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Original Article

Efficacy of alkaline hydrolyzed torula yeast for monitoring *Anastrepha* spp.

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Abstract

Anastrepha fruit flies (Diptera: Tephritidae) are important pests of several crops in the Americas. Alkaline hydrolysis of proteins represents a potential source of novel attractants. Previous studies revealed a higher response by *Anastrepha obliqua* (Macquart) to alkaline hydrolyzed torula yeast compared with other attractants. Under field conditions, captures of *Anastrepha fraterculus* (Wiedemann), *Anastrepha ludens* (Loew), and *Anastrepha serpentina* (Wiedemann) were significantly higher in traps baited with alkaline hydrolyzed torula yeast than with acid hydrolyzed protein (Captor) + borax. Fly attraction to alkaline hydrolyzed torula yeast varied with species but was higher in the 1st week and decreased after 2 or 3 weeks of use in the field. The release of ammonia gas from alkaline hydrolyzed torula yeast and CeraTrap was reduced after 3 weeks, but CeraTrap remained effective in the capture of flies whereas alkaline hydrolyzed torula yeast did not. No improvement in fly captures was observed for aged alkaline hydrolyzed torula yeast when 1% ammonium acetate was added to traps. The addition of propylene glycol or benzalkonium chloride did not improve trap captures over time. Although CeraTrap has advantages in attractiveness and durability, the attraction, simplicity, and low cost make alkaline hydrolyzed torula yeast a promising attractant when compared with the standard Captor + borax in short-term monitoring programs targeted at *Anastrepha* pests.

Introduction

Anastrepha fruit flies (Diptera: Tephritidae) are considered among the major agricultural pests in the Americas due to their direct damage of fruits and because infestations can threaten the commercialization and export of fruit (White & Elson-Harris, 1992). In Mexico, *Anastrepha ludens* (Loew) is an important pest of citrus, *Citrus* spp., and mango, *Mangifera indica* (L.), whereas *Anastrepha obliqua* (Macquart) is an important pest of mango and tropical plum, *Spondias* spp. (Hernández-Ortiz & Aluja, 1993). In addition, *Anastrepha serpentina* (Wiedemann) is considered an important pest of fruits in the family Sapotaceae, such as sapodilla, *Manilkara zapota* (L.) P. Royen (Hernández-Ortiz & Aluja, 1993), and *Anastrepha fraterculus* (Wiedemann) is a complex of cryptic species that mainly infest peach, *Prunus persica* (L.), and guava, *Psidium guajava* (L.) in Mexico (Hernández-Ortíz et al., 2015).

Effective attractants for surveillance programs targeted at *Anastrepha* spp. are key to integrated pest management strategies and could also prove useful for the detection of these pests in other regions where these flies could be a potential threat to fruit crops (IAEA, 2018). Food lures are the only effective attractants for monitoring *Anastrepha* spp. (Epsky et al., 2014), although they are considered weak attractants and can elicit highly variable fly responses depending on the fly's sex, species, age, nutritional and sexual status, protein origin, hydrolysis process, and environmental conditions, among other factors (Epsky et al., 1993, 2004, 2014; Díaz Fleischer et al., 2014). Acid hydrolyzed proteins (Captor) + borax (sodium tetraborate) and torula yeast pellets are the most frequently used food attractants in surveillance programs in the Americas, including Mexico. CeraTrap is a long-lasting enzymatic hydrolyzed animal protein that was initially developed for the medfly *Ceratitis*

capitata L., but became widely used for *Anastrepha* due to its high effectiveness (Lasa et al., 2014, 2015; Flores et al., 2018).

Alkaline hydrolysis of proteins has not been explored for the production of food attractants because this process degrades amino acids, with the exception of tryptophan, so it is not widely used in the food industry. However, an early study by McPhail (1939) reported favorable results in the capture of *Anastrepha striata* (Schiner) when using sodium hydroxide with various types of proteins, including casein, gelatin, animal blood, and bovine hair. Recently, use of alkaline hydrolyzed torula yeast (pH 13.3) resulted in more effective capture of *A. obliqua* than two widely used attractants for this pest, an acid hydrolyzed protein + borax mixture and pellets comprising torula yeast + borax (Lasa & Williams, 2022a). Alkaline hydrolyzed torula yeast is a simple and cheap attractant that has not been tested against other *Anastrepha* pest species, but that may prove valuable for monitoring or mass-trapping control strategies.

The present study compared captures of *A. fraterculus*, *A. ludens*, and *A. serpentina* flies in traps baited with alkaline hydrolyzed torula yeast or traps baited with a standard hydrolyzed protein + borax attractant. Additional experiments on *A. ludens*, *A. serpentina*, and *A. obliqua* compared alkaline hydrolyzed torula yeast with the long-lasting attractant CeraTrap. The amount of ammonia released by traps was measured to elucidate the importance of ammonia as a key component in fly attraction (Bateman & Morton, 1981; Mazor et al., 2002; Lasa & Williams, 2021a). Finally, two formulations of alkaline hydrolyzed torula yeast with propylene glycol and benzalkonium chloride were tested in an effort to improve the stability of the attractant and thereby extend its effective lifespan.

Materials and methods

Trap, attractants, and experiment sites

All experiments were performed using a handmade trap comprising a clear polyethylene terephthalate (PET) bottle of 500 ml capacity in which three holes of 11 mm diameter were drilled at two-thirds of the height. This model trap has been tested on *Anastrepha* spp. and has similar efficacy to yellow-colored commercial traps (Lasa et al., 2013).

Captor 300 (Promotora Agropecuaria Universal, Mexico City, Mexico) and CeraTrap (Bioibérica, Barcelona, Spain) were used as the reference attractants. Captor is an acid hydrolyzed protein widely used to monitor *Anastrepha* spp. in Mexico. This attractant is prepared by mixing 10 ml of Captor 300 with 5 g of borax (J.T. Baker, Mexico City, Mexico) and 235 ml of water (pH 9.1) as indicated by the Mexican phytosanitary authorities (Anonymous, 1999). The enzymatic hydrolyzed protein of animal origin, CeraTrap (pH 6.9), is supplied in liquid form ready to use. In Mexico, both Captor and CeraTrap are commonly used as standard lures for *Anastrepha*. Torula yeast (2.75% wt/vol) (Lake States type B, Lallemand, Montreal, Canada; 49% protein), was alkaline hydrolyzed by the addition of sodium hydroxide pellets (Golden Bell Products, Orange, CA, USA) and adjusted to pH 13.3. Hydrolysis occurred during agitation on a Stable Temp magnetic stirrer (Cole-Parmer, Vernon Hills, IL, USA) at 320 rpm for 24 h, as described previously (Lasa & Williams, 2022). Propylene glycol (99% purity; Droguería Cosmopolita, Mexico City, Mexico) and

benzalkonium chloride (80% purity; Productos Biológicos Hyla, Azcapotzalco, Mexico) were used to increase attractant stability in the final experiment.

Attractant comparisons were performed at two sites that differed in climatic conditions. Experiments with *A. fraterculus* were performed during September–October 2021 in a guava orchard (19°25'8.15"N, 96°58'30.92"W, 1174 m elevation) near the town of San Marcos, Veracruz, Mexico. Experiments with *A. ludens* were performed in November–December 2021 in an abandoned orange (*Citrus sinensis* L.) orchard (19°23'47.46"N, 96°58'27.63"W, 1114 m elevation) in Teocelo village, Veracruz, Mexico. Orange and guava orchards were separated by a distance of 2 km, in an area characterized by a humid climate with a mean temperature of 18–19 °C (range 15–26 °C) and high relative humidity (range 80–100%) at the end of the rainy season.

Experiments with *A. obliqua* were performed in May and July 2022 in a mango orchard close to Jalcomulco (19°19'40"N, 96°45'26"W, 340 m elevation) whereas the attractants were tested against *A. serpentina* in a sapodilla orchard near Apazapan (19°19'35.38"N, 96°43'22.37"W, 317 m elevation), both in Veracruz State, Mexico. No insecticides were applied in any of the experimental orchards to control insect pests. The experimental orchards were separated by 3.8 km in an area characterized by a subtropical-humid climate with a mean temperature of ca. 25 °C (range 21–29 °C) and high mean relative humidity of 82% (range 65–100%) during the months of the experiments.

Alkaline hydrolyzed torula yeast vs. Captor + borax

Three field experiments were performed on the attraction of *A. fraterculus*, *A. ludens*, and *A. serpentina* flies to alkaline hydrolyzed torula yeast compared to Captor + borax. *Anastrepha obliqua* was not included in this first experiment because this species had shown a greater attraction to the alkaline hydrolyzed torula yeast than to the Captor + borax in a previous experiment (Lasa & Williams, 2022). Each orchard was divided into four blocks (25 × 25 m) separated by a distance of at least 20 m. Two PET bottles were baited with 250 ml of attractant, one loaded with Captor + borax and the other loaded with alkaline hydrolyzed torula yeast (pH 13.3). These bottles were hung within each block at a height of 2.5–3 m in orange and guava, and a height of 3.5–4 m in sapodilla using an extendable pole fitted with a hook. Traps within each block were separated by a distance of 10–15 m. Traps were randomly assigned to their initial position within each block and were rotated after a week to the other position to control for positional effects. At each weekly sample, insects were collected from each trap, placed in 70% alcohol and taken to the laboratory to be counted, identified to species, and sorted by sex. After each weekly sample, the attractants were discarded and replaced with new preparations. The experiment lasted 2 weeks as traps were sampled once at each position within each block (n = 8 observations per treatment).

Alkaline hydrolyzed torula yeast vs CeraTrap

Three experiments were performed on the capture of *A. obliqua*, *A. ludens*, or *A. serpentina* flies using alkaline hydrolyzed torula yeast compared to CeraTrap. In this case, *A. obliqua* (instead of *A. fraterculus*) was included in the experiment due its importance as pest in

Mexico and because these attractants had not been compared for this fly species previously (Lasa & Williams, 2022). Experiments were performed in the same orchards described in the previous section, in December 2021 for *A. ludens* and in May 2022 for *A. obliqua* and *A. serpentina*. For this, each orchard was divided into seven blocks (25 × 25 m) for *A. obliqua* and six blocks for *A. ludens* and *A. serpentina*, separated by a distance of at least 20 m. Two PET bottles were baited with 250 ml of CeraTrap or alkaline hydrolyzed torula yeast (pH 13.3). The two traps were placed and rotated at weekly intervals as described in the previous section. Captured insects were collected, counted, and identified to sex. The experiment lasted 3 weeks and attractants were not renewed in order to assess changes in their efficacy over time.

The release of ammonia from attractants after 3 weeks in the field was determined for five of the traps used in each experiment. For this, a 500-ml PET bottle trap was loaded with the attractant liquid that remained in each trap at the end of the experiment (130–210 ml per trap). The bottle with the attractant was placed inside a 5-L opaque glass jar through which filtered air was passed at a flow rate of 150 mL per min. Ammonia gas was collected in a 10-mL distilled water trap reacted with Nessler's reagent and quantified using an ammonia medium range photometer (Hanna Instruments, Woonsocket, RI, USA) at a temperature of 24 ± 1 °C, as described previously (Lasa & Williams, 2021a). The duration of the capture of ammonia ranged from 15 min to 4 h per trap, which resulted in concentrations between 0.1 and 10 mg L^{-1} that fell within the accuracy range of the ammonia photometer. The average release of ammonia from traps was calculated as μg of ammonia per h (μg h^{-1}).

Addition of ammonium acetate to field-aged alkaline hydrolyzed torula yeast.

As a marked reduction of ammonia release from traps was detected for alkaline hydrolyzed torula yeast after 3 weeks of use in the field, we aimed to determine whether the release of ammonia was directly responsible in the observed loss of efficacy for this attractant. Consequently, one experiment was performed on the capture of *A. serpentina* flies in the sapodilla orchard in Apazapan in June 2022. A group of 10 bottle traps was initially loaded with 300 mL of alkaline hydrolyzed torula yeast (pH 13.3). These traps were protected with mosquito netting and hung on trees in the Apazapan orchard for three consecutive weeks. The upper part of the traps was covered by a plastic film to avoid changes in the attractant volume due to evaporation or the entry of rainwater. After this period, these bottle traps were prepared for the experiment by adjusting the attractant volume to 250 mL.

For the experiment, three treatments were prepared: (1) five bottle traps were used with no modification of the 3-week-old alkaline hydrolyzed torula yeast, (2) in the other five bottles, 1% ammonium acetate (2.5 g per trap) was added to the aged alkaline hydrolyzed torula yeast to increase the release of ammonia, and (3) five additional traps were baited with 250 mL of non-aged CeraTrap as a reference treatment. One bottle from each treatment was placed at intervals of 10–15 m in each of five different blocks (25 × 25 m) in the orchard. Blocks were separated by at least 20 m distance. Traps were randomly assigned to their initial position, hung at 3.5–4 m height and were rotated at 24-h intervals to the other positions. Daily captures of flies were placed in 70% alcohol and counted, identified, and sorted by sex

in the laboratory. Attractants were not renewed, and the experiment lasted three consecutive days. At the end of this period, attractants were collected and taken to the laboratory for ammonia quantification as described in the previous section.

Effect of propylene glycol and benzalkonium chloride on attraction over time

A final experiment was performed on *A. serpentina* flies in the sapodilla orchard in Apazapan in July 2022, to determine whether the attraction to alkaline hydrolyzed torula yeast over time could be improved by the addition of stabilizing agents. For this, the capture of flies was compared across four treatments: (1) alkaline hydrolyzed torula yeast alone, (2) alkaline hydrolyzed torula yeast + 10% propylene glycol, (3) alkaline hydrolyzed torula yeast + 240 mg L^{-1} benzalkonium chloride, and (4) CeraTrap as the reference treatment. These concentrations were selected based on the results of previous studies (Thomas et al., 2001). Bottle traps were baited with 250 mL of each attractant and placed in five blocks as described above. Traps were randomly assigned to an initial position within each block and were rotated at weekly intervals. Insects captured each week were placed in 70% alcohol and taken to the laboratory for counting, identification, and sexing. The experiment lasted 3 weeks and attractants were not renewed to determine their efficacy during this period.

Statistical analysis

Fly captures were converted to 'no. flies per trap per day' (FTD) values. Analyses were performed after examining the normality and equality of variances of data using the Shapiro-Wilk and Levene's test, respectively. Mean numbers of *Anastrepha* flies and the percentage of females captured in traps loaded with alkaline hydrolyzed torula yeast or Captor + borax were compared by paired t-tests for pairs of traps within the same experimental block, given their possible spatial correlation. FTD values for the three consecutive weeks of trapping were compared by repeated measures ANOVA after Mauchly's sphericity tests. Means of ammonia releases from traps after 3 weeks in the field were compared by t-test or Welch's t-test for unequal variances. The mean FTD values of trapped *A. serpentina* flies, percentage of females captured, and ammonia release from traps containing CeraTrap or aged alkaline hydrolyzed torula yeast with or without 1% ammonium acetate were subjected to non-parametric Kruskal-Wallis test and means were compared by Dwass-Steel-Critchlow-Fligner (DSCF) pairwise comparisons. Mean FTD values for *A. serpentina* captured weekly over three consecutive weeks in traps baited with CeraTrap or alkaline hydrolyzed torula yeast alone or formulated with propylene glycol or benzalkonium chloride were compared by repeated measures ANOVA after Mauchly's sphericity tests. Low numbers of other small dipterans were captured in traps, but captures were sporadic so were not subjected to analysis and are not considered further. All analyses were performed using the R-based package Jamovi v.2.3.20 (Jamovi, 2022).

Results

Alkaline hydrolyzed torula yeast vs Captor + borax

In total, 770 *Anastrepha* spp. flies were collected in the first field experiment in San Marcos.

Of these, 737 flies (96%) were *A. fraterculus* (591 females, 146 males), 32 were *A. striata* and one fly was *A. ludens*. Only *A. fraterculus* flies were included in the analysis. The FTD value was higher in alkaline hydrolyzed torula yeast than in Captor + borax traps (paired t-test: t = 4.51, d.f. = 7, P = 0.003; Figure 1). The mean (± SE) percentage of females captured was similar for alkaline hydrolyzed torula yeast (75 ± 3.4) and Captor + borax (82 ± 2.7) (t = 1.71, d.f. = 7, P = 0.13).

In the second field experiment in Jalcomulco, in total 117 *Anastrepha* spp. flies were trapped, of which 114 flies (97.4%) were *A. ludens* (96 females, 18 males) and three flies were *A. obliqua*. Only *A. ludens* flies were included in the analysis. The FTD values were higher in alkaline hydrolyzed torula yeast than in Captor + borax (t = 2.64, d.f. = 7, P = 0.033; Figure 1). The mean (± SE) percentage of females captured was similar for alkaline hydrolyzed torula yeast (81 ± 4.2) and Captor + borax (84 ± 6.8) (t = 0.36, d.f. = 7, P = 0.73).

In the third experiment in Apazapan, in total 2487 *Anastrepha* spp. flies were trapped, of which 2207 flies (88.7%) were *A. serpentina* (1661 females, 546 males), 279 flies were *A. obliqua*, and one fly was *Anastrepha pallens* Coquillet. Only *A. serpentina* flies were included in the analysis. The FTD values were higher in alkaline hydrolyzed torula yeast than in Captor + borax (t = 4.19, d.f.=7, P = 0.004) (Figure 1). The mean (± SE) percentage of females captured was similar for alkaline hydrolyzed torula yeast (77 ± 2.4) and Captor + borax (70 ± 2.9) (t = 2.34, d.f. = 7, P = 0.052).

Alkaline hydrolyzed torula yeast vs CeraTrap

For *A. obliqua* the FTD values calculated at weekly intervals along the 3-week study were similar for CeraTrap and alkaline hydrolyzed torula yeast (repeated measures ANOVA, treatment*time: $F_{1,40}$ = 1.164, P = 0.29; Figure 2). In contrast, FTD values were higher for CeraTrap than for alkaline hydrolyzed torula yeast for *A. ludens* (treatment*time: $F_{1,34}$ = 17.67, P < 0.001; Figure 2) and *A. serpentina* (treatment*time: $F_{1,34}$ = 14.68, P < 0.001; Figure 2).

After 3 weeks of exposure in the field, the ammonia released by traps baited with CeraTrap or alkaline hydrolyzed torula yeast were similar for *A. ludens* (t-test: t = 0.659, d.f. = 8, P = 0.53), *A. obliqua* (Welch's t = 1.16, d.f. = 4.96, P = 0.30), and *A. serpentina* (Welch's t = 1.05, d.f. = 4.08, P = 0.35) (Figure 3).

Addition of ammonium acetate to field-aged alkaline hydrolyzed torula yeast

In total, 430 *Anastrepha* spp. flies were collected in this experiment. Of these, 401 flies (93.3%) were *A. serpentina* (272 females, 129 males), and 29 *A. obliqua*. Only *A. serpentina* flies were included in the analysis. The FTD values differed among attractants (Kruskal-Wallis test: H = 31.2, d.f. = 2, P < 0.001). The FTD values were higher in CeraTrap than in traps baited with field-aged alkaline hydrolyzed torula yeast, with or without 1% ammonium acetate (Figure 4A). Addition of ammonium acetate did not improve the numbers of flies captured. The mean (± SE) percentage of females captured was higher for field-aged alkaline hydrolyzed torula yeast (83.3 ± 1.0) than for CeraTrap (66.6 ± 1.9) or field-aged alkaline hydrolyzed torula yeast + 1% ammonium acetate (72.1 ± 4.7) (H = 6.11, d.f. = 2, P = 0.047).

However, these percentages should be viewed with caution as field-aged alkaline hydrolyzed torula yeast captured just six flies, compared to the 372 and 23 flies trapped in CeraTrap and field-aged alkaline hydrolyzed torula yeast + 1% ammonium acetate, respectively.

The ammonia released by traps baited with alkaline hydrolyzed torula yeast + 1% ammonium acetate was higher than ammonia released from traps baited with CeraTrap, and was lowest in traps baited with field-aged alkaline hydrolyzed torula yeast alone (Kruskal-Wallis: H = 12.5, d.f. = 2, P = 0.002; Figure 4B).

Effect of propylene glycol and benzalkonium chloride on attraction over time

In total, 4504 *Anastrepha* spp. flies were captured in this experiment. Of these, 4229 flies (93.9%) were *A. serpentina* (2397 females, 1832 males), 274 *A. obliqua*, and one *A. pallens*. Only *A. serpentina* flies were included in the analysis. The FTD values calculated over the 3-week study varied significantly for CeraTrap compared to all the alkaline hydrolyzed torula yeast formulations (repeated measures ANOVA, treatment*time: $F_{3,56} = 12.7$, $P < 0.001$). Alkaline hydrolyzed torula yeast showed reduced efficacy in captures after 14 or 21 days in the field, irrespective of the addition of polyethylene glycol or benzalkonium chloride (Figure 5). The mean (± SE) percentage of females trapped differed between CeraTrap (61.4 ± 2.0) and alkaline hydrolyzed torula yeast + polyethylene glycol (43.9 ± 6.4) (H = 8.45, d.f. = 3, P = 0.038), but was similar to that of field-aged alkaline hydrolyzed torula yeast (57.8 ± 5.2) or alkaline hydrolyzed torula yeast + benzalkonium chloride (56.0 ± 4.0).

Discussion

The use of protein food lures to monitor fruit flies of the genus *Anastrepha* is necessary because no sexual attractants have yet been developed commercially (Epsky et al., 2014). In general, protein attractants are considered weak and the response of flies to them are variable (Díaz Fleischer et al., 2014; Epsky et al., 2014). Based on many investigations, torula yeast + borax pellets, acid hydrolyzed protein + borax, and CeraTrap prevail as the most common current attractants used for monitoring *Anastrepha* flies in the Americas.

The study of alkaline hydrolyzed proteins has attracted little interest from researchers. Our field experiments demonstrated that alkaline hydrolysis of torula yeast at high pH (13.3) was significantly more effective for the capture of *A. fraterculus*, *A. ludens*, and *A. serpentina* in the short term than the commonly used acid hydrolyzed protein Captor + borax. These results are in accordance with a previous study in which alkaline hydrolyzed torula yeast proved more effective at capturing *A. obliqua* flies than Captor + borax after a 1-week period in mango orchards (Lasa & Williams, 2022).

Alkaline hydrolyzed torula yeast was as attractive as CeraTrap for *A. obliqua* and *A. serpentina* in the 1st week of use but not for *A. ludens.* Species-specific differences in responses to CeraTrap could be explained by reference to previous studies in which the capture of flies was about 10-fold higher using CeraTrap than Captor + borax for *A. ludens* (Lasa et al., 2015), but only ca. 3-fold higher when evaluated with *A. obliqua* and *A. serpentina* (Lasa & Cruz, 2014; Rodríguez et al., 2015). Compared to the initial attraction, fly captures were significantly reduced in the 2nd and 3rd weeks of field use, an effect not

observed for CeraTrap. This reduction in the efficacy of attractants over time could not be directly attributed to a reduced release of ammonia, as CeraTrap also emitted a low level of this gas.

Ammonia has been considered to be a key compound in the attraction of tephritids (Bateman & Morton, 1981; Mazor et al., 1987, 2002). However, there is increasing evidence that ammonia alone does not fully account for the attraction of flies, and other compounds are influential in the attraction response (Matsumoto et al., 1985; Flath et al., 1989; Piñero et al., 2020; Lasa & Williams, 2021a). A ca. 50% reduction of the amount of ammonia released by traps baited with alkaline hydrolyzed torula yeast and Captor + borax was previously reported after 7 days of use in the field, although ammonia was not quantified at later time points (Lasa & Williams, 2022). At 24 h after preparation, alkaline hydrolyzed torula yeast (pH 13.3), released an average of 28.2 µg h^{-1} of ammonia (Lasa & Williams, 2022). This contrasts with the emission from field-aged alkaline hydrolyzed torula yeast after 3 weeks which ranged from 2.7 to 6.3 µg h^{-1} (Figure 3). Similarly, the initial ammonia release by CeraTrap (20.2 µg h^{-1}) was reduced to 3.2–3.4 µg h^{-1} after 3 weeks in the field. Despite this, the mean capture of *Anastrepha* flies, was up to 9-fold higher for CeraTrap than for alkaline hydrolyzed torula yeast after this period of time. These results are a further indication that ammonia is not the main olfactory cue responsible for the attraction of *Anastrepha* species, as stated above. Moreover, the addition of ammonium acetate to the field-aged alkaline hydrolyzed torula yeast did not improve the capture of flies despite a large increase in the release of ammonia from the traps treated with this compound. Similar results were observed for *A. obliqua* and *A. serpentina* when ammonium acetate was added into the standard Captor + borax mixture, or when included as a dry powder inside the trap (Lasa & Williams, 2021b).

Evidence of the decomposition of alkalized torula yeast was not perceived after 7 days of field use but a putrid smell and cloudy appearance typical of microbial decomposition was detected after 2–3 weeks of field use. Microbial proliferation can be an important factor in the loss of efficacy of fruit fly attractants (Green et al., 1960). Putrefaction and turbidity have not been observed in CeraTrap, which presumably contains one or more preservatives that allow it to retain its efficacy for up to 10 weeks (Lasa et al., 2014). Propylene glycol has been used to reduce evaporation, but also as a preservative for captured flies (Thomas et al., 2001). The addition of propylene glycol to torula yeast pellets did not affect the capture of *Anastrepha* flies and improved the stability of this attractant (Thomas & Robacker, 2006). Similarly, the biocidal compound benzalkonium chloride was also evaluated in mixtures of hydrolyzed protein + borax and found to stabilize the attractancy of the lure for several weeks (Lasa & Williams, 2017). In contrast, in the present study the addition of propylene glycol or benzalkonium chloride to alkaline hydrolyzed torula yeast failed to stabilize this attractant over time. We suspect that the loss of attractancy of alkaline hydrolyzed torula is less due to microbial degradation, but rather to rapid volatilization of one or more highly attractive compounds. For example, both protein-borax mixtures and alkaline hydrolyzed torula yeast retained efficacy for about 1 week. During this time, key compounds such as pyrazines (Flath et al., 1989) or other compounds formed in combination with ammonia during the hydrolysis process could be released rapidly from the trap. Once released, these compounds may not be

regenerated in the attractant due the chemical nature of hydrolysis. This could explain why propylene glycol or benzalkonium chloride formulations did not improve the attractancy over time.

Fly captures were biased in favor of females for all species and all the attractants tested, including CeraTrap. The capture of high numbers of females is common in protein baits (Pinero et al., 2002; Conway & Forrester, 2007; Lasa et al., 2013, 2014), as females tend to seek sources of protein as a critical nutrient for ovary maturation and egg production (Aluja et al., 2001; Mangan, 2003).

CeraTrap is superior for catching *Anastrepha* flies and has a longer lasting attraction when compared with other protein compounds. However, alkaline hydrolyzed torula yeast resulted more effective in the capture of *Anastrepha* flies in the 1st week than the acid hydrolyzed protein Captor + borax, a standard attractant commonly used for short monitoring of *Anastrepha* flies in Mexico. Sodium hydroxide is a cheap and readily available compound that is highly effective at protein hydrolysis. A pH of 13.3 can be achieved with a low (ca. 1%) concentration so that alkaline hydrolyzed torula yeast is likely to be cheaper than CeraTrap, protein-borax mixtures, torula yeast + borax pellets, or ammonium-based sachets such as BioLure.

Conclusions

Due to the high attraction of *Anastrepha* spp. flies during the 1st week of use, alkaline hydrolyzed torula yeast could be employed effectively for monitoring purposes although its low stability would hinder its use in mass-trapping control strategies. Future studies should examine alkaline hydrolysis of other proteins and their attractiveness to flies of other tephritid genera such as *Ceratitis* and *Rhagoletis*, that are also monitored using food attractants. Studies on the functional diversity of compounds produced through alkaline hydrolysis of proteins may also provide insights into key volatiles that elicit the attraction of tephritid pests.

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CONFLICT OF INTEREST

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AUTHOR CONTRIBUTION

RL and TW conceived research. RL conducted experiments. RL contributed material. TW and RL analyzed data and conducted statistical analyses. RL and TW wrote the manuscript. RL secured funding. Both authors have read and approved the manuscript.

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Figure Legends

Figure 1 Mean number of flies per trap per day (FTD) captured in traps containing Captor + borax or alkaline hydrolyzed torula yeast (pH 13.3) at weekly intervals in experiments involving *A. fraterculus* (A), *A. ludens* (B) and *A. serpentina* (C). The mean percentage of females trapped in each attractant is included inside columns. Vertical bars indicate SE. FTD values labeled with different letters differ significantly (paired t test, $p < 0.05$).

Figure 2 Mean number of flies per trap per day (FTD) captured in traps loaded with CeraTrap® or alkaline hydrolyzed torula yeast (pH 13.3) in samples taken over three consecutive weeks in field experiments involving *A. obliqua* (A), *A. ludens* (B) and *A. serpentina* (C). Vertical bars indicate SE.

Figure 3 Mean quantities (± SE) of ammonia released by traps containing CeraTrap® and alkaline hydrolyzed torula yeast (pH 13.3) after three weeks in the field in experiments involving *A. ludens* (A), *A. obliqua* (B) and *A. serpentina* (C). NS indicates no significant differences (t-test or Welch's t-test, $p > 0.05$).

Figure 4 Captures of flies and ammonia production in traps baited with CeraTrap or aged hydrolyzed torula yeast with or without the addition of ammonium acetate. (A) Median (line) and interquartile range (box) of *A. serpentina* captured in the different attractants. (B) Median (line) and interquartile range (box) of ammonia quantities released in the different attractants. Flies captured (A) and quantity of ammonia (B) labeled with different letters differed significantly (Kruskal-Wallis, $p < 0.05$).

Figure 5 Mean number of *A. serpentina* flies captured per trap per day (FTD) in traps containing CeraTrap® or alkaline hydrolyzed torula yeast (pH 13.3) alone or formulated with propylene glycol or benzalkonium chloride over three consecutive weeks. Vertical bars indicate SE.

Fig 1

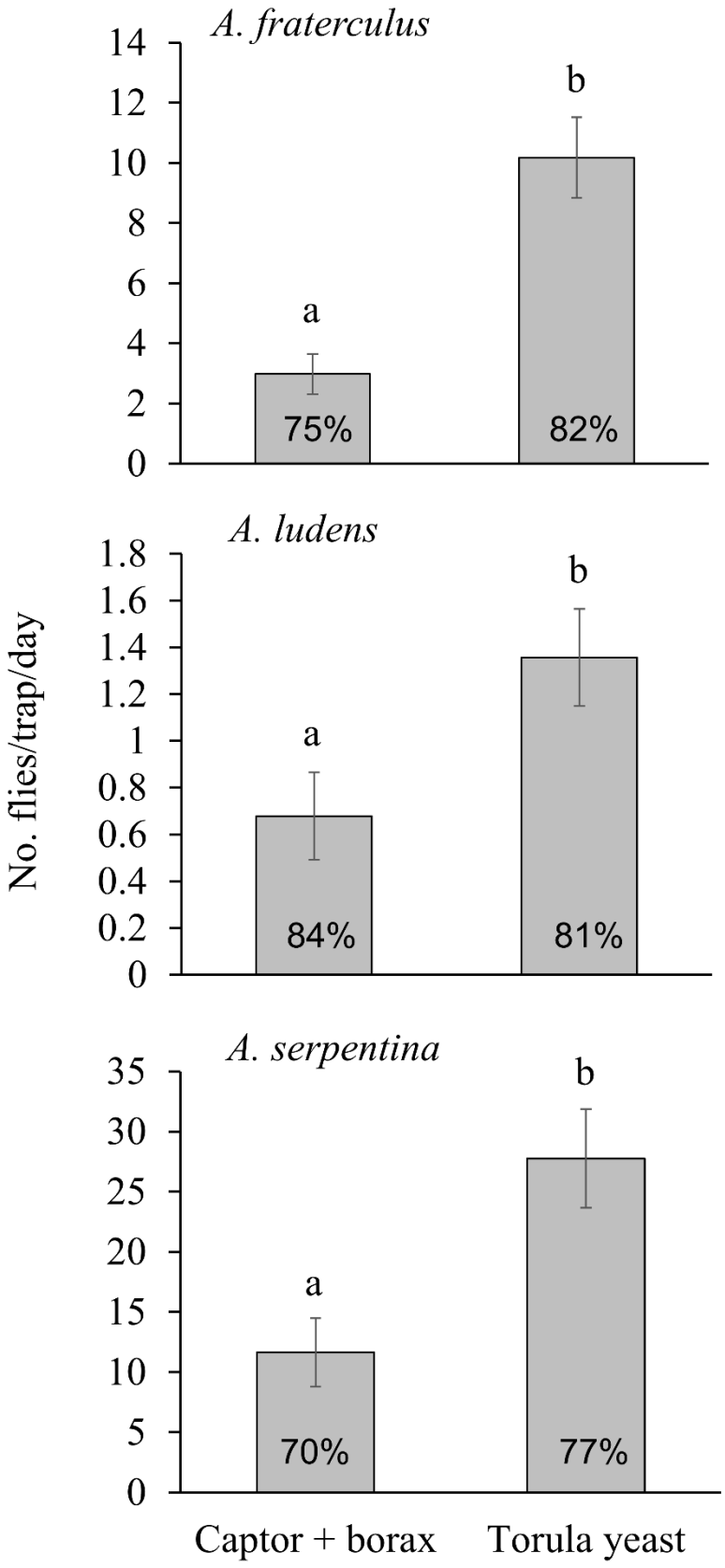
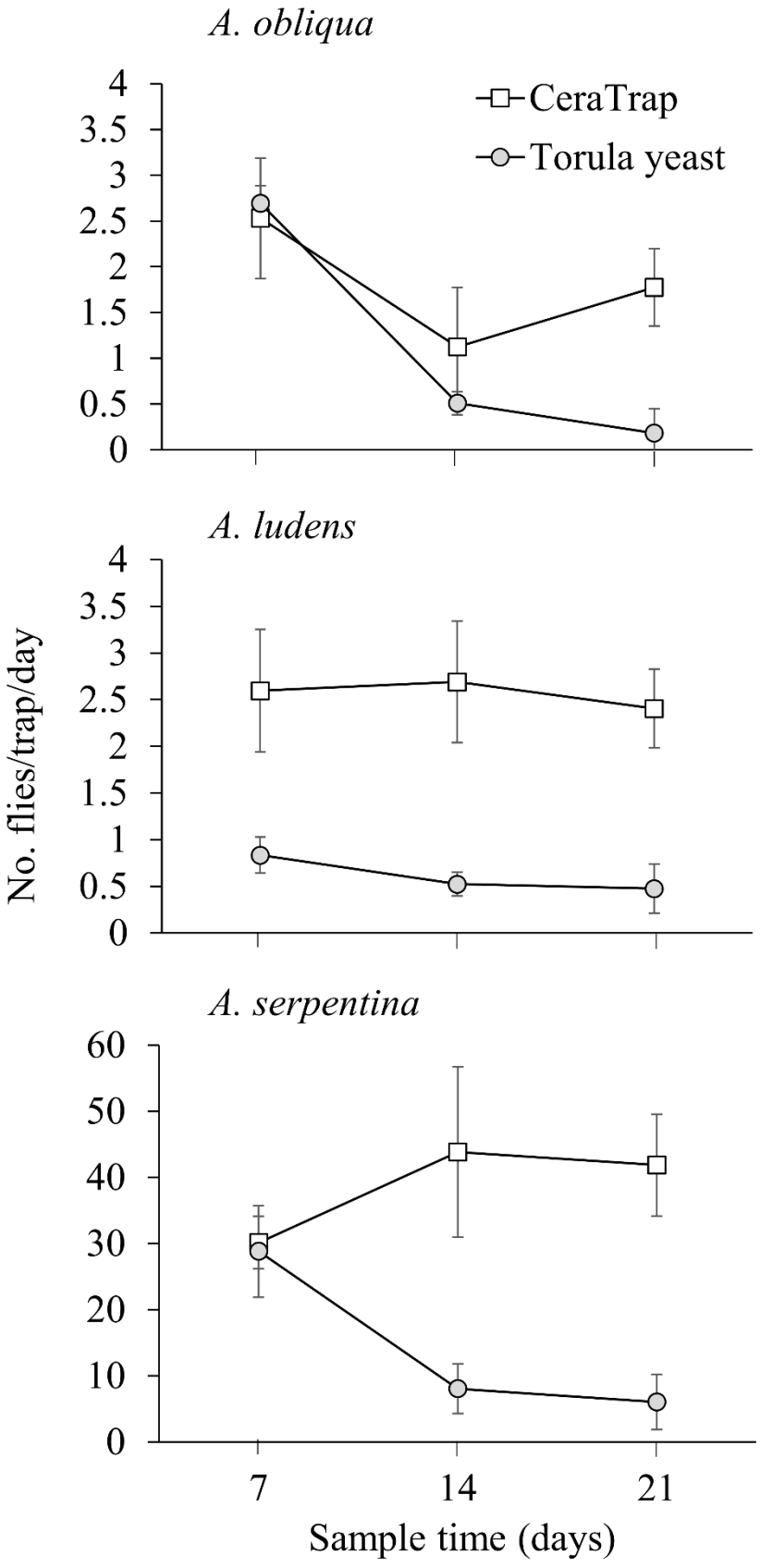

A. fraterculus
14
12
10
8
6
4
2
0
a
b
75%
82%
A. ludens
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
a
b
84%
81%
No. flies/trap/day
A. serpentina
35
30
25
20
15
10
5
0
a
b
70%
77%
Captor + borax
Torula yeast

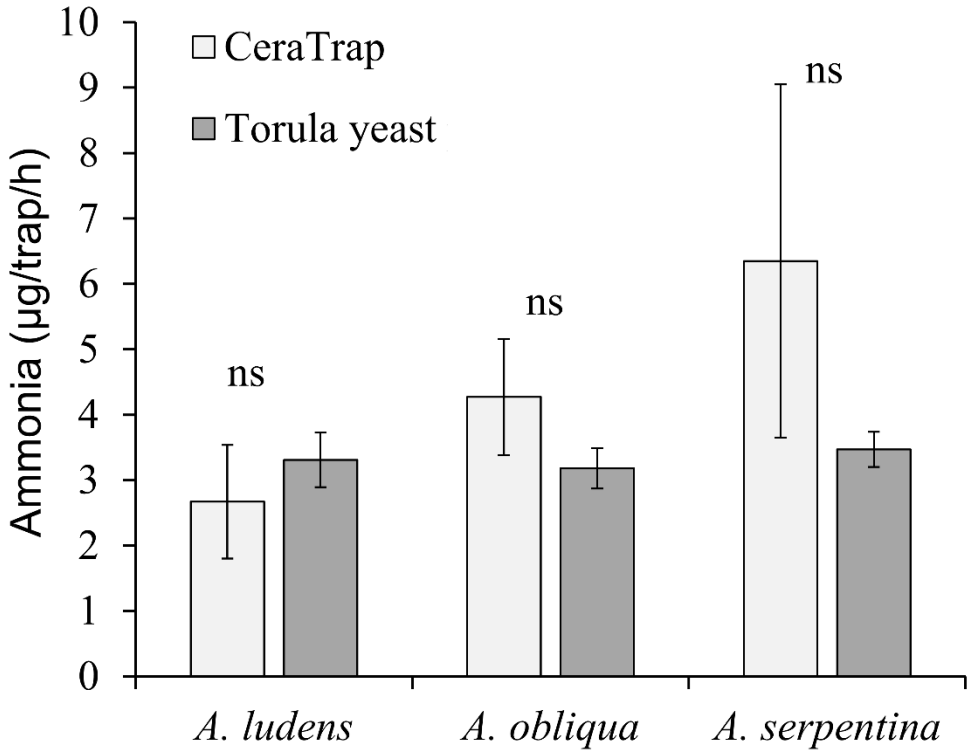
Fig 2


A. obliqua
CeraTrap
Torula yeast
4
3.5
3
2.5
2
1.5
1
0.5
0
A. ludens
No. flies/trap/day
A. serpentina
60
50
40
30
20
10
0
7
14
21
Sample time (days)

1 Fig 3

2

3


10
9
8
7
6
5
4
3
2
1
0
Ammonia (µg/trap/h)
CeraTrap
Torula yeast
ns
ns
ns
A. ludens
A. obliqua
A. serpentina

1 Fig 4

2

3

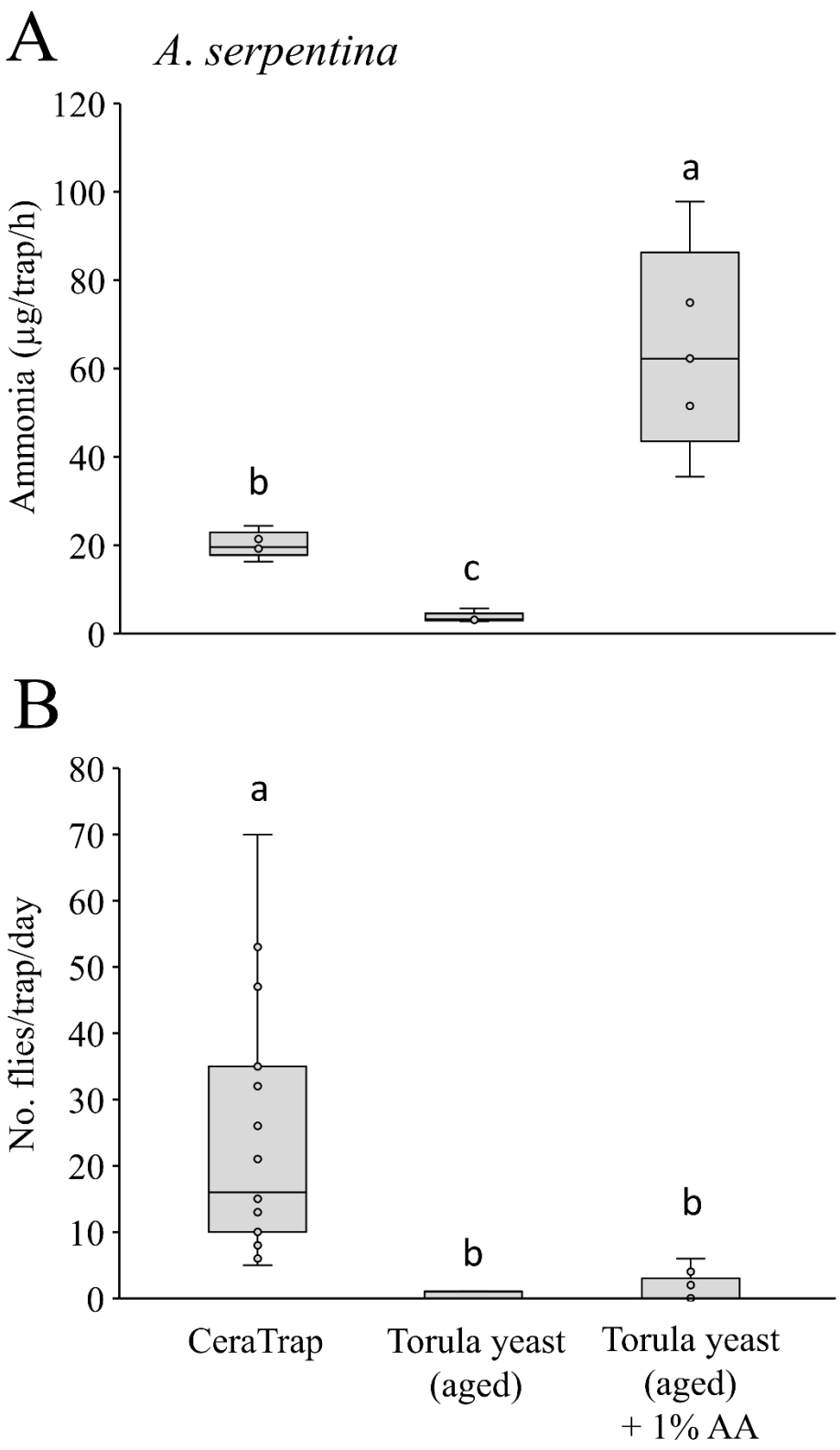
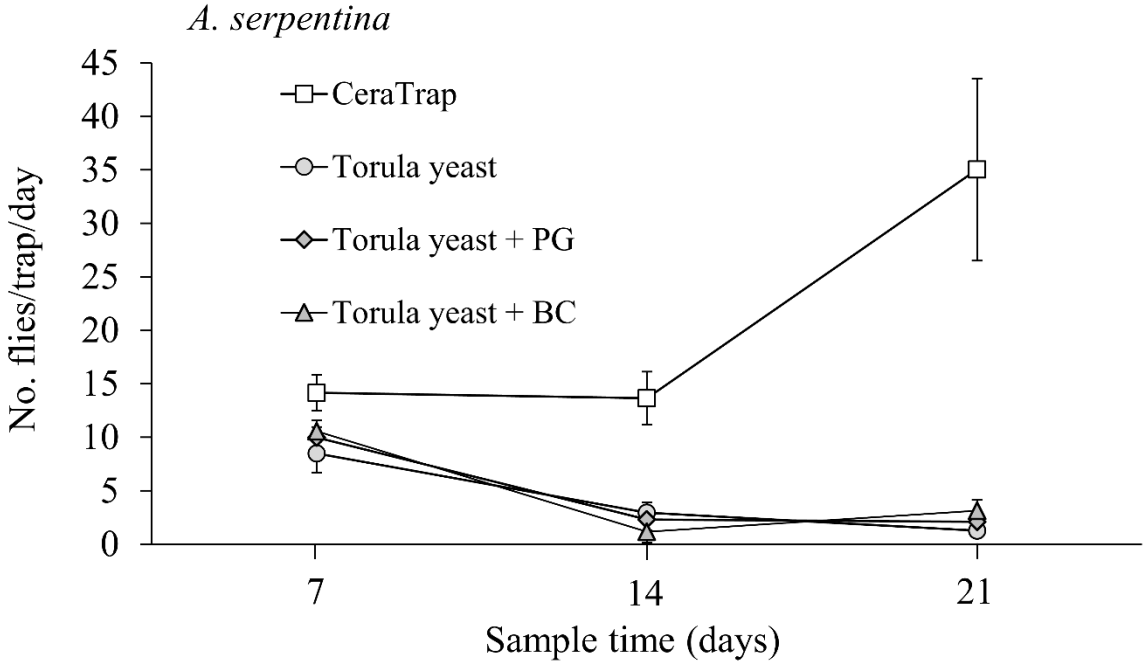

A
A. serpentina
Ammonia (µg/trap/h)
120
100
80
60
40
20
0
a
b
c
B
No. flies/trap/day
80
70
60
50
40
30
20
10
0
a
b
b
CeraTrap
Torula yeast (aged)
Torula yeast (aged) + 1% AA

Fig 5


A. serpentina
45
40
35
30
25
20
15
10
5
0
No. flies/trap/day
CeraTrap
Torula yeast
Torula yeast + PG
Torula yeast + BC
7
14
21
Sample time (days)