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Release rate of ammonia - a key component in the attraction of female mediterranean fruit fly to protein-based food lures.

M. Mazor, A. Peysakhis and G. Reuven

Department of Entomology, ARO, The Volcani Center, Bet-Dagan 50250, Israel

Abstract: The Mediterranean fruit fly, *Ceratitis capitata*, is one of the most injurious pests around the world. The common way to control the fly is via poison-bait sprays directed mainly against the females. Hydrolyzed proteins are the customary, but not satisfactory, bait component. Ammonia is the most significant volatile breakdown product of protein decomposition. The relationship between ammonia emanation and the attraction of the medfly was studied earlier and indicated the dependence of attraction on the rate of release of ammonia. The aim of this study was to quantify the rate of ammonia release for optimal attraction of medfly females as a first step in devising a new and more efficient female-attractive bait based on chemically defined components. The rate of release of ammonia was measured in an ammonia trapping system, which was built for this purpose. The ammonia was released from 1 ml sample in a 3-cm-long x 1.5-cm i.d. glass tube. The most attractive concentration of ammonia solution was 0.01 N. The most attractive rate of release of ammonia calculated from the correlation of increasing concentrations of ammonia solutions and their release rates was 14.3 µg/tube/hour. The use of higher rates of release of ammonia up to ~100 µg/tube/hour may be also adequate for use because although they are somewhat repulsive, they are attractive enough to catch relatively higher numbers of flies.

Key words: Mediterranean fruit fly, *Ceratitis capitata*, Tephritidae, Diptera, food baits, attraction

Introduction

The Mediterranean fruit fly (medfly), *Ceratitis capitata* (Diptera: Tephritidae), is one of the most injurious fruit pests. It is widely distributed and the list of its host range is long and diverse (White and Elson-Harris, 1994). The medfly is a high-priority quarantine pest. The demanding regulations at its export destinations are the cause for the intensive control applications against the medfly in the fruit-growing areas as well as for seeking more efficient and environment friendly ways to control it. Females are the main target for control because they damage fruits and are the dominant factor for multiplication. Female-attractive baits are therefore needed in any applicative system against this pest for both monitoring and direct control.

The need for external supply of protein for ovary maturation is the reason for the attraction of females to protein-based baits. Hydrolyzed proteins are the custom-

ary, but not satisfactory, means to attract the females. The common commercial hydrolyzed proteins baits are black liquids, difficult to handle and variable in content affecting their attraction. The new fruit fly dry bait developed by Heath *et al*. (1995), although easy in use and more efficient then the liquid hydrolyzed proteins (Gazit *et al*., 1998), is limited to traps only. The need for more powerful baits for use in both traps and in bait sprays is a must in the fight against this pest.

Ammonia is the most conspicuous end product of protein decomposition. The relationship between ammonia emanation and the attraction of the medfly was studied earlier (Mazor *et al*., 1987). The quantification of the rate of ammonia release for optimal attraction of medfly females reported here was considered as the first step in devising a new and more efficient female attractive bait based on recognized components.

Materials and Methods

The quantification of the rate of release of ammonia and the attraction of the medfly females were studied in two experimental setups: olfactometer and ammonia trapping system.

Laboratory-reared flies were obtained as pupae from the Citrus Marketing Board of Israel. Pupae and emerging flies were kept in a room with windows, under natural photoperiod conditions and a controlled atmosphere of 26±2^{0}C and 68±2% relative humidity. The behavioral tests were conducted at the same conditions. 200 protein-deprived and mostly unmated 3 - 10-day-old females were placed in an olfactometer described by Gothilf and Galun (1982) for the attraction tests. The flies were offered granulated sugar, and water absorbed on cotton wool. The ammonia stock solution was a concentrated volumetric solution (BDH) adjusted to several concentrations between 0.0001N and 0.1N. 1 ml of the desired ammonia solution was pipetted into a 3-cm-long x 1.5-cm i.d. glass tube. A 10-cm metal wire was joined to the side of the glass tube allowing to insert the bait into the trap. 6 traps, 3 with bait and 3 empty ones as control were suspended alternately from the horizontally rotating wheel of the olfactometer at a rate of 1 complete turn/10 min for 1 hour. At the end of the experiment, the entrance holes of each trap were plugged with a piece of cotton wool and the traps were transferred to the refrigerator for a few minutes to allow the counting of the captured flies. Flies trapped in all 3 baited traps in one olfactometer were considered as one replicate. The attraction of 0.01 N ammonia solution was tested also with 3-10-day old protein-deprived males.

The ammonia trapping system includes a 100 ml round bottom flask containing 1-3, 3-cm-long x 1.5-cm i.d. glass tube(s), with the tested solution (depending on the rate of ammonia release) connected to two consecutive water traps, 16-ml-long x 2-cm i.d. glass tubes containing 10 ml double-distilled water. Each glass tube contained 1 ml of several concentrations between 0.0001N and 0.1N of pure ammonia solution.

The whole system was sunk in a water bath at a temperature of 30^0C. Fresh air was pulled into the trapping system by a vacuum pump at a rate of 100 ml/min. The air was drawn into and through the round glass flask containing the tested material and then through cintered glass filters to the first and the second 10-ml water tubes. Most of the emitted ammonia was caught in the first water tube. The trapping of ammonia lasted 1 to 8 hours (depending on the rate of release of ammonia).

The amount of ammonium ion in the water was determined by a colorimetric phenol chlorite method (Solorzano, 1969) and was calculated as ug ammonia released from 1 ml experimental material per hour.

Results and discussions

The rate of ammonia release affected the attraction of the flies. The most attractive ammonia solution, 0.01 N, caught per olfactometer during 1 hour the highest proportion of females, namely an average of 58% of the 200 females (calculated from Fig. 1). The observed rate of release of ammonia from this solution was 17.1 ± 3.5 µg/tube/hour. The calculated rate of release of ammonia from this ammonia solution was 14.3 µg/tube/hour, based on the equation $y=22040x^2+802.31x+4.0506$ describing the correlation between increasing concentrations of ammonia solution and the measured release rates of ammonia from them (Fig. 2). The slope of the curve representing attraction against increasing rates of release of ammonia is very steep up to the most attractive point, while the subsequent descent is gradual (Fig. 3). The correlation between these two parameters up to this highest point fits best a logarithmic function (Fig. 4). The most attractive solution, 0.01 N, was attractive also to males, but the ratio between the catches of females to males in response to this solution was twice as much. A comparison between the catches of female and male medflies by 0.01N ammonia solution showed that 117 ± 23 females and 54 ± 13 males were captured ((n=64, n=16 respectively). Although the aim of the bait development is toward a more female-selective bait, evaluating the degree of male catches should be considered.

A source that releases ammonia at the optimal rate of release may serve as the principal component of female attractive food-lure baits. Only rates of release higher than the most attractive rate that was measured in our system, namely ~14 µg/tube/hour should be taken into account, because of the large differences in attraction due to small changes in concentrations at lower release rates. The use of higher rates of release of ammonia up to ~100 µg/tube/hour may be adequate for use because although they are repulsive to some extent they are attractive enough to catch relatively higher numbers of flies.

In spite of the optimal captures (58%) as a response to a specific rate of release of ammonia, it is doubtful whether this can be still be improved, a point yet unknown.

The females that took part in these experiments were protein-deprived and mostly unmated because they were introduced into the olfactometers when they

were 2-day-old. Preliminary results showed that equal numbers of females responded equally to ammonia at different ages when they were continuously protein-deprived. The ammonia probably symbolized a signal for the presence of protein. This point should and will be tested in the near future. Other components that may induce attraction in females in different physiological states such as mature fruit lures are the target for further study.

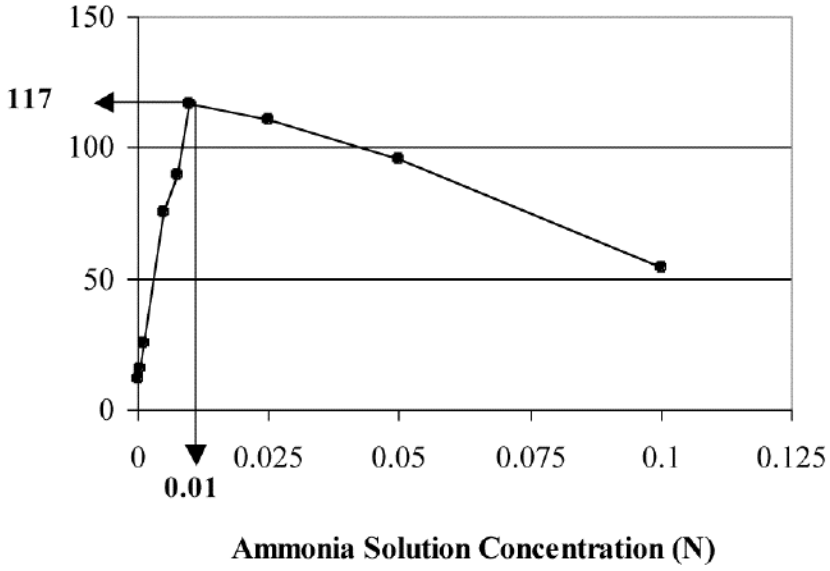

Figure 1. Female catches in response to increasing concentrations of ammonia solutions. (n=16)

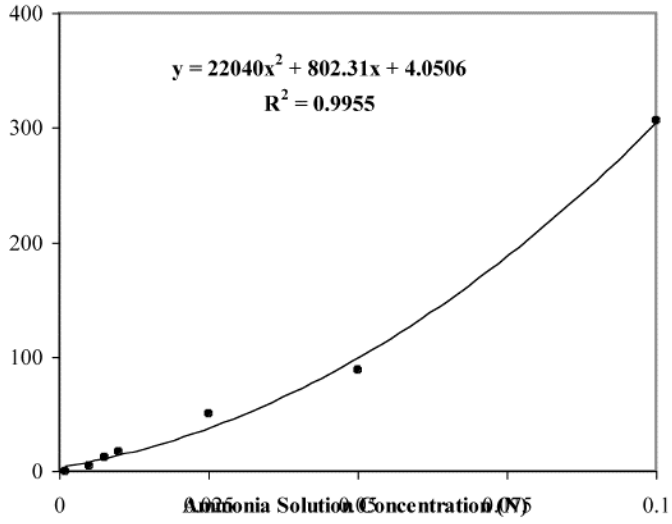

Figure 2. The rate of release of ammonia from increasing concentrations of ammonia solution. (n=12).

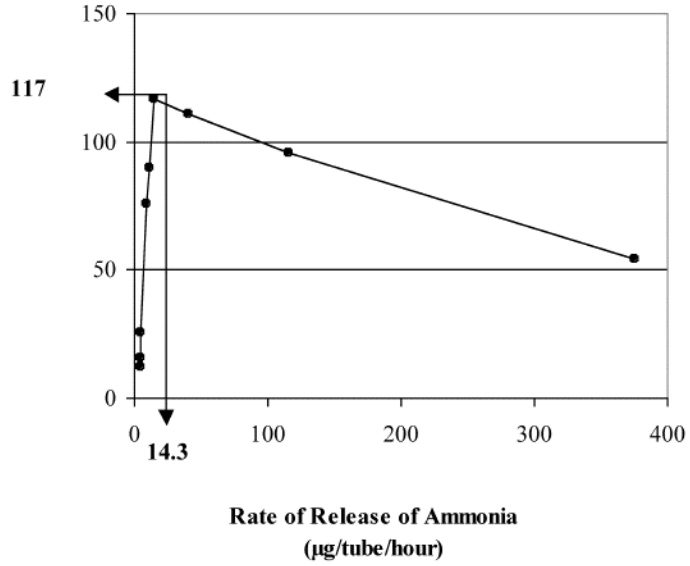

Figure 3. Female catches in relation to calculated increasing rates of release of ammonia. (n=16).

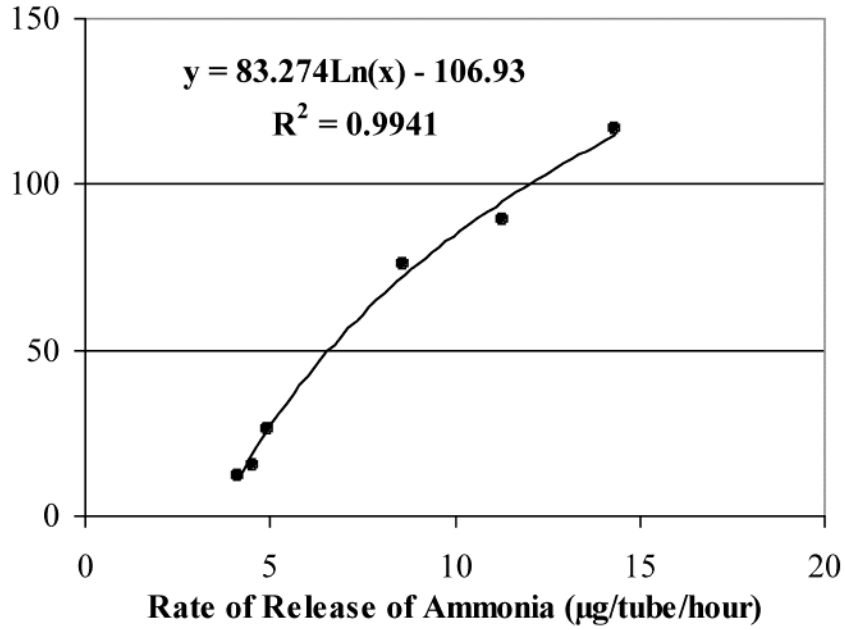

Figure 4. The relationship between female catches and the calculated rate of release between the lowest and the highest points of attraction. (n=16).

The relationship between the rate of ammonia release and attraction of females was tested with the medfly, but since most, if not all fruit flies adult females share the same need for external supply of protein for egg production and survival (Tsipsipis, 1989;

Jacome *et al*., 1999), such studies for other fruit fly species may contribute to the development and improvement of more efficient baits for them.

The next step of this study is to translate its results into practical use. A device that will release ammonia at the desired optimal rate of release that will be fitted for application in traps and use in poison bait sprays will be developed. The requirements needed for trap application are different from those needed for poison-bait spray. Bait for trap should be only attractive, while bait for poison-bait spray should be also phagostimulative. Another difference is the application phase which is liquid for sprays, whereas a solid phase is much more convenient in trap application.

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