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## CONSUMPTION OF BAIT SOLUTIONS BY ANASTREPHA SUSPENSA

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#### Abstract

Nu-Lure and other protein solutions were presented to Anastrepha suspensa in J-tubes and consumption was quantified spectrophotometrically. In choice comparisons, flies consumed more or equal water compared to Nu-Lure and more Nu-Lure compared to Bragg's Liquid Aminos, corn steep liquor, NZ case, pepticase, Solulys, soy hydrolysate, Torula yeast, whey, and yeast enzymatic hydrolysate. Consumption of protein solutions was one-half or less than 0.2 M sucrose, the positive control. The addition of 0.2 M sucrose or 0.2 M fructose to Nu-Lure did not increase the consumption of Nu-Lure compared to the consumption of sucrose alone, suggesting that Nu-Lure negates the phagostimulant properties of sucrose and possibly fructose for A. suspensa. If higher consumption rates of a bait/toxicant mixture is a goal, 0.2 M sucrose would be a better choice than the protein solutions tested, including Nu-Lure.

#### RESUMEN

Se suministró Nu-Lure así como otras soluciones protéicas a individuos de Anastrepha suspensa en tubos "J" y se cuantificó por via fotométrica el consumo de estas proteinas durante un intervalo adecuado de tiempo. En las comparaciones seleccionadas se observó que el consumo de agua se mantuvo igual o superior al de Nu-Lure; asimismo, el consume de esta proteina fue superior al observado para Amino Líquido de Bragg, licor de maiz, NZ case, pepticase, Solulys, hidrosilato de soya, levadura de Torula y suero e hidrosilato enzimático de levadura. En general, el consumo de soluciones protéicas se mantuvo por debajo de la mitad del correspondiente al control positivo de sacarosa 0.2 M. La adición de sacarosa o fructosa (ambos a la concentración de 0.2 M), a Nu-Lure no incrementó el consumo de dicha proteina en comparación con el consume de azúcar, lo que sugiere que Nu-Lure podría eliminar las propiedades fagoestimulantes de la sacarosa y posiblemente tambien de la fructosa en A. suspensa. En aquellos casos en que se desea alcanzar velocidades de consumo mas elevadas de agentes tóxicos mezclados con el sebo correspondiente por parte de A. suspensa, la solución de sacarosa 0.2 M podría constituir una mejor alternativa que las soluciones protéicas prepadas, incluído el Nu-Lure.

Translation provided by the authors.

Nu-Lure7, a commercially available, corn protein hydrolysate (Miller Chemical and Fertilizer Corp., P.O. Box 333, Hanover, PA 17331) is combined with malathion for the management of *Anastrepha suspensa* (Loew) in Florida (Nigg et al. 2004a). The 20% malathion/80% Nu-Lure mixture is described as a bait/pesticide and may be applied by air or by ground equipment (Nigg et al. 2004a). We attempted to attract and kill approximately 20,000 *A. suspensa* in the greenhouse with this mixture without success (H. N. Nigg & S. E. Simpson, personal observation).

Others have studied the attractiveness of Nu-Lure to fruit flies under various conditions in trapping studies (Epsky et al. 1993, 1999; Heath et al. 1994; Katsoyannos et al. 1999; Fabre et al. 2003). Although consumption was not determined, Nu-Lure appeared to be an attractant to A. suspensa and other tephritidae in those studies. In *A. suspensa* management programs, Nu-Lure/malathion is applied as a droplet to surfaces. There is an assumption by scientists, growers, and the public that these pesticide-laden bait droplets are consumed by the fly with resultant mortality. Our greenhouse observation appears to be the sole contrary observation to this supposition.

If we could increase the consumption of Nu-Lure, the amount of pesticide added to NuLure could be reduced on a 1:1 basis. That is, if consumption were doubled, pesticide concentration could be halved. Our initial efforts on bait improvement were feeding requirements (Nigg et al. 2004c), development of an individual fly consumption method (Nigg et al. 2004b), and determination of sugar consumption (Nigg et al. 2006). With our development of an accurate method for monitoring individual *A. suspensa* consumption

(Nigg et al. 2004b), the premise that Nu-Lure was consumed by *A. suspensa* could be evaluated.

The purpose of this study was to quantify the consumption of Nu-Lure and other protein solutions by adult *A. suspensa*.

## MATERIALS AND METHODS

#### Insects

Anastrepha suspensa pupae were shipped overnight from the Florida Department of Agriculture and Consumer Services (Division of Plant Industry, Gainesville, FL) fly-rearing facility. The ziplock bags in which they were shipped were opened, the pupae were gently manipulated by hand, and the bags were resealed and placed in a refrigerator at 4°C. This procedure allowed for gas exchange and resulted in better adult emergence. Flies destined to be tested at 24 h were held in the refrigerator as pupae for 48 h before being placed in emergence cages. Flies destined to be tested at 6 d of age were held in the refrigerator as pupae for 24 h. This procedure allowed coordination of fly emergence so experiments could be conducted Monday through Friday. Flies were allowed to emerge into cages that were  $30 \times 30 \times$ 30 cm (Bioquip, Inc., Gardena, CA) and were tested as immature (24-h) and sexually mature (6-d) flies. Flies were fed yeast, sugar, and water according to Nigg et al. (1994, 1995) in their emergence cages. Once adult emergence began, the pupae were removed to an empty cage, emergence was allowed to continue for 12 h, and all remaining pupae were discarded. This procedure resulted in flies 1-2 and 6-7 d old on the day of an experiment. Twenty-four h prior to an experiment, flies were selected directly from their emergence cage. Only active flies with normal wings were transferred by grasping one wing and placing the fly into a 950 mL translucent plastic container. Flies were provided only on agar patty for water for 16 h prior to an experiment.

The consumption of solutions by flies was studied in cages by allowing flies to feed for 45 min (Nigg et al. 2004a). Each cage contained 5 males and 5 females and was treated as a replicate. Five positive control cages, presented with 0.2 M sucrose plus 0.1% cresol red in a J-tube, were included in each trial (Nigg et al. 2006). If the flies in the positive control did not average 2.5 µL or greater consumption over 45 min, the entire data set for that week was discarded. This procedure eliminated 1 data set during these experiments.

Nu-Lure was obtained from Miller Chemical and Fertilizer Corp. (P.O. Box 333, Hanover, PA 17331); whey protein (W-1500) from bovine milk, pepticase (P1192), N-Z-Case M (C7585), and soy protein acid hydrolysate (S-1674) were from Sigma Chemical Company (P.O. Box 14508, St. Louis, MO 63178); sodium caseinate (spray dried)

and hydrolyzed casein (HCA411) from American Casein Company (Burlington, NJ 08016-4123); yeast hydrolysate enzymatic (103304), corn gluten meal (960015), and Torula yeast (903085) from MP Biomedicals, LLC (1263 South Chillicothe Road, Aurora, OH 44202); soy protein (Prolisse) from Cargill Health & Food Technologies (15407 McGinty Road W., Wayzata, MN 55391); and Bragg Liquid Aminos (Live Food Products, Inc., Box 7, Santa Barbara, CA 93102) from a local supermarket. Solulys was from Roquette America, Inc. (1417 Exchange St., P.O. Box 6647, Keokuk, IA 52632-6647).

## Consumption Quantification

Flies were allowed to feed for 45 min as this is the time for maximum initial consumption (Nigg et al. 2004b). Quantification of consumption was according to Nigg et al. (2004b). Briefly, flies were presented with protein solutions containing 0.1% fluorescein or 0.1% cresol red in 5-mL J-tubes. Solutions containing 0.1% cresol red or 0.1% fluorescein are consumed equally by these flies (Nigg et al. 2006). Different dyes allowed the direct comparison of two solutions in the same fly (Nigg et al. 2004a). Consumption was measured by extracting each fly in 0.1 M NaOH and quantifying the dye spectrophotometrically, cresol red at 573 nm and fluorescein at 491 nm (Nigg et al. 2004b).

## **Experiment One**

This experiment was designed to directly compare the consumption of NuLure with other protein solutions. Two J-tubes with different solutions were presented in each treatment cage for 45 min. Consumption of 10% Nu-Lure was compared to distilled water and to 10% solutions of the proteins listed above except for Solulys which was tested as packaged. There were 5 replicates of each treatment. All flies were included in the statistical analysis of this experiment whether they had fed or not. To calculate the mean for each replicate, the sum of each solution by sex and cage (replicate) was divided by the number of that sex in the cage.

## Experiment Two

Our previous work showed that *A. suspensa* readily consumed 0.2 M sucrose so we compared its consumption to consumption of NuLure (Nigg et al. 2006). This experiment indirectly compared the consumption of NuLure, water, and 0.2 M sucrose. A single J-tube was presented in each cage for 45 min. Treatments were 10% Nu-Lure plus 0.1% cresol red or glass-distilled deionized water plus 0.1% fluorescein or 0.2 M sucrose plus 0.1% fluorescein, or 0.2 M sucrose in 10% Nu-Lure plus 0.1% fluorescein. After 45 min, flies were pro-

cessed and consumption was quantified as described above. There were 5 replicates of each treatment.

### **Experiment Three**

Sugars are phagostimulants for many insects (Hagen & Finney 1950; Peacock & Fisk 1970; Sutherland 1971; Ma & Kubo 1977; Friend 1981; Cobbinah et al. 1982; Doss & Shanks, Jr. 1984; Mochizuki et al. 1985; Shanks & Doss 1987; Ladd 1988; Schmidt & Friend 1991; Allsop 1992; Sharma 1994; Soetens & Pasteels 1994; Shields & Mitchell 1995; Yazawa 1997; Saran & Rust 2005), including A. suspensa (Nigg et al. 2006). This experiment examined the influence on the consumption of NuLure by the addition of sucrose, fructose, valine, or sodium tetraborate to 10% Nu-Lure. Two J-tubes containing different solutions were presented in each treatment cage for 45 min. The choice comparisons for experiment 3 were as follows: (1) 10% Nu-Lure plus 0.1% cresol red vs. distilled water plus 0.1% fluorescein; (2) 10% Nu-Lure plus 0.1% cresol red vs. 0.2 M sucrose plus 0.1% fluorescein; (3) 10% Nu-Lure plus 0.1% cresol red vs. 10% Nu-Lure in 0.2 M sucrose plus 0.1% fluorescein; (4) 0.2 M sucrose plus 0.1% cresol red vs. 10% Nu-Lure in 0.2 M sucrose plus 0.1% fluorescein; (5) 10% Nu-Lure plus 0.1% fluorescein vs. 10% Nu-Lure in 0.2 M fructose plus 0.1% cresol red; (6) 10% Nu-Lure plus 0.1% fluorescein vs. 10% Nu-Lure plus 0.05 M valine plus 0.1% cresol red; (7) 10% Nu-Lure in 0.2 M sucrose plus 0.1% cresol red vs. 10% Nu-Lure in 0.2 M sucrose plus 0.05 M valine plus 0.1% fluorescein; and (8) 10% Nu-Lure plus 0.1% fluorescein vs. 10% Nu-Lure in 5% sodium tetraborate plus 0.1% cresol red. There were 5 replicates of each comparison except there were 10 replications for 10% Nu-Lure vs. distilled water and for 10% Nu-Lure vs. 10% Nu-Lure in 0.2 M sucrose. We compared statistically the percent of flies that did not feed, flies that fed only on one of the solutions, and flies that fed on both solutions. We examined in detail the consumption of flies that fed on both solutions.

## Statistics

A replicate for all experiments is the mean of a cage by sex. For example, a five-replicate experiment is 5 cages. The means of the 5 cages by sex are the basis for the means and variation of each treatment. Standard deviation is used throughout. Means in Table 2 were compared with paired t-tests  $\alpha=0.05,\,0.01,\,$  or 0.001 (Microsoft Office Excel 2003). Means in Tables 3, 4, and 5 were statistically compared by analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test at  $\alpha=0.05$  (SAS Institute 2001).

#### RESULTS AND DISCUSSION

The means and standard deviations of the consumption of the sucrose positive controls by males were 2.50  $\pm$  0.31  $\mu L$  (range 2.07-3.08  $\mu L)$  and by females 3.27  $\pm$  0.74  $\mu L$  (range 2.15-4.43  $\mu L)$ . There were no statistical differences week to week in the consumption of sucrose by the sucrose control flies except for one week with less than 2.5  $\mu L/fly$ ; that data set was discarded.

The pH of the protein solutions ranged from a low of 3.79 (Nu-Lure in 0.2 M sucrose + 0.05 M valine) to 7.12 (EZ Case M), a factor that may affect attractiveness (Flath et al. 1989; Heath et al. 1994), and ranged from completely soluble to insoluble (Table 1). The 10% Nu-Lure sugar and Nu-Lure valine solutions pHs ranged from 3.80 to 3.78. The pH of 10% Nu-Lure + 5% sodium tetraborate was 8.01. Materials that were insoluble and unsuitable for a liquid bait were Torula yeast, Prolisse, and sodium caseinate (Table 1).

## **Experiment One**

No fly consumed Nu-Lure only. The percentage of flies feeding ranged from 36-100% compared to sucrose controls at 98-100%. Male and female flies consumed about 5× more water compared to Nu-Lure, although 24-h fly consumption was low (Table 2). There was no difference in the consumption of Braggs liquid amino acids vs. Nu-Lure for 6-d flies (Table 2); more NuLure was consumed by 24-h flies. Six-day flies preferred Nu-Lure compared to corn steep liquor; there were no differences for 24-h flies (Table 2). Nu-Lure was preferred to NZ Case and pepticase by 24-h and 6d flies (Table 2). Nu-Lure was preferred over Solulys by 6-d males only (Table 2). Nu-Lure was preferred over soy protein hydrolysate only by 24h females (Table 2). Nu-Lure was preferred over Torula yeast except by 6-d males (Table 2). Whey protein was consumed less than Nu-Lure by 24-h males and 6-d females (Table 2). Nu-Lure was preferred over yeast hydrolysate by 24-h flies, but not by 6-d flies (Table 2). The important point about Table 2 data is the less than 2.0 µL average consumption of protein solutions, actually most below 1.0 µL, compared to an average sucrose control consumption of 2.50 µL for males and 3.27 μL for females.

## Experiment Two

With the discovery in Experiment 1 that the consumption of protein solutions was low compared to the sucrose controls, we designed Experiment 2 to examine a no-choice comparison of Nu-Lure, sucrose, and water. Experiment 2 no-choice consumption data are presented in Table 3. For males, the percent feeding was not different across solutions (Table 3). For 6-d females, the

TABLE 1. SOLUBILITY AND PH OF PROTEIN SOLUTIONS

| 10% type                                 | Solubility        | pН   |  |
|--|-------------------|------|--|
| Corn steep liquor                        | Slight sediment   | 3.98 |  |
| EZ Case M                                | Soluble           | 7.12 |  |
| Hydrolyzed casein                        | Stable suspension | 5.02 |  |
| Liquid amino acids                       | Soluble           | 5.58 |  |
| Nu-Lure                                  | Soluble           | 3.82 |  |
| Nu-Lure in 0.2 M fructose                | Soluble           | 3.82 |  |
| Nu-Lure + 5% sodium tetraborate          | Soluble           | 8.01 |  |
| Nu-Lure in 0.2 M sucrose                 | Soluble           | 3.80 |  |
| Nu-Lure in 0.2 M sucrose + 0.05 M valine | Soluble           | 3.79 |  |
| Nu-Lure plus 0.05 M valine               | Soluble           | 3.78 |  |
| Pepticase                                | Soluble           | 6.96 |  |
| Prolisse                                 | Thick suspension  | 6.96 |  |
| Sodium caseinate                         | Not soluble       | 6.22 |  |
| Solulys                                  | Slight sediment   | 3.98 |  |
| Soy protein acid hydrolysate             | Some sediment     | 5.70 |  |
| Torula yeast                             | Not soluble       | 6.31 |  |
| Whey                                     | Some sediment     | 5.38 |  |
| Yeast hydrolysate enzymatic              | Some sediment     | 5.60 |  |

percent feeding on water was lower than the other solutions, but the amount of water consumed was not different than Nu-Lure or Nu-Lure plus sucrose. For males and females, the amount of 0.2 M sucrose consumed was  $2\times$  to  $5\times$  greater than water, Nu-Lure, or Nu-Lure plus sucrose (Table 3). The addition of sucrose to Nu-Lure did not enhance its consumption compared to Nu-Lure alone (Table 3).

#### **Experiment Three**

Experiment 3 examined the choices flies made in their consumption of the solutions in Experiment 2 (Table 2) and the possible improvement of Nu-Lure consumption. The percentage of flies that fed ranged from 36 to 100% (data not presented). For most experiments, the percent feeding was 70% or more (data not presented). Only the flies that fed on both solutions were included in these analyses.

When comparing the quantities consumed, there was no difference between Nu-Lure and water (line 1, Tables 4 and 5). This is the same result as in Table 3, that is, no difference between the consumption of Nu-Lure and the consumption of water.

Flies fed more on sucrose than on 10% Nu-Lure; this reached statistical significance with 6d females (line 2, Tables 4 and 5). The addition of sucrose to Nu-Lure led to more consumption of sucrose/Nu-Lure compared to Nu-Lure alone for 24-h females only (line 3, Tables 4 and 5).

Valine improved Nu-Lure and Nu-Lure in 0.2 M sucrose consumption by 6-d, but not 24-h males and females (line 6, Tables 4 and 5). Although more NuLure plus 0.2 M sucrose was consumed

when valine was added, this reached statistical significance only with 6-d females (line 7, Tables 4 and 5). The addition of 5% borax to 10% Nu-Lure did not improve its consumption (line 8, Tables 4 and 5), a combination known to increase Nu-Lure attractiveness to Anastrepha spp. (Heath et al. 1994). Our interpretation of these data is that the addition of NuLure to 0.2 M sucrose decreased the consumption of sucrose and the inclusion of NuLure in a comparison decreased the consumption of solutions in general. If we total the consumption of flies in Tables 4 and 5, we can compare these totals to the consumption of sucrose controls. Overall, male sucrose controls averaged 2.5 µL/fly; females 3.27 µL/fly. By comparison, 24h males consumed 3.41 µL/fly; females 5.81 µL for the Nu-Lure/sucrose comparison (line 2, Table 4). This is the only set of totals for Table 4 that meet or exceed the control average. Sucrose control consumption was exceeded by males in Table 5 (line 2); females consumed 3.28 µL/fly (line 2, Table 5). In some cases, sucrose may overcome a deterrent effect of a substance (Shields & Mitchell 1995), but apparently not with Nu-Lure and A. suspensa.

Ninety-eight to 100% of the sucrose positive controls fed over the 10 weeks of these experiments (data not presented). There were no differences in the consumption of water and 10% Nu-Lure by 24-h and 6-d males and females (Table 3). In the water-Nu-Lure comparison, an average of 92% of males and 96% of females in the sucrose checks fed with a mean consumption of 3.24  $\pm$  0.21  $\mu L$  (males) and 3.89  $\pm$  0.33  $\mu L$  (females). The consumption of the protein solutions was generally less than one-half of the consumption of the 0.2 M sucrose controls. The addition of Nu-Lure to

374

Table 2. Anastrepha suspensa consumption of protein solutions (µL, Experiment 1).

|     | Comparison                         | 24-h        |  |             | 6-d   |             |  |             |   |
|-----|------------------------------------|-------------|--|-------------|---|-------------|--|-------------|---|
|     |                                    | % feeding   | Male   | % feeding   | Female  | % feeding   | Male   | % feeding   | Female  |
| 1.  | Nu-Lure<br>Water                   | 87 ± 12     | 0.14 ± 0.12***<br>0.59 ± 0.19                  | 53 ± 23     | $0.04 \pm 0.04$ *<br>$0.16 \pm 0.12$          | 56 ± 26     | 0.17 ± 0.07*<br>1.10 ± 0.56                    | 92 ± 11     | 0.37 ± 0.11*<br>1.75 ± 0.84                   |
| 2.  | Nu-Lure<br>Braggs Liquid Aminos    | $52 \pm 34$ | $0.33 \pm 0.21^*$<br>$0.05 \pm 0.04$           | 88 ± 18     | $0.67 \pm 0.26**$<br>$0.28 \pm 0.16$          | $100 \pm 0$ | $0.45 \pm 0.28 \text{ NS}$<br>$0.24 \pm 0.20$  | $96 \pm 9$  | $0.42 \pm 0.33 \text{ NS}$<br>$0.49 \pm 0.24$ |
| 3.  | Nu-Lure<br>Corn Steep Liquor       | $64 \pm 17$ | $0.10 \pm 0.11 \text{ NS}$<br>$0.11 \pm 0.03$  | $72 \pm 17$ | $0.27 \pm 0.24 \text{ NS}$<br>$0.10 \pm 0.03$ | $100 \pm 0$ | $0.65 \pm 0.32^*$<br>$0.06 \pm 0.06$           | $100 \pm 0$ | $0.63 \pm 0.24$ **<br>$0.04 \pm 0.03$         |
| 4.  | Nu-Lure<br>NZ Case                 | $56 \pm 9$  | $0.23 \pm 0.14 \text{ NS} $<br>$0.03 \pm 0.04$ | $60 \pm 32$ | $0.62 \pm 0.50^*$<br>$0.03 \pm 0.05$          | $91 \pm 12$ | $0.99 \pm 0.22***$<br>$0.02 \pm 0.04$          | $100 \pm 0$ | 1.76 ± 0.45***<br>0.33 ± 0.15                 |
| 5.  | Nu-Lure<br>Pepticase               | $100 \pm 0$ | $0.40 \pm 0.13***$<br>$0.01 \pm 0.03$          | $96 \pm 9$  | $0.83 \pm 0.30$ **<br>$0.12 \pm 0.05$         | $84 \pm 26$ | $0.61 \pm 0.29** \\ 0.11 \pm 0.07$             | $100 \pm 0$ | 1.34 ± 0.41**<br>0.26 ± 0.17                  |
| 6.  | Nu-Lure<br>Solulys                 | $56 \pm 26$ | $0.11 \pm 0.10 \text{ NS}$<br>$0.10 \pm 0.10$  | $100 \pm 0$ | $0.49 \pm 0.41 \text{ NS}$<br>$0.46 \pm 0.10$ | $100 \pm 0$ | $0.72 \pm 0.18***$<br>$0.03 \pm 0.03$          | 96 ± 9      | $0.52 \pm 0.34 \text{ NS}$<br>$0.20 \pm 0.21$ |
| 7.  | Nu-Lure<br>Soy Protein Hydrolysate | 48 ± 11     | $0.38 \pm 0.32 \text{ NS}$<br>$0.09 \pm 0.07$  | $76 \pm 17$ | $1.39 \pm 0.90$ *<br>$0.09 \pm 0.09$          | $64 \pm 9$  | $0.38 \pm 0.20 \text{ NS}$<br>$0.13 \pm 0.09$  | $76 \pm 17$ | $0.84 \pm 0.47 \text{ NS}$<br>$0.28 \pm 0.31$ |
| 8.  | Nu-Lure<br>Torula Yeast            | $100 \pm 0$ | 0.88 ± 0.21**<br>0.04 ± 0.09                   | $100 \pm 0$ | $1.07 \pm 0.47*$<br>$0.33 \pm 0.11$           | $36 \pm 22$ | $0.28 \pm 0.18 \text{ NS} $<br>$0.07 \pm 0.08$ | 88 ± 18     | 1.91 ± 0.31***<br>0.20 ± 0.09                 |
| 9.  | Nu-Lure<br>Whey                    | $36 \pm 22$ | $0.21 \pm 0.18 \text{ NS}$<br>$0.03 \pm 0.02$  | $48 \pm 27$ | $0.95 \pm 0.69$ *<br>$0.05 \pm 0.03$          | $93 \pm 12$ | $0.91 \pm 0.36**$<br>$0.07 \pm 0.11$           | $80 \pm 20$ | $0.86 \pm 0.82 \text{ NS}$<br>$0.09 \pm 0.07$ |
| 10. | Nu-Lure<br>Yeast Hydrolysate       | $56 \pm 33$ | $0.14 \pm 0.09$ *<br>$0.01 \pm 0.01$           | $100 \pm 0$ | $0.84 \pm 0.23***$<br>$0.004 \pm 0.01$        | 88 ± 18     | $0.34 \pm 0.15 \text{ NS}$<br>$0.37 \pm 0.12$  | 88 ± 18     | $0.50 \pm 0.24 \text{ NS}$<br>$0.59 \pm 0.48$ |

Mean ± standard deviation, n = 5; means are different at \*0.05, \*\*0.01, and \*\*\*0.001 by paired t-tests (Microsoft Office Excel 2003) or NS = not significantly different.

Table 3. No-choice consumption of water, 10% Nu-Lure plus 0.2 M sucrose, 10% Nu-Lure and 0.2 M sucrose by Anastrepha suspensa (Experiment 2).

|                                | % feeding             | Mean ± SD<br>μL per fly<br>consumed | % feeding              | Mean ± SD<br>μL per fly<br>consumed |  |
|--------------------------------|-----------------------|-------------------------------------|------------------------|-------------------------------------|--|
|                                | 24-h Male             |                                     | 24-h Female            |                                     |  |
| DDI water                      | 66 ± 24 a*            | 0.51 ± 0.44 b                       | 48 ± 39 b              | $0.70 \pm 0.52 \mathrm{b}$          |  |
| 10% Nu-Lure plus 0.2 M sucrose | $80 \pm 20 \text{ a}$ | $0.45 \pm 0.12 \mathrm{b}$          | $74 \pm 19 \text{ ab}$ | $0.44 \pm 0.20 \text{ b}$           |  |
| 10% Nu-Lure                    | $68 \pm 30 \text{ a}$ | $0.78 \pm 0.23 \text{ b}$           | $96 \pm 9 \text{ a}$   | $1.11 \pm 0.34 \text{ b}$           |  |
| 0.2 M sucrose                  | 76 ± 17 a             | $2.10 \pm 0.33$ a                   | $84 \pm 17 \text{ ab}$ | $2.68 \pm 0.77$ a                   |  |
|                                | 6-d                   | 6-d Male                            |                        | 6-d Female                          |  |
| DDI water                      | 52 ± 33 a             | 0.69 ± 0.36 b                       | 20 ± 0 b               | $0.13 \pm 0.07 \text{ b}$           |  |
| 10% Nu-Lure plus 0.2 M sucrose | $96 \pm 9 \text{ a}$  | $0.78 \pm 0.29 \text{ b}$           | $100 \pm 0 a$          | $0.80 \pm 0.13 \text{ b}$           |  |
| 10% Nu-Lure                    | $65 \pm 25 \text{ a}$ | $1.61 \pm 0.37 \text{ b}$           | $82 \pm 10 \text{ a}$  | $0.84 \pm 0.59 \mathrm{b}$          |  |
| 0.2 M sucrose                  | $76 \pm 33 \text{ a}$ | $2.57 \pm 0.70$ a                   | $84 \pm 17 a$          | $2.79 \pm 1.43$ a                   |  |

<sup>\*</sup>Means by age and sex followed by the same letter are not statistically different by ANOVA followed by Tukey's HSD test,  $\pm = 0.05$ , n = 5. SD = standard deviation.

a consumption comparison appears to decrease the total consumption of both solutions (Tables 4 and 5). One possibility for our data is that *A. suspensa* self-selected an optimal diet (Hagen & Finney 1950; Waldbauer & Friedman 1991). *Anastrepha suspensa* seems to prefer sugar as an immature fly and protein when sexually mature (Nigg et al. 1995). Our previous data suggested

that 6-d-old females would have preferentially consumed protein (Nigg et al. 1995). However, in the present study, both sexually mature and immature flies preferentially consumed 0.2 M sucrose (Table 3). This said, the goal here was an increase in consumption so that pesticide quantity might be reduced. The mechanism of the increase might be studied in the future.

Table 4. Choice comparison of Nu-Lure consumption by 24-h Anastrepha suspensa (Experiment 3).

|     |  | Mean ± SD<br>μL per fly consumed | Mean ± SD<br>μL per fly 6-d |  |
|-----|--|----------------------------------|-----------------------------|--|
| Fee | eding category                               | 24-h male                        | 24-h female                 |  |
| 1.  | 10% Nu-Lure                                  | $0.46 \pm 0.21 \text{ NS}$       | 0.82 ± 0.13 NS              |  |
|     | Water  | $0.59 \pm 0.29$                  | 0.67 ± 0.13                 |  |
| 2.  | 10% Nu-Lure                                  | $1.19 \pm 0.34 \text{ NS}$       | $1.93 \pm 0.33 \text{ NS}$  |  |
|     | 0.2 M sucrose                                | $2.22 \pm 2.82$                  | $3.88 \pm 1.92$             |  |
| 3.  | 10% Nu-Lure                                  | $0.36 \pm 0.13 \text{ NS}$       | $0.48 \pm 0.15$ *           |  |
|     | 10% Nu-Lure in 0.2 M sucrose                 | $1.39 \pm 0.85$                  | $1.40 \pm 0.52$             |  |
| 4.  | 0.2 M sucrose                                | $1.13 \pm 0.95 \text{ NS} $      | $0.67 \pm 0.29 \text{ NS}$  |  |
|     | 10% Nu-Lure in 0.2 M sucrose                 | $0.14 \pm 0.05$                  | $0.24 \pm 0.16$             |  |
| 5.  | 10% Nu-Lure                                  | $0.36 \pm 0.16 \text{ NS}$       | $0.48 \pm 0.26 \text{ NS}$  |  |
|     | 10% Nu-Lure in 0.2 M fructose                | $1.37 \pm 1.03$                  | $2.19 \pm 1.62$             |  |
| 6.  | 10% Nu-Lure                                  | $0.17 \pm 0.06 \text{ NS}$       | $0.44 \pm 0.20 \text{ NS}$  |  |
|     | 10% Nu-Lure in 0.05 M valine                 | $1.16 \pm 1.14$                  | $1.30 \pm 0.76$             |  |
| 7.  | 10% Nu-Lure in 0.2 M sucrose                 | $0.35 \pm 0.16 \text{ NS}$       | $0.66 \pm 0.28 \text{ NS}$  |  |
|     | 10% Nu-Lure in 0.2 M sucrose + 0.05 M valine | $1.79 \pm 0.88$                  | $2.32 \pm 1.92$             |  |
| 8.  | 10% Nu-Lure                                  | $0.31 \pm 0.12^{**}$             | $0.52 \pm 0.38 \text{ NS} $ |  |
|     | 10% Nu-Lure in 5% borax                      | $0.54 \pm 0.18$                  | $0.77 \pm 0.43$             |  |

Means are significantly different at \*0.05, \*\*0.01, \*\*\*0.001. Rows with the same number were compared statistically. SD = standard deviation.

Table 5. Choice comparison of Nu-Lure consumption by 6-d Anastrepha suspensa (Experiment 3).

|     |  | Mean $\pm$ SD $\mu L$ per fly consumed | Mean ± SD<br>μL per fly 6-d |  |
|-----|--|--|-----------------------------|--|
| Fee | eding category                               | 6-d Male                               | 24-h Female                 |  |
| 1.  | 10% Nu-Lure                                  | $0.75 \pm 0.22 \text{ NS}$             | $1.03 \pm 0.27 \text{ NS}$  |  |
|     | Water  | $1.39 \pm 0.73$                        | $1.41 \pm 0.46$             |  |
| 2.  | 10% Nu-Lure                                  | $0.80 \pm 0.49 \text{ NS}$             | $1.21 \pm 0.51^*$           |  |
|     | 0.2 M sucrose                                | $2.20 \pm 0.76$                        | $2.07 \pm 0.57$             |  |
| 3.  | 10% Nu-Lure                                  | $0.70 \pm 0.55 \text{ NS}$             | $1.15 \pm 0.73 \text{ NS}$  |  |
|     | 10% Nu-Lure in 0.2 M sucrose                 | $1.32 \pm 0.95$                        | $0.89 \pm 0.78$             |  |
| 4.  | 0.2 M sucrose                                | $0.30 \pm 0.47 \text{ NS}$             | $0.88 \pm 1.09 \text{ NS}$  |  |
|     | 10% Nu-Lure in 0.2 M sucrose                 | $0.37 \pm 0.07$                        | $0.80 \pm 0.30$             |  |
| 5.  | 10% Nu-Lure                                  | $0.30 \pm 0.20 \text{ NS}$             | $0.51 \pm 0.14 \text{ NS}$  |  |
|     | 10% Nu-Lure in 0.2 M fructose                | $1.13 \pm 0.61$                        | $1.96 \pm 1.21$             |  |
| 6.  | 10% Nu-Lure                                  | $0.20 \pm 0.12^{**}$                   | $0.56 \pm 0.15**$           |  |
|     | 10% Nu-Lure in 0.05 M valine                 | $2.09 \pm 0.55$                        | $3.71 \pm 0.71$             |  |
| 7.  | 10% Nu-Lure in 0.2 M sucrose                 | $0.31 \pm 0.17 \text{ NS}$             | $0.61 \pm 0.36$ *           |  |
|     | 10% Nu-Lure in 0.2 M sucrose + 0.05 M valine | $0.50 \pm 0.47$                        | $2.34 \pm 0.95$             |  |
| 8.  | 10% Nu-Lure                                  | $0.65 \pm 0.16 \text{ NS}$             | $0.84 \pm 0.48 \text{ NS}$  |  |
|     | 10% Nu-Lure in 5% borax                      | $1.00 \pm 0.25$                        | $0.62 \pm 0.20$             |  |

Means are significantly different at \*0.05, \*\*0.01, \*\*\*0.001. Rows with the same number were compared statistically. SD = standard deviation

A bait must be both attractive and readily consumed. Maximum consumption is desirable in order to reduce pesticide while maintaining effectiveness. For consumption, our data suggest that Nu-Lure and other tested protein solutions are inappropriate as *consumed* baits for *A. suspensa* and could be replaced by 0.2 M sucrose.

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