

EFFECTS OF SUCROSE IN ADULT DIET ON MORTALITY OF MALES OF *ANASTREPHA SUSPENS*A (DIPTERA: TEPHRITIDAE)

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ABSTRACT

Survival of adult male Caribbean fruit flies, *Anastrepha suspensa* (Loew) fed sucrose and protein in the form of hydrolyzed brewers yeast was studied under greenhouse conditions. Flies fed either a 3:1 mixture of sucrose and protein (optimal) or just sugar from the day of adult eclosion showed no appreciable mortality during the 14-day test period. However, flies fed just protein, or those that were not provided with sugar or protein showed rapid rates of mortality, with 50% mortality occurring at 1.87 and 1.53 days, respectively, and 95% mortality occurring at 2.8 and 2.5 days. Switching flies from the optimal diet to either the protein-only diet or nothing at 7 or 11 days after emergence resulted in values of 50% and 95% mortality, respectively, that were similar to those for flies reared from eclosion on either just protein or nothing. No significant mortality occurred among males maintained on the optimal or sugar-only diets or when flies were shifted from the optimal diet to only sugar at either day 7 or 11 after emergence. These data demonstrate that the flies have an absolute requirement for carbohydrate in the adult diet. Additionally, the results indicate that the flies are incapable of converting of amino acids from protein hydrolysate into precursors useful for generating metabolic energy in sufficient amounts to sustain life.

Key Words: Caribbean Fruit Fly, dietary sucrose, carbohydrate, protein.

RESUMEN

La sobrevivencia de los machos adultos de la mosca del Caribe alimentados con sucrosa y proteína en la forma de levadura hidrolizada de cerveza fue estudiada bajo condiciones de invernadero. Las moscas alimentadas con una mezcla de 3:1 sucrosa y proteína (óptima) o solo azúcar desde el día de la eclosión del adulto no mostraron mortalidad apreciable durante el período de pruebas de 14 días. Sin embargo, las moscas alimentadas solo con proteína, o las moscas no proveídas con azúcar o proteína mostraron una tasa rápida de mortalidad, con 50% mortalidad ocurriendo a los 1.87 y 1.53 días, respectivamente, y 95% mortalidad ocurriendo a los 2.8 y 2.5 días. Cambiando las moscas de una dieta óptima a cualquier de las dietas de solo proteína o nada a los 7 o 11 días después de la emergencia resultó en valores de mortalidad de 50% y 95%, respectivamente, que fueron similares a los datos para las moscas criadas desde la eclosión en la dieta de sola proteína o nada. No mortalidad significativa ocurrió entre los machos mantenidos en las dietas óptima o de solo azúcar o cuando la dieta de las moscas fue cambiada de la dieta óptima a la dieta de solo azúcar a los 7 o 11 días después de la emergencia. Estos datos demuestran que las moscas tienen un requisito absoluto para el carbohidratado en la dieta del adulto. Además, los resultados indican que las moscas no fueron capaces de convertir los aminoácidos de proteína-hidrolizada a los precursores útiles para generar la energía metabólica en cantidades suficientes para mantener la vida.

The Caribbean fruit fly, *Anastrepha suspensa* (Loew), became established in south Florida in the early 1960s and spread rapidly through most of southern and central Florida. The rapid spread of the pest was due to its host range which includes at least 80 different fruit and vegetable hosts commonly found in Florida (Swanson & Baranowski 1972). Although many of the host plants are not of economic importance, the ability to infest over-ripe grapefruit and oranges has resulted in quarantine restrictions being placed on shipment of not only citrus but also other fruits including tomato, bell pepper, lychee, mango, avocado, guava and carambola (Greany & Riherd 1993). In order to overcome shipment restrictions

the state of Florida developed and implemented the Caribbean Fruit Fly Pest Management System (CFFPMS) that has resulted in state certification of "Fly Free Zones" (Greany & Riherd 1993). Although the CFFPMS has been effective and Florida fruit is being shipped around the world, eradication of the fly would significantly improve the agricultural economics of citrus and vegetable production in Florida.

One of the most effective and environmentally sound methods for eradication of pest insects is the sterile insect technique (SIT) pioneered by Knippling (1955). It was first implemented effectively to eradicate the screwworm fly from Florida (Baumhover et al. 1955; Knippling 1959). SIT has

been demonstrated to be an effective tool for suppression and eradication of a number of species of tephritid flies, particularly when coupled with other control techniques (Kakinohana et al. 1997; Steiner et al. 1965, 1970; Wong et al. 1992). Indeed, SIT commonly is used for both direct and prophylactic control of Mediterranean (*Ceratitis capitata* (Wiedemann)) and Mexican (*Anastrepha ludens* Loew) fruit flies in the continental United States and it has been tested for control of the Caribbean fruit fly (Burditt et al. 1975; Holler & Harris 1993). Control is achieved in SIT by mass release of sterile males which mate with wild females. Females that mate with sterile males do not produce viable eggs, and, over time, this results in population decline and possibly eradication. The key to optimizing efficacy of SIT is to produce sterile males that compete as well as, or out compete, wild males in mating opportunities with wild females. Although SIT is an effective population management tool, it is expensive in terms of both money and time. Thus, cost benefits must be clearly defined in order to balance the needs for effective control with funding constraints. One of the more significant costs associated with SIT protocols for the Caribbean fruit fly is the need to hold mass reared adult flies for as many as 7 days prior to release because the mass-produced strains require time to become sexually mature (Teal et al. 2000, and references therein). Minimizing the costs associated with adult holding while still optimizing reproductive performance of the flies is clearly a key element in improving efficacy of SIT management protocols. One way to do this is to minimize the expense associated with feeding adult flies prior to release (see Martinez et al. 1987). Although it is well known that these flies require food as adults and that reproduction and effective sexual signaling are positively impacted by consumption of both carbohydrate and protein (Epsky & Heath 1993; Landolt & Davis-Hernandez 1993; Landolt & Sivinski 1992) little is known about the absolute necessities for either protein or sugar in the adult diet. We were interested in determining if adult males of the Caribbean fruit fly required sugars, protein or both for survival. The following reports the results of studies demonstrating that carbohydrate is absolutely essential for adult survival.

MATERIALS AND METHODS

Insect Cultures

Caribbean fruit flies were obtained as pupae from a culture maintained at the Division of Plant Industry, Florida Department of Agriculture and Consumer Services, Gainesville, Florida. Pupae were housed in screen cages in a greenhouse maintained at 23-27°C and 50-70% RH, and natural light conditions (Teal et al. 2000). Newly

eclosed adults were removed daily, separated by sex and placed into cages immediately after emergence. Females were not used and males were held in a greenhouse that contained no adult female flies and maintained under the above conditions. All flies were provided with water dispensed from a cotton wick pushed through the top of a covered plastic cup containing 25 ml of water. Water cups were changed weekly.

Diets vs Survival

For initial studies we assessed the effects of providing adult males with only water or water plus diets containing either sugar plus protein, only sugar or only protein. The sugar plus protein diet was composed of a cake containing a 3:1 mixture of sucrose and brewers yeast hydrolysate (ICN Biochemicals, #103304). This diet was considered to be the "optimal diet" and was used as the standard diet to which all other diets were compared. The sugar-only diet was a sugar cube and the protein-only diet was a cake composed of yeast hydrolysate. Groups of 25 males were caged in 30-cm³ screen cages on the day of eclosion and provided with one of the above diets. Survivorship was recorded every 24 h. We also conducted an experiment in which we maintained the males on the optimal diet and then switched to only water, or sugar-, or protein-only diets on either 7 or 11 days after eclosion. We compared the survival of flies that were switched to new diets with those maintained on the optimal diet. A one way analysis of variance was used to test the effect of diet on survival, and separate analyses were conducted for flies that were 0, 7, or 11 d old at the time they were switched to the test diets. Fisher's least significant difference test ($P = 0.05$) was used for separation of means.

RESULTS AND DISCUSSION:

Results of studies in which we provided males with only water or water plus diets containing either sugar plus protein (optimum diet), or only sugar, or only protein from the day of adult eclosion showed that sugar was absolutely essential for adult survival. Flies fed water only or water plus protein all died within 96 h but essentially all flies provided with water plus either only sugar or sugar plus protein lived for the duration of the experiment (Tables 1 to 3). In fact, in other experiments in which we fed flies sugar and water from the day of eclosion we found no significant mortality for as long as three weeks ($n = 6$ groups, 25 males; mean mortality at 21 days = $6.7\% \pm 3.67$). Transferring flies to protein plus water, or water only, after feeding for 7 days on the optimal diet resulted in significant mortality (ca. 50%) on day 9, and more than 85% males were dead by the end of the 10th day (Tables 1 and 2). However,

TABLE 1. MEAN SURVIVORSHIP OF FLIES ON EACH DAY AFTER BEING FED ONLY PROTEIN. FLIES WERE EITHER PROVIDED WITH ONLY PROTEIN FROM THE DAY OF ECLOSION OR SWITCHED FROM THE OPTIMAL SUGAR PLUS PROTEIN DIET TO ONLY PROTEIN ON EITHER THE SEVENTH OR ELEVENTH DAY AFTER ADULT ECLOSION. ALL FLIES WERE PROVIDED WITH WATER.

Days after feeding only protein	Mean survivorship of flies fed only protein from day of eclosion*	Mean survivorship of flies switched to protein on day 7*	Mean survivorship of flies switched to protein on day 11*
0	100 A	100 A	100 A
1	91.5 A	94.8 A	92.4 A
2	33.0 B	39.7 B	8.0 C
3	8.5 C	8.7 C	2.4 C
4	1.5 C	1.0 C	0.0 C

*Means in the same column or row followed by the same letter are not significantly different by Fisher's least significant difference test ($P = 0.05$) applied after ANOVA indicated differences among the means.

flies switched to sugar plus water or maintained on the optimal diet had no significant mortality through day 15 (Table 3). Similarly transferring flies from the optimal diet on the 11th day to protein plus water or only water resulted in greater than 90% mortality on day 13 and essentially all were dead by day 14 (Tables 1 and 2). Mortality was negligible for males maintained on the optimal diet or when switched to sugar plus water through day 15 (Table 3). When we compared survival with respect to time after switching diets we found that survival at each day was no different for flies maintained on protein only from eclosion or if they were transferred from the optimal to protein on either day 7 or 11 (ANOVA followed by Fisher's LSD, $P = 0.05$) (Table 1). However, when flies were transferred to only water on day 7 fewer flies died on the second day after transfer than if they were transferred to water on day 11 (ANOVA followed by Fisher's LSD, $P = 0.05$) (Table 2). Survivorship at other ages was not different. There was no significant mortality when flies were maintained on either the optimal diet or when switched to sugar plus water at 11 days.

A number of studies have demonstrated that the addition of protein to a carbohydrate source is important for optimizing reproductive success in

adult *Anastrepha* species. For example, protein dietary supplements have been shown to have positive effects on ovarian maturation and fecundity in females of *A. serpentina*, *A. ludens*, and *A. obliqua* (Jacome et al. 1999; Aluja et al. 2001a; Mangan 2003) and improves sexual performance of males of at least four species including *A. suspensa* (Epsky & Heath 1993) *A. serpentina*, *A. striata*, and *A. obliqua*. (Aljua et al. 2001b). Thus, dietary protein appears to be an important component for reproductive success in all *Anastrepha* spp. However, apart from reports that sugars are necessary dietary requirements (Bateman 1972), important for long term survival (Jacome et al. 1999), and that starvation, by removal of both protein and carbohydrate sources from the adult diet, results in rapid mortality, there is little information on the dietary need of sugar alone or if protein can take the place of sugar as an adult nutrient. Results of our study indicate clearly that carbohydrates are absolute dietary requirements, not only among adult males that have not reached sexual maturity, but also among sexually mature adult males. The results demonstrate that adult male Caribbean flies carry few resources forward from pupation to the adult stage that can be tapped for energy utilization and store only lim-

TABLE 2. MEAN SURVIVORSHIP OF FLIES ON EACH DAY AFTER ALL FOOD WAS WITHHELD. FLIES WERE EITHER STARVED FROM THE DAY OF ECLOSION OR SWITCHED FROM THE OPTIMAL SUGAR PLUS PROTEIN DIET TO NO FOOD ON EITHER THE SEVENTH OR ELEVENTH DAY AFTER ADULT ECLOSION. ALL FLIES WERE PROVIDED WITH WATER.

Days after removing food	Mean survivorship of flies starved from day of eclosion*	Mean survivorship of flies switched to no food on day 7*	Mean survivorship of flies switched to no food on day 11*
0	100 A	100 A	100 A
1	81 B	90.7 A, B	85.6 A, B
2	21.5 D	54.6 C	8.0 D, E
3	4.0 E	5.8 D, E	1.6 E
4	0.0 E	0.5 E	0.0 E

*Means in the same column or row followed by the same letter are not significantly different by Fisher's least significant difference test ($P = 0.05$) applied after ANOVA indicated differences among the means.

TABLE 3. MEAN SURVIVORSHIP OF FLIES ON EACH DAY AFTER BEING FED ONLY SUGAR. FLIES WERE EITHER PROVIDED WITH ONLY SUGAR FROM THE DAY OF ECLOSION OR SWITCHED FROM THE OPTIMAL SUGAR + PROTEIN DIET TO JUST SUGAR ON EITHER THE SEVENTH OR ELEVENTH DAY AFTER ADULT ECLOSION. ALL FLIES WERE PROVIDED WITH WATER.

Days after feeding sugar only	Mean survivorship of flies starved from day of eclosion*	Mean survivorship of flies switched to no food on day 7*	Mean survivorship of flies switched to no food on day 11*
0	100	100	100
1	100	100	100
2	100	99.8	100
3	100	98.6	98
4	99.3	96.6	94.7

*Means in the same column or row are not significantly different in an ANOVA or Fisher's least significant difference test ($P = 0.05$). Mean survivorship of flies fed protein plus sugar for the first 4 days was 98.67%; for 11 days it was 99.6%, and for 15 days it was 99.6%.

ited energy reserves as adults despite being provided with far more food, in the form of hydrolyzed yeast protein, than is required. This suggests strongly that flies maintain only limited supplies of glycogen in muscle and fat body tissue and have limited capacity to convert lipids from the fat body into energy. Perhaps more surprising is the apparent inability of flies to convert dietary protein resources to energy in the absence of carbohydrate as is evidenced by the fact that flies provided with only protein or switched from the complete diet to protein alone at 7 or 11 days exhibited the same rates of survival as males provided with water only. This is intriguing given that the protein fed to males was rich in amino acids such as proline, alanine, aspartate, and glutamate which are readily converted by some insects to pyruvate or oxaloacetate or α -ketoglutarate for use in the Krebs cycle. Indeed, complete catabolism of one mole of proline can yield 14 moles of ATP and in some Diptera, such as the Tsetse fly (*Glossina morsitans*, Westwood), proline is the major source for ATP generation (Bursell 1981). This suggests strongly that transamination of normally important amino acids is not a primary method of substrate production for the Krebs cycle of these flies.

The absolute requirement for sugar and inability of the flies to utilize protein for metabolic energy may reflect a physiological adaptation to environmental conditions. In the tropics, where these flies evolved, sugars from fruit and nectar are available at all times of the year but protein is a relatively limited resource (Bateman 1972; Hendrichs et al. 1993). Indeed, protein dietary supplements have been shown to have positive effects on ovarian maturation and fecundity in *A. serpentina*, *A. ludens*, and *A. obliqua* (Jacome et al. 1999; Aluja et al. 2001a; Mangan 2003) indicating that dietary protein is a critical component for egg production and, consequently, reproductive success in all *Anastrepha* species. Thus, the flies have probably developed a physiological strategy in

which they utilize the most available food source, sugars, to insure survival, and take advantage of limited protein resources when available to achieve sexual maturity (Bateman 1972).

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