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Benzalkonium Chloride Provides Remarkable Stability to Liquid Protein Lures for Trapping *Anastrepha obliqua* (Diptera: Tephritidae)

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Abstract

Hydrolyzed protein lures are widely used to monitor fruit fly pests but are rapidly degraded by microbial activity and must be replaced frequently. To improve the stability of lures, the quaternary ammonium biocide, benzalkonium chloride (BC), was evaluated in mixtures with two hydrolyzed proteins commonly used to monitor *Anastrepha* spp. The mean number of *Anastrepha obliqua* adults captured during six consecutive weeks using Captor + borax with the addition of 240 mg BC/liter, not renewed during the test, was similar to Captor + borax that was replaced at weekly intervals and was more effective than Captor + borax without BC. Numbers of *A. obliqua* flies captured in 30% CeraTrap diluted in water containing 240 mg BC/liter were similar to those caught in traps baited with Captor + borax or 30% CeraTrap without BC in the first 9 d of evaluation but was significantly more effective than both lures after 56 d. After >2 mo of use, 30% CeraTrap containing 240 mg BC/liter remained as effective as newly prepared 30% CeraTrap. The addition of BC to lures reduced surface tension of liquid lures by ~40–50%. However, when BC was increased to 720 mg BC/liter, only a small additional reduction in surface tension was observed and higher concentrations of BC did not increase capture rates. These findings could contribute to reduced costs for trapping networks and the development of long-lasting formulations of liquid protein lures for bait stations and mass-trapping targeted at major tephritid pests.

Key words: Quaternary ammonium, mass trapping, monitoring

Food lures represent one of the best attractants for monitoring and control of tephritid fruit pests of the genus *Anastrepha*, for which pheromone-based lures are not available. Even though several dry lures based on ammonium salts have been evaluated for *Anastrepha* spp., results have been contradictory, although in most cases liquid proteins have proved to be more effective than dry baits, especially in tropical areas (Heath et al. 1995, 1997; Epsky et al. 2003; Pingel et al. 2006; Conway and Forrester 2007; Thomas et al. 2008). Liquid protein lures have been used for more than 70 yr, although these products degrade rapidly in the field and require replacement usually at intervals of 1 or 2 wk. For example, aqueous solutions of 1% hydrolyzed protein can quickly change color and become putrefied in the field, increasing the attraction of nontarget insects and resulting in the disintegration of captured flies in the liquid, which hinders their subsequent identification. To address this problem, López and Hernández-Becerril (1967) evaluated 76 chemicals as additives for liquid proteins and concluded that borax was the best compound, favoring catches and reducing the rate of protein degradation. Borax has therefore become the most common product mixed with proteins worldwide because, in addition to its low cost and some preservative effect, the mixture increases the pH of the bait lure and promotes the emission of volatiles that improve attraction (Lopez and Henández-Becerril 1967, Bateman and Morton 1981, Flath et al. 1989, Epsky et al. 1993, Heath et al. 1994). However, the inclusion of borax in liquid lures rarely provides more than 2 wk of protection against protein degradation and does not afford long-lasting attraction needed for mass-trapping purposes.

In recent years, the commercial protein lure, CeraTrap, has proved to be highly attractive for several *Anastrepha* species (Lasa and Cruz 2014, Lasa et al. 2015, Rodriguez et al. 2015) and, in contrast to other protein lures, this lure retains high stability and remains attractive during periods exceeding more than 4–6 wk (Lasa et al. 2014, 2015; Lasa and Cruz 2014). This has favored its use in mass-trapping control strategies that were not previously possible with liquid protein lures (Lasa et al. 2014). Protein stability of this product under field conditions appears to be related to the presence of preservatives in the formulation, which prevent microbial growth and limit product degradation over time. In a previous field test, the attraction of *Anastrepha ludens* (Loew) to 50% CeraTrap diluted in water, remained at similar levels as those observed for undiluted product. However, microbial degradation was evident after 3 wk under field conditions (Lasa et al. 2015), suggesting that this effect could be a result of the dilution of the preservatives present in the commercial formulation.

Quaternary ammonium compounds are biocidal compounds that are widely used due to a combination of properties: they have broad spectrum microbiological activity (including bactericidal, fungicidal, and virucidal activity), activity over the entire pH range, low human toxicity, are nonvolatile, have high aqueous solubility, high surface activity, low cost, and are colorless and odorless (Schaeufele 1984). Since its discovery in 1935, benzalkonium chloride (BC), has been one of the most commonly used quaternary ammonium compounds worldwide, although new generation variants of these compounds have been developed subsequently (Schaeufele 1984). BC is commonly used in cleaning products, swimming pool algaecides, sanitizers, and topical antiseptics (Schaeufele 1984) and is also used to inhibit microbial growth in products such as eyedrops, nasal sprays, and cosmetics (Liebert 1989).

This study aimed to assess the effect of low concentrations of BC when mixed with protein lures. This effect was firstly evaluated with Captor + borax. An additional experiment evaluated the attraction of this protein when mixed with three different concentrations of BC. Finally, the long-term attraction of diluted CeraTrap containing BC was evaluated and compared with the same dilution of CeraTrap and a standard Captor plus borax treatment. The capture efficacy of diluted CeraTrap in mixtures with BC was also compared with newly diluted CeraTrap under field conditions. A final test evaluated the surface tension of protein lures with or without the addition of BC.

Experimental Methods

Trap, Lures, and Biocide

Two different commercial lures were used in field experiments: Captor 300 (Promotora Agropecuaria Universal, Mexico City, Mexico) and CeraTrap (Bioibérica, Barcelona, Spain). Captor is a chemically hydrolyzed protein of plant origin that is prepared by mixing 10 ml of the commercial product, supplied at a concentration of 360 g active ingredient (a.i.)/liter, with 5 g of borax (J. T. Baker, Mexico City), and 235 ml water, as indicated in the Mexican Federal Standard for this kind of fruit fly lure (Anonymous 1999). In contrast, CeraTrap is an enzymatically hydrolyzed protein of animal origin commercially supplied at a concentration of 5.9% for use without dilution.

The MS2 fruit fly trap (Fitozoosanitaria S.A. de C.V., Texcoco, Mexico) was used in all experiments. This trap is a two-component bottle-shaped device comprising a yellow base covered with a transparent top that has three 11-mm diameter circular holes spaced 5-cm apart. This trap has been supplied in Mexico to hold 250 ml of CeraTrap lure. The biocide use in experiments was BC (80% purity, Productos Biológicos Hyla, Azcapotzalco, Mexico).

Efficacy of BC in Long-Term Attraction Of Captor + Borax Lure

A test was performed during May–July 2016 in a mixed area of mango, *Mangifera indica* L. and tropical plum, *Spondias purpurea* L., located near Jalcomulco (N 19° 19′ 40.29″; W 96° 45′ 25.73″) in central Veracruz State, Mexico. Jalcomulco is characterized by a subhumid warm climate with mean temperatures between 25.7 and 27.4°C (average over 20 yr) during the months of June–September when the tests were performed. Precipitation varies between 161 and 200 mm/mo during this period. The objective of this experiment was to evaluate the efficacy of BC in extending the duration for which each lure remained attractive. For this, a concentration of 240 mg BC/liter of benzalkonium chloride was initially selected based on its biocidal effect at this concentration (Heinzel 1999) and also because high repellency to flies was observed in preliminary trials when used at a concentration of 8000 mg a.i./liter, representing 1% of the commercial product (Supplemental Data [online only]).

Seven different blocks, about 20–40 m apart, contained three MS2 bait station traps each baited with 250 ml of one of three different treatments: 1) 4% Captor + borax that was replaced every 7 d as indicated in the Mexican standard formula (Anonymous 1999), 2) 4% Captor + borax that was not replaced with fresh lure during the 6-wk test period, and 3) 4% Captor + borax with the addition of 240 mg BC/liter that was also not replaced with fresh lure during the test. After weekly servicing of each trap, the volume of lures was maintained at 250 ml by the addition of 15–25 ml of Captor + borax or Captor + borax + 240 mg BC/liter with a batch of the respective lure mixture prepared at the beginning of the test for this purpose and maintained under laboratory conditions, that was used to topup traps during the complete experiment. In the treatment in which Captor + borax mixtures were replaced at weekly intervals, the lure was prepared the day before use in the field. Traps were hung at random points on mango trees at 3–3.3 m height within a distance of 12–15 m between traps. Traps were checked every 7 d and rotated weekly, and this continued for the 6-wk experimental period. Captured insects were placed in 70% ethanol and transported to the laboratory where they were counted and identified to species and sex.

Attraction of Captor + Borax with Different Concentrations Of BC

Considering the high capture levels observed in Captor + borax + BC at the beginning of the previous experiment, a new trial was performed to evaluate the effects of different concentrations of BC in the Captor + borax mixture. This test was performed in June 2016 in a mango–tropical plum area located at a distance of 5 km from the previous experiment (N 19°20′42.89″; W 96°46′15.52″). Four MS2 bait station traps were placed in each of six blocks, about 20 m apart, within the orchard area. Each trap was baited with 250 ml of one of four different treatments: 1) 4% Captor + borax, as the standard lure, 2) 4% Captor + borax with 80 mg BC/liter, 3) 4% Captor + borax with 240 mg BC/liter, and 4) 4% Captor + borax with 720 mg BC/liter. All traps were initially baited with 250 ml of lure. The standard Captor + borax lure without BC was renewed every 8 d. The Captor + borax mixture used to replace this treatment was prepared the day before use in the field. Treatments containing BC were not renewed, but the volume was maintained at 250 ml, to make up for evaporational losses, by the addition of 15–40 ml of the corresponding lure at each inspection. Batches of Captor + borax containing each concentration of BC were prepared once at the start of the experiment and used to topup traps as necessary. Traps were randomly hung at heights of 2.5–3.5 m above the ground on different trees within a block, at a distance of 12–15 m between traps. Traps were checked every 4 d and rotated at each inspection throughout the 16-d experimental period (one time point per position). Captured insects were transported to the laboratory in 70% ethanol, counted, and identified to species and sex.

Efficacy of BC in Extending Attraction to Diluted CeraTrap

A new test was developed during July–September 2016 to evaluate the effect of BC on the duration of attraction to diluted CeraTrap. This trial was performed in the same six blocks of mango-tropical plum orchard described previously in the second experiment. Three treatments were compared: 1) 4% Captor + borax, 2) 30% CeraTrap diluted in water, and 3) 30% CeraTrap diluted in water with 240 mg BC/liter. Traps were placed on 8 July 2016, and captured flies were counted and identified every 3 d during a 9-d period. Traps were not topped-up with lures after service. During this time, traps were rotated clockwise so that each trap was at each position for one 3-d period, to avoid position effects. Traps then remained in the orchard without revision until 24 August, at 47 d after initial deployment. At this moment, flies trapped in each treatment were collected, placed in 70% ethanol, counted, and identified. After this period, traps were maintained in the orchard during an additional period of 18 d (24 August to 11 September), during which time traps were revised and rotated one position every 6 d. This final evaluation period with trap rotation, allowed an estimation of fly attraction of lures after the prolonged period of exposure to field conditions.

Comparison of Field-Exposed 30% CeraTrap + BC Versus New 30% CeraTrap

The 30% CeraTrap + BC lure that had been exposed to field conditions in the previous experiment was collected from all traps and mixed together in a plastic bottle with a total volume of 800 ml (mean 53% recovery). The product was stored in the laboratory during 1 wk before use in the following experiment. Lures that did not contain BC in the previous experiment were discarded because they had developed a putrefied odor and had very low attraction to flies at the end of the previous experiment. A trial started on 19 September 2016 to compare the remaining attraction of the 30% CeraTrap + BC that had been exposed to field conditions for 2 mo, with a newly prepared dilution of 30% CeraTrap. The trial was conducted in an area of 0.8 ha in the same mango-tropical plum orchard used previously in the second trial. A total of six MS2 traps, three baited with 250 ml of each lure, were hung randomly on different trees with a distance of at least 10 m between traps. Traps were initially positioned at random and subsequently randomly changed to a new position at inspection. Traps were checked every 3 d during a period of 9 d. Captured flies were taken to the laboratory in 70% ethanol, counted, and identified to species and sex.

Surface Tension of Lures with or Without BC

The surface tension of liquid lures could affect their ability to retain flies that enter the trap. Surface tension was determined by the drop number method using Traube's stalagmometer technique. This method is often used to measure the relative surface tension in relation to the surface of a standard liquid, usually distilled water (Findlay 1945). Several water and liquid protein formulations (Table 4) were measured in relation to the surface tension of distilled water at 20°C, which has a surface tension of 72.8 dyne/cm. Samples of 200 ml of the protein lures were prepared using potable (bottled) water that was used to prepare the lures tested previously. A Traube´s stalagmometer (Alamo, Madrid, Spain) was cleaned, dried, and mounted vertically using a burette stand for measurements. After filling the stalagmometer, the number of drops between two marked positions was counted serially for all liquids. Five samples were measured for each liquid lure. Surface tension was obtained following the formula: surface tension of protein lure, ST1 = ST2 ($n2/n1$)·(d1/d2), where $n1$ is no. of drops of protein lure, d1 indicates density of protein lure at room temperature (20°C), ST2 denotes surface tension of distilled water at 20°C, $n2$ is no. of drops of water, and d2 is density of distilled water at 20°C.

The density of each protein lure was measured using a calibrated micropipette and a precision electronic balance to an accuracy of 0.1 mg with five samples of 1 ml each.

Statistical Analyses

Total numbers of *Anastrepha obliqua* adults trapped in each trap were transformed to flies per trap per day (FTD) and were transformed $\sqrt{(x + 0.5)}$ to stabilize variance or normalized by rank transformation before being subjected to a factorial two-way analysis of variance (ANOVA) with treatment and block as factors. Time (weeks) was not included as a factor because preliminary analyses indicated that FTD values did not vary significantly over time. Separation for all ANOVA analyses was performed by Tukey's honestly significant difference. FTD values during the 9- to 47-d period in experiment 3, during which traps were not rotated, were rank transformed and subjected to one-way ANOVA. A *t*-test was used to compare field-exposed 30% CeraTrap + BC versus new 30% CeraTrap. Surface tension of all measured lures was normalized by rank transformation and subjected to one-way ANOVA. Analyses were performed using SPSS v.17 (SPSS Inc., Chicago, IL). The percentage of females captured was analyzed by fitting generalized linear models with a binomial error distribution specified in GLIM 4 (Numerical Algorithms Group 1993). Minor overdispersion was taken into account by scaling the error distribution where necessary (Crawley 1993). The significance of changes in model deviance due to treatment effects following sequential removal of terms was interpreted with reference to the χ^2 distribution or the F distribution in the case of scaled analyses (Crawley 1993). Means were compared by *t*-test. The standard errors (SEs) of binomially distributed means are asymmetrical and are shown as range of SE.

Results

Efficacy of BC in Long-Term Attraction of Captor Plus Borax Lure

A total of 2434 *Anastrepha* fruit flies were collected in traps over the 6-wk period. Of these, the majority (99.8%) was *A. obliqua,* which were used for FTD calculations. Of the *A. obliqua* adults, only 17 flies (<1%) had degraded and were identified by the presence of their wings, so their sex could not be determined. Among the sexed flies, 71.1% were females and 28.9 % males.

The FTD values differed significantly among lures (F = 4.78; df = 2, 105; P = 0.010). The mean FTD value of Captor + borax with BC was over 40% higher than observed when Captor + borax mixture was renewed on a weekly basis, although this difference was not significant (Table 1). A significant block effect was observed (F = 17.01; df = 6, 105; P < 0.001), but no significant effect was observed for the block*lure interaction (F = 1.42; df = 12, 105; P = 0.169). The Captor + borax lure that was not renewed had a lower mean capture than that of Captor + borax + BC, although it was not significantly different to the mean capture of the renewed Captor + borax treatment (Table 1). The cumulative FTD value for *A. obliqua* was higher with Captor + borax + BC than with the other treatments (Fig. 1). The percentage of females trapped in the Captor + borax with BC was female biased (63.1% female) but was significantly lower than when BC was not present in the lure (F = 19.88; df = 2, 124; P < 0.001) (Table 1).

Table 1. Mean FTD (± SE) and percentage of females (range of SE) of *Anastrepha obliqua* and total flies trapped during a 6-wk experiment Captor + borax lures, with or without benzalkonium chloride (BC)

Lure	Mean FTD	% Females	Total no. flies
Captor + borax, replaced weekly	2.6 ± 0.3 ab	77.2 (75.3–78.4) a	766
Captor + borax + BC[a], not replaced	3.5 ± 0.5 a	63.1 (60.7–65.4) b	1035
Captor + borax, not replaced	2.1 ± 0.3 b	76.9 (75.0–78.7) a	628

Mean FTD values in columns followed by the same letter were not significantly different (ANOVA; Tukey test, $P > 0.05$). Mean percentages of females were compared by *t*-test (GLM; $P > 0.05$, scale parameter 1.42).
[a]BC was included at 240 mg/liter.

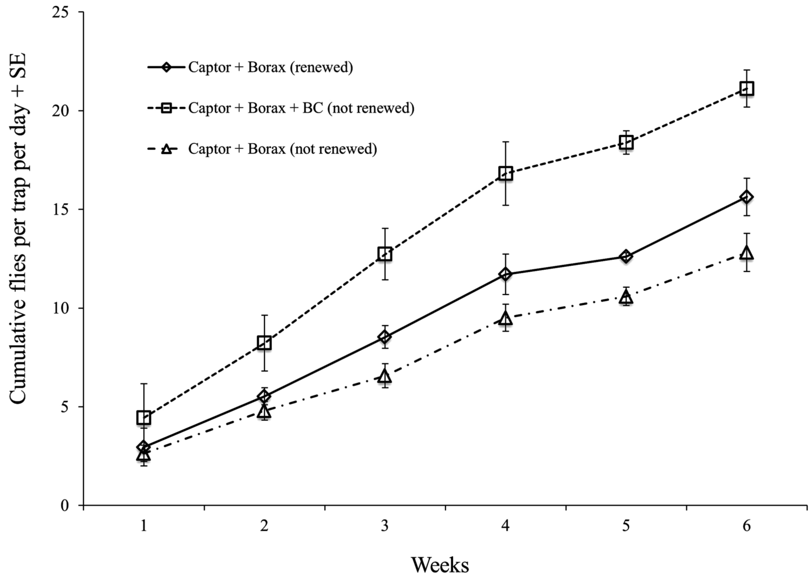

Fig. 1. Cumulative number of *Anastrepha obliqua* flies captured per trap per day (FTD ± SE) with Captor + borax lure replaced weekly and Captor + borax with and without addition of BC (240 mg/liter), that were not replaced during a 6-wk trial in a mango and tropical plum orchard.

Effect of BC Concentration on Attraction of Captor + Borax

A total of 2187 fruit flies were collected in traps over the 16-d experiment, 99.9% of which were *A. obliqua*. Only nine flies were identified by the presence of their wings and their sex could not be determined. Among the sexed flies, 62.8% were females and 37.2 % males. The mean FTD values did not differ significantly among treatments ($F = 0.024$; df = 3, 72; $P = 0.995$) (Table 2). That is, the treatments involving Captor + borax with BC at three different concentrations were as attractive as the standard Captor + borax renewed at weekly intervals (Table 2). As observed in the previous trial, the percentage of females trapped was ~10% lower in treatments containing BC ($F = 3.42$; df = 3, 95; $P < 0.05$), compared with the standard Captor + borax treatment (Table 2).

Efficacy of BC on Long-Term Attraction to Diluted CeraTrap

A total of 3619 fruit flies were collected in traps over the 9-wk experimental period. Almost all captured flies were *A. obliqua* (99.5%), whereas a small numbers of *Anastrepha serpentina* and *A. ludens* (total 17 flies) were also captured but were not considered in FTD calculations. Among the trapped *A. obliqua* flies, sex could not be determined in nine flies that were identified by the presence of their wings. Among the sexed flies, 62.8% were females and 37.2% males. At the beginning of the experiment, from day 1 to day 9, the mean *A. obliqua* FTD value differed significantly among lures ($F = 3.44$; df = 2, 36; $P = 0.043$) with a higher capture in traps baited with 30% CeraTrap than in traps baited with Captor + borax, although no significant differences were observed between 30% CeraTrap with BC and the other two lures (Table 3). After an additional period of 47 d, the mean FTD values of the 30% CeraTrap lure, with or without BC, were significantly superior to that of Captor + borax ($F = 8.02$; df = 2, 15; $P = 0.004$). FTD values did not differ significantly between CeraTrap lures with or without BC during this period (Table 3). Finally, the captures obtained at the end of the experiment (47 to 56 d), indicated that 30% CeraTrap + BC was superior to the other two lures ($F = 8.40$; df = 2, 36; $P = 0.001$) (Table 3). No significant differences were observed among lures in the percentage of trapped females during the trial (1–9 d period; $\chi^2 = 4.44$; df = 2; $P = 0.108$; 9–47 d period; $F = 1.803$; df = 2, 15; $P > 0.05$; 47–56 d period; $F = 2.24$; df = 2, 45; $P > 0.05$).

Residual Attraction of Degraded 30% CeraTrap Versus New 30% CeraTrap

In the final test, only 71 *A. obliqua* flies were trapped during the 9 d of assay, with a total of 38 females and 33 males. No significant differences were observed in the FTD value in the degraded 30% CeraTrap + BC treatment (1.11 ± 0.3) when compared with new 30% CeraTrap (1.52 ± 0.2) ($t = 1.35$; df = 16; $P = 0.195$). The percentage of females in traps baited with the new 30% CeraTrap (22.6%, range of SE: 14.4–33.7%) was significantly lower than the percentage of females (32.3%, range of SE: 21.6–45.3%) in

Table 2. Mean FTD (± SE) and percentage of females (range of SE) and total flies of *Anastrepha obliqua* trapped for Captor + borax mixed with one of three different concentrations of benzalkonium chloride (BC) during a 16-d trial

Lure	Mean FTD	% Females	Total flies trapped
Captor + borax	5.2 ± 0.6 a	70.2 (67.5–72.8) a	482
Captor + borax + 720 mg BC/liter	5.9 ± 1.0 a	62.4 (59.4–65.3) b	545
Captor + borax + 240 mg BC/liter	6.1 ± 1.1 a	61.4 (58.4–64.3) b	559
Captor + borax + 80 mg BC/liter	6.5 ± 1.4 a	58.8 (55.7–61.8) b	598

Mean FTD values in columns followed by the same letter were not significantly different (ANOVA; Tukey test, $P > 0.05$). Mean percentages of females were compared by t-test (GLM; $P > 0.05$, scale parameter 1.57).

Table 3. Mean FTD (± SE) and percentage of females (range of SE) of *Anastrepha obliqua* captured by three different lures that were not replaced during a period of 56 d under field conditions

	From day 1 to day 9		From day 9 to day 47[a]		From day 47 to day 56	
	Mean FTD	% Females	Mean FTD	% Females	Mean FTD	% Females
Captor + borax	4.8 ± 0.8 b	58.7 (55.4–61.7) a	0.9 ± 0.3 b	57.2 (53.6–60.7) a	0.7 ± 0.2 b	75.5 (67.3–82.2) a
30% CeraTrap + BC[b]	6.3 ± 0.9 ab	67.0 (64.1–69.8) a	4.3 ± 1.1 a	60.4 (56.8–63.8) a	4.3 ± 1.2 a	84.0 (78.3–88.7) a
30% CeraTrap	7.9 ± 1.2 a	64.0 (60.9–66.9) a	2.3 ± 0.4 a	55.2 (51.6–58.8) a	1.5 ± 0.5 b	72.3 (64.0–80.0) a

Mean FTD values in columns followed by the same letter were not significantly different (Tukey test, $P > 0.05$). Mean percentages of females were compared by *t*-test (GLM; $P > 0.05$; 1–9 d period, scale parameter 1.00; 9–47 d period, scale parameter 1.33; 47–56 d period, scale parameter 1.59).

[a]Traps were not rotated during this period.

[b]Benzalkonium chloride (BC) was included at 240 mg/liter.

Table 4. Mean (±SE) surface tension of distilled and potable water and different protein baits with or without the presence of benzalkonium chloride (BC)

Lures	Surface tension (dyne/cm)
Distilled water	72.8
Potable water	80.1 ± 1.7 f
Potable water + 80 mg BC/liter	43.7 ± 1.1 de
Potable water + 240 mg BC/liter	35.1 ± 0.3 a
Potable water + 720 mg BC/liter	27.9 ± 0.2 a
Potable water + Captor + Borax	76.2 ± 1.5 f
Potable water + Captor + Borax + 80 mg BC/liter	42.6 ± 0.8 cd
Potable water + Captor + Borax + 240 mg BC/liter	40.4 ± 0.3 c
Potable water + Captor + Borax + 720 mg BC/liter	37.6 ± 0.3 b
Ceratrap (undiluted)	58.5 ± 0.5 e
30% diluted CeraTrap	75.8 ± 1.8 f
30% diluted CeraTrap + 240 mg BC/liter	37.7 ± 0.2 b

Means in columns followed by the same letter were not significantly different (Tukey test, $P > 0.05$). Nontransformed means shown.

traps baited with degraded 30% CeraTrap + BC ($\chi^2 = 8.64$; df = 1; $P = 0.003$).

Effect of BC on Surface Tension of Lure Formulations

An important reduction in the surface tension was measured when BC was included in potable water or in both types of protein lures that were evaluated in this study ($F = 127.62$; df = 10, 44; $P < 0.001$) (Table 4). In the Captor + borax lures, a reduction of ~44% of the surface tension was observed with the inclusion of 80 mg BC/liter, but only a 3 to 5% additional reduction was observed when the concentration was increased to 240 and 720 mg BC/liter, respectively. Similar results were observed in potable water with an initial reduction of ~45% in the surface tension following the addition of 80 mg BC/liter and with a 9 to 10.7% additional reduction when the concentration was increased to 240 and 720 mg BC/liter, respectively. In this case, the presence of proteins in water seemed to interact with BC in modifying the final surface tension of the lure, as observed in our results. CeraTrap diluted in water with the addition of 240 mg BC/liter had near identical surface tension as Captor + borax with 720 mg BC/liter.

Discussion

Liquid protein lures are frequently used to monitor pestiferous flies of the genus *Anastrepha* but are also recommended for trapping other tephritid species of the genera *Bactrocera*, *Ceratitis*, and *Dacus*, such as *Bactrocera oleae* (Gmelin), *Bactrocera cucumis* (French), *Ceratitis capitata* (Wiedemann), *Ceratitis cosyra* (Walker), and *Dacus ciliatus* (Loew), among others (IAEA 2013). Compounds are usually included as preservatives in liquid protein lures, with borax the most common one (Epsky et al. 2014). A handful of alternative compounds have been tested including 10% propylene glycol which was shown to be a useful preservative for trapped flies (Thomas et al. 2001). However, apart from their value in preserving flies for later identification, little information is available on the use of preservatives for liquid protein lures. Our results revealed that low concentrations of BC provided long-lasting stability to liquid protein lures that resulted in retaining their attractiveness for periods of at least 6 wk under tropical field conditions.

The addition of BC in protein lures slightly improved catch levels of *A. obliqua* as a result of the lower surface tension of the liquid that probably prevented flies escaping once they had entered the traps. The low surface tension of liquid lures that contained BC (between 38 and 43 dyne/cm) was also evident because dead flies were commonly found in the bottom of the liquid, whereas in liquid lures without BC (70–75 dyne/cm) dead flies were mostly found floating on the liquid surface. The sinking of the flies to the bottom of the liquid phase also reduces the probability that trapped flies would be eaten by ants, something that was observed among the partial remains of flies that could not be sexed in experiments (n = 27), of which almost all were observed in treatments without BC (n = 26).

It appeared that BC, at the concentrations used in our tests (80–720 mg BC/liter), did not deter or negatively influence captures of *A. obliqua*. It is important to consider that, in our experiments, it was not possible to separate the attraction effect and the capture effect, the later of which may be influenced by the surface tension of the liquid. To examine this, it would be necessary to perform specific tests focused on the influence of different BC concentrations on the attraction to protein lures, using laboratory olfactometers or wind tunnels.

López and Hernandez-Becerril (1967) tested the effect of high concentrations of BC (1000–60000 mg a.i./liter) and other chemicals as protein lure preservatives but did not report details of their results, except to mention that catches of the Mexican fruit fly, *A. ludens* (Loew) were considerably lower in the presence of BC. The elevated BC concentrations that they employed are likely to have reduced the capture of flies, as we observed in mixtures of Captor + borax with 8000 mg BC/liter (Supplemental Data [online only]). Moreover, López and Hernandez-Becerril (1967) used protein at 1% concentration compared to the 4% concentration that we employed. It was clear to us that during mixture preparation, the smell of protein lure at concentrations of 4–10% masked the smell of BC, at least to human olfactory perception (RL, personal observations).

Field trials were performed in Jalcomulco, a region characterized by a warm and humid climate with an average temperature of approximately 26°C and a rainy season that lasts between June and November. These conditions greatly favor protein lure degradation. Nevertheless, under these conditions, the presence of BC maintained captures of *A. obliqua* by both Captor and diluted CeraTrap lures over periods of at least 6 wk. When BC was included with Captor + borax, this lure was as effective as Captor + borax lures that were replaced at weekly intervals, even after 6 wk of use under field conditions. Moreover, after more than 8 wk of field use, 30% CeraTrap + BC was as effective as newly prepared 30% CeraTrap and significantly more effective than 30% diluted CeraTrap or Captor + borax following 8 wk of exposure to field conditions.

Microbial contamination in lures was not monitored during trials, but no signs of microbial proliferation were observed in protein lures containing BC, even after 8 wk in the field. BC belongs to the group of cationic surfactants that have the ability to attach to the cell membrane and integrate into the membranes of the microbiota. Such membrane integration is sufficient to perturb growth and, at levels used for antiseptic formulations, is sufficient for cell death (Gilbert and Moore 2005). We believe that the biocidal properties were responsible for the stability of protein lures with BC. Notably, Captor + borax with BC had a similar smell (for us) during the period of the field trial, whereas Captor + borax without BC developed a strong smell of putrefaction after 2 wk in the field, presumably due to the action of microbial contaminants. In the case of 30% diluted CeraTrap with BC, the smell was stable during the whole test, whereas 30% diluted CeraTrap without BC began to have a detectable odor of protein degradation after 6–9 d and had a highly putrid odor after 47 d in the field. Earlier studies on protein lures also reported rapid proliferation of the microbiota (Gow 1954), that resulted in the loss of lure attractiveness to fruit flies (Green et al. 1960). Lure degradation is affected by the ambient temperature and the type of microorganisms that enter the trap, many of them associated with the insects that are captured. The higher the insect catch, the greater abundance and diversity of contaminants that can degrade the bait. Information in the published literature on how microbial activity can influence attraction indicates that some specific microbes can improve attraction but not others (Epsky et al. 2014). Moreover, Gow (1954) reported that the addition of antibiotics to lures to inhibit mold growth and avoid putrefaction resulted in an improved period of attraction. This author also evaluated 1000 mg BC/liter but concluded that it significantly decreased attraction to lures.

Precise evaluation of volatile emission in lures containing BC, using gas chromatography and mass spectrometry techniques, should be of interest in the future as homogeneous emission of volatiles over time is important to the effectiveness of trapping programs. A concentration of 240 mg BC/liter was sufficient for long-term stability in our tests, but it is possible that this concentration could be further reduced while maintaining the duration of lure stability under field conditions. Higher concentrations of BC tended to result in protein precipitation, which we observed in mixtures containing 720 mg BC/liter but not at 80 or 240 mg BC/liter. Fortunately, cost is not an impediment for the use of BC in lures as 1 liter BC (80% purity) costs ~US$12, which is equivalent to less than US$0.005 per lure at the concentration evaluated.

Interestingly, when BC was mixed with the Captor protein, there was a small but significant reduction in the capture of females, something that was also observed in the second experiment. This effect was not observed with CeraTrap. Further tests are required to elucidate the basis for this effect with Captor or other protein lures. The reason behind the absence of this effect in CeraTrap is unclear, although it may be related to the presence of quarternary ammonium compounds in the commercial formulation of CeraTrap, which remains to be determined.

We conclude that these results could allow growers and phytosanitary authorities to greatly reduce trapping costs and favor the use of effective tephritid trapping networks. Additional trials are required to validate this technology, principally in fine tuning the concentration of BC or other quaternary ammonium compounds for improved protein lure stability. This study reveals the value of incorporating quaternary ammonium compounds such as BC into liquid protein lure formulations for mass trapping purposes and bait stations for tephritid flies. This technology may also find applications in the monitoring or control of other insect pests that are trapped with liquid protein baits.

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Supplementary Data

Supplementary data are available at *Journal of Economic Entomology* online.

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