenous and exogenous facts affect the catch rates of *A. obliqua* flies (Arredondo et al. 2014; Díaz-Fleischer et al. 2009). Thus, the difference found in our study in the attraction of insects by hydrolyzed protein between the field and the laboratory can be explained by endogenous factors in the field, in which the age of insects, their feeding, and sexual maturity all were uncontrolled, in contrast to our laboratory experiment.

Studies have shown that female fruit flies require higher amounts of amino acids than males, because ingestion of amino acids is necessary for maximal egg development (Fontellas & Zucoloto 1999; Cresoni-Pereira & Zucoloto 2001; Aluja et al. 2001). Those studies support the findings of Arredondo et al. (2014) and Díaz-Fleischer et al. (2009), which found greater attraction of *A. obliqua* females to different protein attractions in field trials than males of the same species. However, our study showed similar attraction of males and females for the different treatments in the tests conducted in wind tunnel. Again, these results may be explained by the fact that under field conditions there is no control of the physiological conditions of the captured flies, while in controlled tests in a wind tunnel, both females and male flies had received a protein-based diet and were at most 5 d old and thus sexually immature.

Insects are attracted to food baits through the volatiles emitted by them (Hagen et al. 1976; Miller & Haarer 1981). In this work, using HS-SPME technique, VOCs from each treatment were extracted and 10 compounds identified. The compounds methional, benzaldehyde, phenylacetaldehyde, and 2-furanmethanol were detected in BioAnastrepha® and already had been reported in previous work involving VOCs of hydrolyzed protein (Buttery et al. 1983). However, there has as yet been no chemical assessment of volatile extracts from yeast. Only benzaldehyde, phenylacetaldehyde, and ethylester L-isoleucine were found in both types of food bait.

Our study demonstrates that the yeast extract Bionis YE MF® was attractive to *A. obliqua* in both the laboratory and the field, whereas sugar was not attractive alone or when mixed with baits. Moreover, the hydrolyzed protein BioAnastrepha® proved to be attractive only in laboratory tests. The differences observed between the tests using wind tunnel and in field catches reinforces the importance of the joint use of both of these tests, taking advantage of the controlled conditions of laboratory tests, and also exploring the effects of environmental conditions in fruit orchards. The chemical compounds that have been identified in both food baits need to be assessed individually to better understand their real influence in attracting fruit flies, along with the possibility of enhancing this attractiveness through synthetic blends.

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