Influence of Adding Borax and Modifying pH on Effectiveness of Food Attractants for Melon Fly (Diptera: Tephritidae)

P. F. DUYCK, P. ROUSSE, P. RYCKEWAERT, F. FABRE, AND S. QUILICI

UMR Peuplements végétaux et bio-agresseurs en milieu tropical, CIRAD Pôle de Protection des Plantes (3P), 7 Chemin de l'IRAT, 97410 Saint-Pierre, France

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ABSTRACT The melon fly, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), is the most damaging pest of cucurbits in Reunion Island. The influence of adding borax and modifying pH on the effectiveness of different food attractants for both sexes of the melon fly is analyzed by a release–recapture method in field cages. Adding borax to protein hydrolysates Nulure and Buminal strongly reduced their attractiveness for *B. cucurbitae*. Acidification of 5% Buminal solution (from pH 6 to pH 3) doubled its attractiveness for melon fly. Conversely, Torula yeast at pH 10.5 was significantly more attractive than the standard Torula yeast at pH 9 (28% of captured flies compared with 17%). However, a further pH increase of the yeast solution does not improve its attractiveness. The results are discussed in relation to other studies on pH modification of various baits for Tephritidae.

KEY WORDS *Bactrocera cucurbitae*, Tephritidae, food attractants, borax, pH

THE MELON FLY, *Bactrocera cucurbitae* (Coquillett) (Diptera: Tephritidae), is an important pest on Cucurbitaceae in most countries of Asia, the New Guinea region, many Pacific island countries, some countries of Africa, the Middle East, and some Indian Ocean islands (White and Elson-Harris 1992, Hollingworth et al. 1996). In Reunion Island, *B. cucurbitae* is the major pest on cucurbits and requires intensive application of cover sprays with broad-spectrum insecticides (Vayssières and Carel 1999; P. Ryckewaert, personal communication).

Effective food attractants for fruit flies are needed for monitoring or detection purposes and for bait-spray techniques (i.e., mixture of an attractant and an insecticide applied as spot-spray, Roessler 1989). Proteins are necessary for female fruit flies and allow their egg maturation (Hagen and Finney 1950), even though protein-based attractants work on males, too (Heath et al. 1994, Fabre et al. 2003). Effective bait sprays techniques for fruit fly control are important for integrated control methods against cucurbit pests. In a previous study, Fabre et al. (2003) compared the attractiveness of six commercially available protein hydrolysates on *B. cucurbitae* and showed that Buminal (Bayer SA, Puteaux, France), the only registered protein hydrolysate in France, was the least attractive for this species. Buminal is commonly used in bait sprays against the olive fruit fly, *Bactrocera oleae* (Gmelin), in the Mediterranean basin (Prota 1983) and against *Ceratitis* spp. in Reunion Island (Quilici 1993). Nulure (formerly, Protein Insect bait No. 7 [PIB-7], Miller Chemical & Fertilizer Corporation, Hanover, PA) is also widely used in bait sprays (Roessler 1989). Both Nulure and Buminal are frequently used in traps for monitoring or detection purposes, in aqueous solutions with borax in McPhail traps (Campos et al. 1989, Wakabayashi and Cunningham 1991, Heath et al. 1994). Borax is used to prevent the decomposition of trapped flies (Lopez and Hernandez Becerill 1967). For the same purpose, standard Torula yeast pellets, already containing borax, are also largely used (Epsky et al. 1994).

Several baits such as Buminal, Nulure, Solbait, Corn Steep Water, Pinnacle, or Hymlure are already commercially available in different countries. Various studies have shown that certain additives may improve the attractiveness of some of these baits (Lopez et al. 1971; Epsky et al. 1993, 1994; Heath et al. 1994, 1997). Borax may improve the attractiveness of protein baits in traps for various Tephritidae (Bateman and Morton 1981, Heath et al. 1994), but to our knowledge no studies have been carried out on the influence of borax on bait attractiveness for *B. cucurbitae*. Modification of pH, most frequently alkalization, is also known to influence the attractiveness of the baits (Bateman and Morton 1981, Flath et al. 1989, Epsky et al. 1994).

In this study, the influence of adding borax to Buminal or Nulure on the attractiveness for the melon fly was evaluated. The influence on the attractiveness for the melon fly of modifying the pH of Buminal, Nulure, and standard Torula yeast solutions was also examined.

Materials and Methods

This study was carried out between April and August 2001, in the CIRAD experimental station of Saint-Pierre (Reunion Island, Indian Ocean, France).

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Flies. Wild pupae of *B. cucurbitae* were collected in June 2000 from infested pumpkins, *Cucurbita maxima* Duchesne, in three localities of Reunion Island (Petite Ile, Bassin Martin, and Piton Saint-Leu). Adult flies obtained from these samples were reared under controlled conditions of 25 ± 2°C, 70 ± 20% RH, and a photoperiod of 12:12 (L:D) h. They were given free access to granulated sugar complemented with enzymatic yeast hydrolysate (ICN Biomedicals, Aurora, OH) and water. Experiments were conducted with the F2 laboratory-reared flies. Three times a week, for 1 h, zucchinis, *Cucurbita pepo* L., were offered to the flies as an oviposition substrate. Zucchinis were then placed in a plastic box (6 by 9 by 18 cm), containing dehydrated potatoes, which was itself placed inside a larger plastic container (25 by 12 by 8 cm), the bottom of which was covered by a layer of sand to allow pupation of mature larvae. Pupae were then collected and, from the onset of emergence, adults were confined in 40 by 40 by 40-cm cages. All flies used in our experiments were 15–25 d old and were assumed to be sexually mature (Vargas et al. 1984, Hollingworth et al. 1996), which has been verified by some ovarian dissections (unpublished data).

Field Cages and Traps. The methods were similar to those developed for the comparison of different protein hydrolysates by Fabre et al. (2003), by using a release–recapture method in field cages.

The experiments were conducted in cylindrical mesh-screened field cages (2.5 m in height by 3 m in diameter, Synthetic Industries, Gainesville, FL). Twenty potted plants of *Cucurbita moschata* Duchesne ('Martinica') were placed in each cage to create seminatural conditions and to homogenize the distribution of the flies in the field cage (unpublished data). Protein hydrolysates were poured into plastic McPhail traps (Dome Trap, Agrisense, United Kingdom) with the bottom part painted in black to minimize attraction by visual stimuli. Each trap contained 200 ml of one of the different attractant solutions tested. In each cage, four traps were suspended on an H-shaped base, 50 cm above the plant canopy and placed along a 1-m radius imaginary circle centered in the field-cage. A fifth trap, placed near the center of the cage and receiving only water, was used as a control.

Experiments took place only during sunny days. Each day of the experiment, four cages were used simultaneously: two for females and two for males. In each cage 250 adults were released at 0700 hours local time to allow flies to disperse. For each replicate, at 0800 hours, four traps with the different attractants were placed in the cage in random order and every 2 h a circular permutation (quarter turn) of the traps position was carried out to reduce any influence of trap position. At 1600 hours, the flies collected from each trap were counted and those remaining in the cage were recaptured to allow for another trial the day after. A trial was run during two days which gave four replicates (a replicate is one cage per day) by sex for a complete experiment. Fresh baits and naive flies were used at the beginning of each replicate. Percentages of the solutions are expressed in volume by volume, except for borax for which they are expressed in weight.

Effect of Borax. In choice trials, the relative attractiveness of Buminal (Miller Chemical & Fertilizer Corporation) with 0, 1, 5, and 10% borax (disodium tetraborate decahydrate, $Na_2B_4O_7$ 10 H_2O, Prolabo, Fontenay sous bois, France) was compared in experiment 1, whereas the relative attractiveness of Nulure (Miller Chemical & Fertilizer Corporation) (5%) with 0, 1, 5, and 10% borax was compared in experiment 2. Borax was dissolved in boiling water, and after cooling, was added to the protein hydrolysate. The pH of each protein–borax solution was measured just after preparing the solutions with a laboratory pH meter (Accumet model 25, OSI, Paris, France). Three replicates were made for each pH determination.

Effect of pH Modification. Because borax is known to affect the pH of the solutions, we investigated whether its effect on the attractiveness was only due to pH modification. Buminal (5%) and Nulure (5%) were alkalized with sodium hydroxide in experiments 3 and 4 respectively, to reach target pH of 6, 7, 8, 9 and 4, 6, 8, 10, respectively. In experiments 5 and 6, Torula yeast was alkalized with sodium hydroxide to reach target pH of 9, 9.5, 10, 10.5 and 10.5, 11, 11.5, 12, respectively. Buminal (5%) and Torula yeast (Agrisense, Mid Glamorgan, United Kingdom) (two pellets per 200 ml) were acidified with nitric acid in experiments 7 and 8 respectively, to reach target pH of 6, 5, 4, 3 and 9, 7, 5, 3, respectively. No attempt was done to acidify Nulure, because the initial pH of this product was already very low (pH 3.53). Nitric acid and sodium hydroxide were used because they enabled us to modify efficiently the pH of the solution with a limited quantity of product. Moreover, sodium hydroxide has previously been used in other studies to alkalize attractants for fruit flies (Sharp 1987). Previous curves of volume of nitric acid or sodium hydroxide plotted against pH were prepared in preliminary trials to determine the quantity of nitric acid or sodium hydroxide needed to reach a particular pH value.

Statistical Analysis. Because our purpose was to compare the relative effectiveness of the products, the data analyzed for each cage were the total number of flies caught in one trap during 1 d divided by the total number of flies caught in all traps during the same day. Data were transformed by arcsine (square root x) to stabilize the variance before analysis. They were analyzed by a three-way analysis of variance (ANOVA) with the first order interactions (StatSoft-France 1997). The three factors were products (studied factor, five levels), sex (studied factor, two levels), and replicate (controlled factor, four levels). When the F value was significant ($P < 0.05$), a Tukey's mean separation test was used.

Results

For all experiments, neither replicate nor sex nor the three first order interactions had any significant influence on the results. Conversely, the factor products always showed a significant influence on the re-

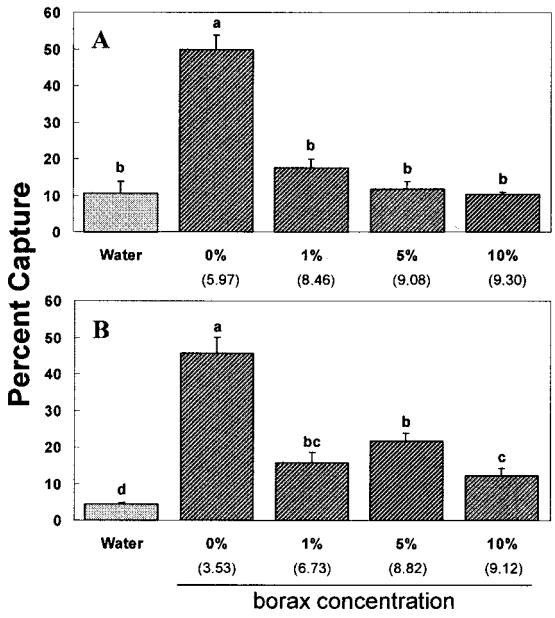

Fig. 1. Mean percentage of male and female *B. cucurbitae* captured in McPhail traps baited with protein hydrolysates and various concentrations of borax: (A) Buminal 5% + borax (experiment 1). (B) Nulure 5% + borax (experiment 2). pH values of bait mixture are indicated in parenthesis under borax concentration values. Bars headed by the same letter within a graph are not significantly different (Tukey's mean separation test on arcsine [sqrt x]-transformed data, nontransformed means presented). Bars without hatches are water controls.

sults (at least between treatments and control). Because the relative attractiveness of each product was the same for each replicate and for both sexes, the mean percentages of capture were only compared among products. Mean percentage of recaptured flies in the different experiments was 54 ± 17% for males and 58 ± 19% for females.

Effect of Borax. Adding borax to protein hydrolysates Buminal and Nulure strongly reduced their attractiveness for *B. cucurbitae* (Fig. 1A and B). Attractiveness of Buminal with 1, 5, or 10% borax was not significantly different from that of water ($F = 29.65$; df = 4, 12; $P < 10^{-5}$). Adding 1, 5, or 10% borax to Nulure was less attractive than Nulure alone ($F = 34.67$; df = 4, 12; $P < 10^{-5}$). However, Nulure with borax was significantly more attractive than water. Nulure with 10% borax was less attractive than with 5% borax.

Effect of pH Modification. Alkalization of Buminal reduced its effectiveness ($F = 7.00$; df = 4, 12; $P < 0.005$) (Fig. 2A). There was an insignificant reduction in attractiveness between the Buminal pH six (original bait) and the Buminal pH 7. The decrease in attractiveness was more drastic when pH value increased to pH 8 and pH 9; at these two pH values, the Buminal solutions were not significantly more attractive than the control. Unlike Buminal, the attractiveness of Nulure did not decline with increasing pH ($F = 25.74$; df = 4, 12; $P < 10^{-5}$) (Fig. 2B). There was no significant difference between original (pH 4) and alkalized Nulure.

Results of experiment 5 show that Torula yeast alkalized to a certain extent is more attractive for *B. cucurbitae* than standard Torula yeast ($F = 23.47$; df =

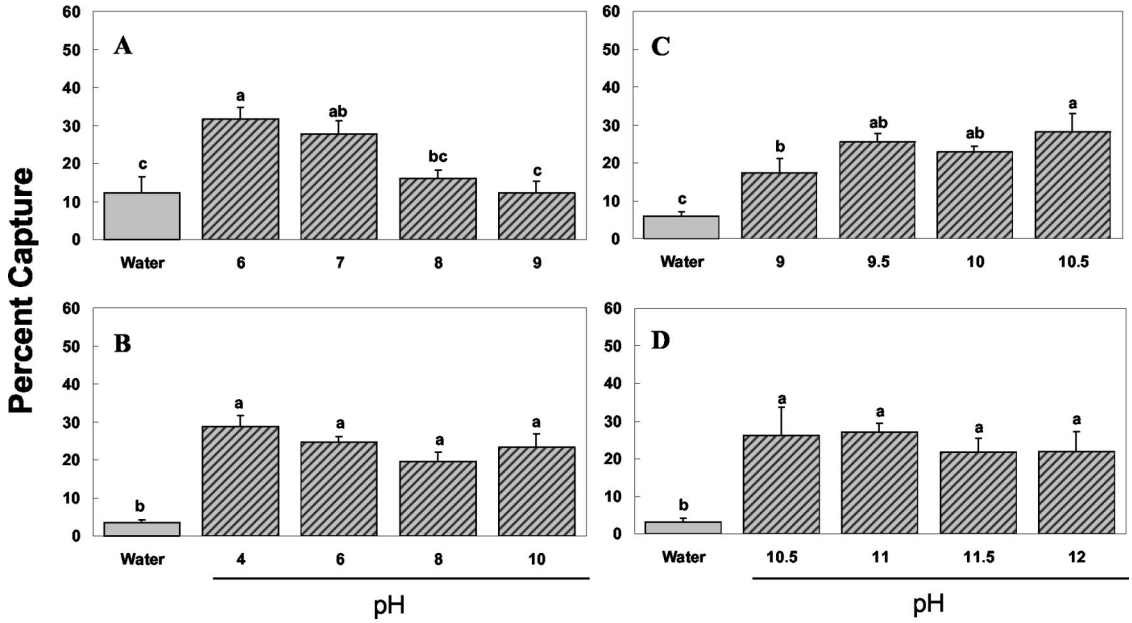

Fig. 2. Mean percentage of male and female *B. cucurbitae* captured in McPhail traps baited with food attractants alkalized to reach various pH values: (A) Buminal 5% + NaOH (experiment 3); (B) Nulure + NaOH (experiment 4); (C) Torula + NaOH (experiment 5); and (D) Torula + NaOH (experiment 6). Bars headed by the same letter within a graph are not significantly different (Tukey's mean separation test on arcsine [sqrt x] transformed data, nontransformed means presented). Bars without hatches are water controls.

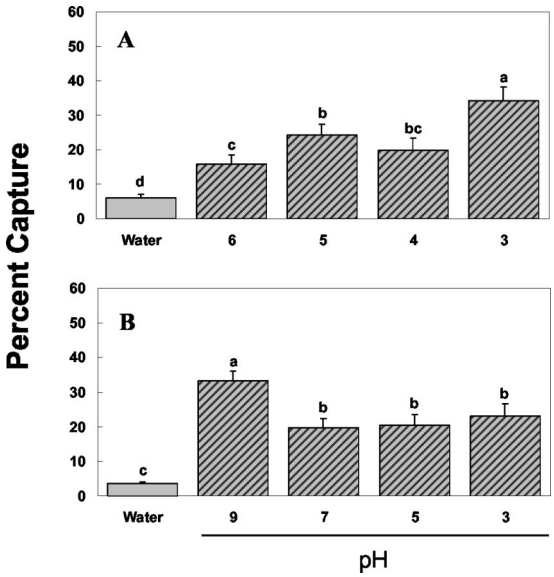

Fig. 3. Mean percentage of male and female *B. cucurbitae* captured in McPhail traps baited with food attractants acidified to reach various pH values: (A) Buminal 5% + HNO_3 (experiment 7) and (B) Torula + HNO_3 (experiment 8). Bars headed by the same letter within a graph are not significantly different (Tukey's mean separation test on arcsine [sqrt x]-transformed data, nontransformed means presented). Bars without hatches are water controls.

4, 12; $P < 10^{-4}$) (Fig. 2C). Torula yeast at pH 10.5 was significantly more attractive than standard Torula yeast at pH 9 (responsible for 28% of recaptured flies compared with 17%). However, a further increase of the pH of the Torula yeast solution, induces no further improvement of its attractiveness ($F = 19.87$; df = 4, 12; $P < 10^{-4}$). In experiment 6, we found no significant difference among several Torula yeast solutions at pH 10.5, 11, 11.5, and 12 (Fig. 2D).

Modification of the pH of a Buminal 5% solution from pH 6 to pH 3 doubled its attractiveness for *B. cucurbitae* (Fig. 3A). Buminal at pH 3 was significantly more attractive than all other treatments compared during experiment 7 ($F = 30.86$; df = 4, 12; $P < 10^{-5}$). However, results of experiment 8 showed that acidified Torula yeast became less attractive than the standard solution (pH 9), the three acidified Torula (pH 7, 5, and 3) being not significantly different among them ($F = 33.73$; df = 4, 12; $P < 10^{-5}$) (Fig. 3B).

Discussion

Our study shows that the addition of borax decreases the attractiveness of some commercial protein hydrolysates to *B. cucurbitae.* Similarly, Lopez and Hernandez Becerill (1967) demonstrated in a field experiment that the attractiveness of PIB-7 to *Anastrepha ludens* (Loew) was lowered when borax was added to the solutions. In contrast, Heath et al. (1994) showed that adding borax (1–10%) to Nulure solutions increased their attractiveness for *Ceritatis capitata* (Wiedemann) up to the highest concentration tested (10%). For *A. ludens,* these authors showed that the optimal concentration of borax when added to Nulure was 3% and that the attractiveness subsequently decreased with increasing concentrations up to 10%. The influence of borax on attractiveness of protein hydrolysate solutions seems to vary depending on the tephritid species (or at least on the subfamily) studied.

This result may have important consequences on trapping results as borax is frequently added in traps during fruit fly monitoring or population dynamic studies to prevent protein hydrolysates from decaying and also reduces the capture of other insects (Lopez and Hernandez Becerill 1967). When borax is added to the solutions, populations of *B. cucurbitae* might be underevaluated compared with other tephritid species that are not repelled by this product.

The pH of protein hydrolysates increases with raising borax concentration (Fig. 1). The lower attractiveness of the protein hydrolysates for *B. cucurbitae* when borax is added is probably not only due to alkalization, because adding sodium hydroxide to Nulure does not reduce its attractiveness. The final pH of an attractant may be important for its attractiveness, but the product with which we modify the solution may have its own effect especially when added in large quantity.

Several studies on *Anastrepha striata* Shiner (McPhail 1939), *Bactrocera tryoni* (Froggatt) (Bateman and Morton 1981), and *Anastrepha suspensa* (Loew) (Sharp 1987) have shown that the attractiveness of different enzymatic hydrolysates for these species can be improved by increasing the pH. Mazor et al. (1987) studied the attractiveness of alkalized Nasiman (a protein hydrolysate from Israel) and Buminal for *C. capitata.* The attractiveness increased with the pH of the solutions up to a certain limit (pH 8.5 for both Nasiman and Buminal), whereas further increase of pH decreased the attractiveness of the solutions. Our results with Buminal show that *B. cucurbitae* behaves differently from *C. capitata* in this respect though the attractiveness of Torula yeast for melon fly was improved by alkalization up to pH 10.5. Similarly *B. cucurbitae* shows opposite responses to acidification of Buminal or Torula. The changes of attractiveness for *B. cucurbitae* with modifying pH were very different when these two products (a yeast and a protein hydrolysate) were used, which is not surprising because they probably differ greatly in their chemical composition. Determining better attractive mixtures would require a definition of an optimum pH for each species and for each bait.

The pH of an attractant, and hence its attractiveness for a given fruit fly, will often be naturally modified after several days in a trap. Heath et al. (1994) showed that the pH of a NuLure + 1% borax solution could considerably increase after 7 d of field use. This could have an effect in certain studies realized on a long period in which the traps are exposed for 1 wk (Epsky et al. 1993, 1994; Heath et al. 1994) or 2 wk (Lopez et al. 1971).

The situation differs if the objective is to control flies (bait sprays), as a solution of low or high optimal

pH may be used in a trapping system, whereas phytotoxicity problems may prevent its use for bait sprays. In this respect, further experiments would be needed before our results could be used for that purpose (e.g., repulsiveness or pH modification by the insecticide).

Another difficulty comes from the fact that two other species of Dacini, *Dacus* (*Didacus*) *ciliatus* Loew and *D.* (*Dacus*) *demmerezi* (Bezzi), attack cucurbits in Reunion Island (Vayssières 1999, Fabre et al. 2003), although these two species cause less damage than *B. cucurbitae* (Vayssières and Carel 1999). The optimal baits may be different for the three species.

The results of our study should be useful for optimizing trapping systems for both sexes of the melon fly. A possible application of these results for bait sprays with protein hydrolysate or yeast would require further field experiments.

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