Antennal and Behavioral Responses to Putrescine and Ammonium Bicarbonate in the Caribbean Fruit Fly (Diptera: Tephritidae)

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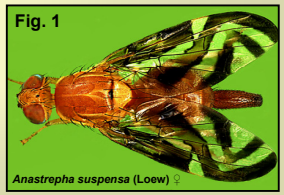
INTRODUCTION

Improved attractants are needed for *Anastrepha* fruit flies, including the Caribbean fruit fly (Fig. 1). Once restricted to the Bahamas and Greater Antilles, this invasive tephritid is now permanently established in south Florida, where it is a quarantine pest of citrus and a production pest of guava. Current monitoring programs utilize a two-component lure that emits ammonia and putrescine (1,4-diaminobutane), both regarded as similar protein cues that result in female-biased attraction.

To better understand tephritid olfactory ecology, we initiated a research program that integrates electroantennography (EAG) and developmental physiology with behavioral response to olfactory attractants. In this study, we used EAG and flight tunnel bioassays to quantify response to putrescine and ammonium bicarbonate lures, presented alone and in combination. We also evaluated the effect of female reproductive state on antennal response to these lures.

Fig. 1



MATERIALS & METHODS

Lures and Insects. Two commercially available lures were used for EAG and flight tunnel tests: ammonium bicarbonate, NH_4HCO_3 (AgriSense-BCS, MidGlamorgan, UK) and putrescine, $NH_2(CH_2)_4NH_2$ (Suterra, Bend, OR). *Anastrepha suspensa* were obtained from a laboratory colony maintained at the USDA-ARS, Miami, FL. Female maturity status was assessed according to a six-stage classification system developed previously (Kendra et al. 2006).

Electroantennography. Antennal signals were recorded with a Syntech EAG system (Hilversum, The Netherlands) (Fig. 2A) using methods previously reported (Kendra et al. 2005a,b; 2008). Whole fly heads, with antennae extended, were mounted between electrodes (Fig. 2B) and placed under a stream of purified air (Fig. 2C). Lures were placed in 250-ml gas-tight glass bottles fitted with septum port lids. Using gas-tight syringes, volumetric doses of saturated vapor were withdrawn from the test bottles, injected into the airstream, and delivered to the antennae (Fig. 2D). EAG responses (Fig. 2E) were measured initially in units of mV, then normalized to percent responses relative to a standard reference chemical (20 µl 2-butanone saturated vapor).

Fig. 2

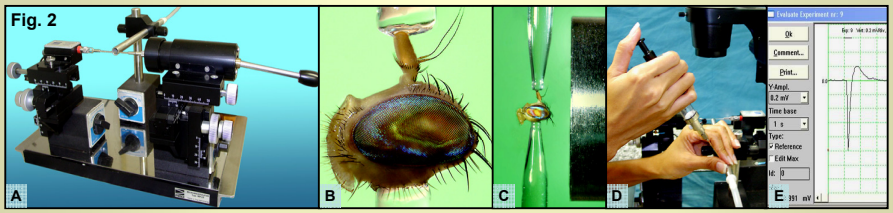
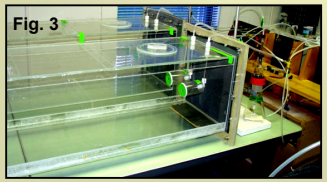


Fig. 3

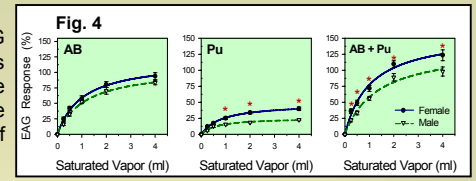


Flight Tunnel Bioassays. Two-choice tests were conducted in Plexiglas wind tunnels (Fig. 3). Odor source was external, entering the tunnel through plastic traps containing a toxicant panel. For each test, 15-25 mature flies were released at the downwind end, and the number captured was recorded after 20 hrs. Sexes were tested separately, tests were replicated 10 times, and position of choices was switched between trials.

RESULTS & DISCUSSION

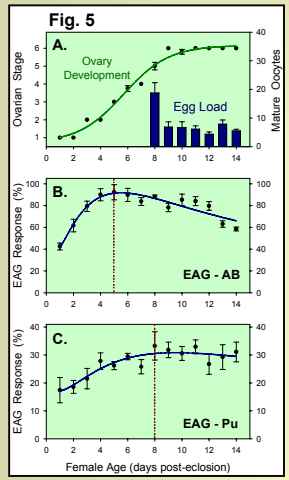
EAG Dose-Response Profiles.
Over a range of doses evaluated (Fig. 4), EAG response to ammonium bicarbonate (AB) was equivalent for both sexes, but female response was greater than male response to putrescine (Pu) at higher doses, and to a 1:1 mixture of both lures (AB+Pu) at all doses tested.

Fig. 4



* Doses with significant difference between male and female response (*t*-test, $P \le 0.05$)

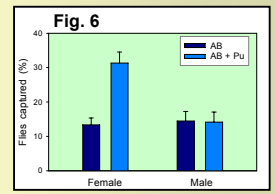
Fig. 5



Female Maturation.
Dissection of ovaries from females 1-14 days old (Fig. 5A) indicated that flies were sexually immature (ovarian stages 1-4) for the first 7 days after emergence. They reached maturity at day 8 (stage 5) when peak egg load (mature oocytes/ovary) was observed. The ovipositional phase (stage 6) began on day 9, as reflected by a significant drop in mean egg load.

Female EAG Temporal Profiles.
EAG responses to fixed 2-ml doses of AB and Pu were not constant, but varied according to female physiological state (Fig. 5B-C). Peak response to AB (Fig. 5B) was recorded from immature females with ovaries actively undergoing vitellogenesis (stages 2-4). However, peak response to putrescine (Fig. 5C) was measured from sexually mature females (stages 5-6). Apparently, the two semiochemicals serve slightly different functional roles as protein-feeding cues for female *Anastrepha*.

Fig. 6



Behavioral Response.
In flight tunnel bioassays with mature flies (Fig. 6), more females were captured with AB+Pu than with AB alone. This difference was not observed in males, resulting in a higher female:male ratio in captures with AB+Pu (3:1) versus AB alone (1:1).

CONCLUSIONS

Our results suggest that (1) ammonia and putrescine, two protein-based attractants, are detected by separate olfactory receptors in *A. suspensa*, (2) olfactory response to these semiochemicals is dependent upon female reproductive state, and (3) the putrescine component is responsible for the female-biased attraction observed with the two-component lure (Kendra et al. 2009).

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