An Artificial Diet for the Sugarcane Aphid (Melanaphis sacchari Zehntner) (Hemiptera: Aphididae) with Potential Uses for in Vitro Toxicological Studies

Authors: Toledo-Hernández, Erubiel, Hernández-Velázquez, Víctor Manuel, and Peña-Chora, Guadalupe

Source: Florida Entomologist, 101(3) : 395-398

Published By: Florida Entomological Society

URL: https://doi.org/10.1653/024.101.0317

An artificial diet for the sugarcane aphid (*Melanaphis sacchari* Zehntner) (Hemiptera: Aphididae) with potential uses for in vitro toxicological studies

*Erubiel Toledo-Hernández[1], Víctor Manuel Hernández-Velázquez[1], and Guadalupe Peña-Chora[2,]**

Abstract

The sugarcane aphid, *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), is an important insect pest of sorghum and sugarcane crops throughout the United States and Mexico. In 2013, its capacity for crop destruction of these 2 commodities amounted to millions of dollars. Currently, no artificial diets are available that can be used for in vitro screening of potential bioactive substances toxic to this aphid species. The objective of our work, reported herein, was to evaluate liquid artificial diets for use in such in vitro screening bioassays. We evaluated a diet originally developed for the green peach aphid (*Myzus persicae* Sulzer [Hemiptera: Aphididae]) and another for the whitefly (*Bemisia argentifolii* Bellow and Perring [Hemiptera: Aleyrodidae]). *Melanaphis sacchari* did not survive on the *M. persicae* diet, whereas about 46% survived on the whitefly diet that contained 30% sucrose and 5% yeast extract during the 10 d trial. Further tests then were conducted to determine if sucrose alone at concentrations of 15, 30, and 50% would be a sufficient artificial diet. We found that *M. sacchari* survival at 10 d post ingestion was greatest (87%) when the diet contained 30% sucrose alone. This solution represented the best diet option for use for in vitro toxicological screening bioassays for the sugarcane aphid.

Key Words: survival; sucrose; insect pest; sorghum; toxicological assays

Resumen

El pulgón de la caña de azúcar *Melanaphis sacchari* Zehntner (Hemiptera: Aphididae), es un insecto plaga importante de cultivos de sorgo y caña de azúcar de los Estados Unidos de Norteamérica y México. En el 2013, el daño ocasionado en estos dos cultivos ascendió a millones de dólares. Actualmente, no hay dietas artificiales disponibles que puedan ser usadas de manera in vitro con la finalidad de realizar la búsqueda de toxinas bioactivas potenciales contra esta especie de áfido. El objetivo de nuestro trabajo, reportado aquí, fue evaluar dietas artificiales líquidas para su uso in vitro en bioensayos de selección. Nosotros evaluamos una dieta originalmente desarrollada para el pulgón verde del melocotonero (*Myzus persicae* Sulzer [Hemiptera: Aphididae]) y otra para la mosquita blanca de la hoja plateada (*Bemisia argentifolii* Bellow y Perring [Hemiptera: Aleyrodidae]). Durante los 10 d de prueba *M. sacchari* no sobrevivió en la dieta de *M. persicae*, mientras que en la dieta de la mosquita blanca de la hoja plateada sobrevivió un 46%, la cual contiene 30% de sacarosa y 5% de extracto de levadura. Se realizaron pruebas posteriores para ver si la sacarosa sola al 15, 30 y 50% serían suficientes para una dieta artificial. Encontramos que la supervivencia de *M. sacchari* en el décimo 10 d pos-ingesta con la dieta que contenía 30% de sacarosa fue la mayor (87%). Esta dieta representa la mejor opción para llevar a cabo bioensayos toxicológicos de selección in vitro contra el pulgón amarillo.

Palabras Clave: supervivencia; sacarosa; insecto plaga; sorgo; ensayos toxicológicos

Melanaphis sacchari Zehntner (Hemiptera: Aphididae), also known as the sugarcane aphid, is an insect pest of African origin that is currently distributed worldwide (Singh et al. 2004). This aphid species adversely affects approximately 20 agricultural crops belonging to the family Gramineae. In addition to causing direct damage to the plant, the aphid also can act as a vector for plant pathogens, including millet red leaf, sugarcane yellow leaf, and sugarcane mosaic viruses (Singh et al. 2004).

In the United States, sugarcane aphid was first reported in 1977 in Florida, and by 1999 had spread into Louisiana (Mead 1978; White et al. 2001). In 1988, Denmark (1988) reported the presence of *M. sacchari* feeding on sorghum in Florida; at that time it was not considered economically important. In 2013, the aphid appeared in Liberty County, Texas, and eventually spread to 38 counties in 4 states (Bowling et al. 2016). In that same year, the sugarcane aphid was reported from Tamaulipas, Mexico, as well as other northern states in Mexico (Rodríguez-del-Bosque & Teran 2015). By the fall of 2015, *M. sacchari* had spread to approximately 400 counties in 17 states of the United States. In Mexico, this pest species was found in all of the sorghum producing regions of the country (Rodríguez-del-Bosque & Terán 2015; Bowling et al. 2016).

The rapid expansion and colonization of the sugarcane aphid in 2 of the largest sorghum producers in the world (i.e., the United States and Mexico) present problems regarding its management (Villanueva et al.

[1]Universidad Autónoma del Estado de Morelos, Centro de Investigación en Biotecnología, Av. Universidad 1001, Col. Chamilpa, CP 62209, Cuernavaca, Morelos, Mexico; E-mail: erubielth@hotmail.com (E. T. H.); vmanuelh@uaem.mx (V. M. H. V.)
[2]Universidad Autónoma del Estado de Morelos, Centro de Investigaciones Biológicas, Av. Universidad 1001, Col. Chamilpa, CP 62209, Cuernavaca, Morelos, Mexico; E-mail: penacg@uaem.mx (G. P. C.)
*Corresponding author; E-mail: penacg@uaem.mx

2014). If field populations of this pest were to develop resistance to any of the pesticides used for their control, those inherited characteristics could spread rapidly to subsequent generations due to the high rate of sugarcane aphid reproduction (Sarwar 2015). This issue emphasizes the necessity of developing screening assays to identify alternative or novel bioactive substances for this pest. For example, developing in vitro toxicological assays for the sugar cane aphid would be a useful tool to determine insecticidal properties of various secondary plant metabolites and identify promising candidate molecules for further development for this pest (De Geyter et al. 2012; Raga & Sato 2011). Currently, there is no standard artificial diet for *M. sacchari* to accomplish this task. Because of the importance of the sugarcane aphid to the agricultural regions of the United States and Mexico, development of screening assays is needed urgently. Therefore, the aim of this study was to evaluate various liquid artificial diets for eventual use in *M. sacchari* in vitro toxicological assays.

Materials and Methods

INSECTS

Native populations of *M. sacchari* were collected from sorghum crops in the state of Morelos, Mexico (18.7669°N, 98.8970°W) and maintained on sorghum, variety M-550 under greenhouse conditions at 70% RH. This plant variety (Majestic Seeds Co., Hodges, South Carolina, USA) is reported to be tolerant of infestation by *M. sacchari*. Infested plants were placed in cages (90 cm^3) covered with mesh of 650 µm (DM Agromallas S.A. de C.V. Group, Culiacan, Sinaloa, Mexico). In addition, each plant was covered with a fine fabric (cylinder of 40 cm diam x 50 cm tall) to exclude parasitoids and predators, as well as to prevent migration of aphids to adjacent plants.

ARTIFICIAL DIETS

One diet we evaluated was originally developed for *Myzus persicae* Sulzer (Hemiptera: Aphididae) (Mittler & Koski 1976) and consisted of 15% sucrose (Reasol, Iztapalapa, Mexico City, Mexico); 2.5 g casein hydrolysate (Sigma-Aldrich, 22090-500G, St. Louis, Missouri, USA); 2% yeast extract (MCD Lab, Tlalnepantla, Mexico City Mexico, Mexico); 123 mg $MgSO_4$ $7H_2O$ (Jalmek, San Nicolas de los Garza, Nuevo Leon, Mexico); 100 mg ascorbic acid (Sigma-Aldrich, A0278-25G, St. Louis, Missouri, USA); 10 mg niacin (Sigma-Aldrich, N0761-100G, St. Louis, Missouri, USA); 5 mg calcium pantothenate (Sigma-Aldrich, C8731-25G, St. Louis, Missouri, USA); 2.5 mg pyridoxine (Sigma-Aldrich, P5669-5G, St. Louis, Missouri, USA); 2.5 mg thiamine (Sigma-Aldrich, T1270-25G, St. Louis, Missouri, USA); and 1.5 mg K_2HPO_4 $3H_2O$ (Jalmek, San Nicolas de los Garza, Nuevo Leon, Mexico).

The second diet evaluated was originally developed for the whitefly, *Bemisia argentifolii* (Hemiptera: Aleyrodidae) (Jancovich et al. 1997), and consisted of 30% sucrose and 0.5% yeast extract (MCD Lab., Tlalnepantla, state of Mexico, Mexico) as a nitrogen source. Both diets were prepared with distilled water (adjusted to pH 7.0 with NaOH or HCl) and sterilized at 121 °C for 20 min at 15 psi.

In addition, diets containing 15, 30, and 50% sucrose only (Reasol, Iztapalapa, Mexico City, Mexico) were evaluated. To each of these 3 diets we added 4.4 mL per L of 10% formalin and 7.3 mL per L of 15% choline chloride, to prevent the growth of fungi and bacteria.

FEEDING CHAMBERS

The feeding chamber used in this study was the same as reported by Torres-Quintero et al. (2013) and was fashioned out of two 40 mm ht × 20 mm diam translucent disposable plastic cups (Envases Cuevas, S.A. de C.V, Xalostoc, state of Mexico, Mexico) (Fig. 1). Into 1 cup, 2 mL of diet was pipetted then sealed with Parafilm® (Sigma-Aldrich, BR701605, St. Louis, Missouri, USA). The bottom of the second cup was removed and the top part inverted onto the cup with diet. A strip of parafilm was used to seal the cups together. Aphids were then placed on top of the parafilm of the cup containing diet using a camel's hair brush. Both cups were covered with a piece of fine mesh fabric and affixed firmly with 2 rubber bands (Fig. 1G). Finally, the feeding chamber was inverted and placed on a plastic plate. Humidity in the chamber was maintained by placing a piece of wet cotton beneath it. All materials used to build the feeding chamber were sterilized with UV light prior to construction.

EVALUATION OF SURVIVAL AND FECUNDITY

Fifteen fourth instar *M. sacchari* were placed in each diet chamber. To evaluate the survival time that aphids could survive without food in the chambers (i.e., negative control), only bottled tap water was provided. Mortality was recorded every 24 h for 10 d and the number of newborn aphids recorded during this period. First instar nymphs were transferred, using a camel's hair brush, to new chambers containing the same diet as adults. Nymphal survival and developmental time were recorded daily. Feeding chambers were maintained at 29 ± 1 °C with a photoperiod of 14:10 h (L:D) at 70 ± 2% RH.

STATISTICS

Preliminary analysis of all mean data was conducted using normality and equal variance tests as reported by Manikandan (2010). Percent data were converted to decimal number for analyses (%/100 = 0.00). Mean number of aphid births and survival data were analyzed by 1-way ANOVA, whereas the pair-wise mean multiple comparison Holm Sidak post hoc test was used to determine differences at $P = 0.05$ (Reyes et al. 2015). Sigma Plot 11.0 software (Systat Software, Inc., San Jose, California, USA) was used for all statistical analyses. Non-transformed means are presented in figures.

Results

Melanaphis sacchari did not survive on the *M. persicae* diet, whereas an overall cumulative average of about 46% survived on the sucrose/yeast (whitefly) diet during the 10 d trial (Table 1). Percent survival of aphids from all 3 sucrose-only diets ranged from about 60% to slightly < 100% (Fig. 2). The negative control (water only) resulted in

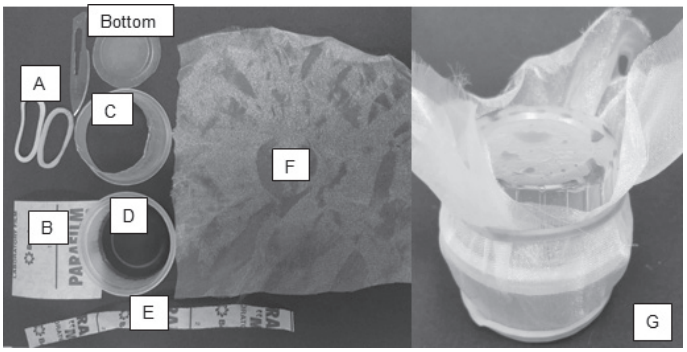

Fig. 1. Construction of the feeding chamber: (A) rubber bands, (B) parafilm, (C) bottom removed from 1 plastic cup, (D) plastic cup with diet, (E) parafilm strip, (F) fine fabric mesh, (G) completed chamber.

Table 1. Cumulative mean (± SE) survival of *Melanaphis sacchari* during a 10 d feeding evaluation of 15, 30, and 50% sucrose and 30% sucrose supplemented with 0.5% yeast diets.

Diets	Survival (%)[a]
Sucrose 30%	92.9 ± 4.1a
Sucrose 50%	84.9 ± 8.7a
Sucrose 15%	54.3 ± 30.5b
Sucrose 30%/yeast 0.5%	46.3 ± 27.1b
Tap water (control)	0.0 ± 0.0*

[a]Means with a different letter are significantly different, Holm Sidak multiple comparison test (P = 0.05). *Complete mortality reached on the third d.

Table 2. Daily birth average (± SE) and percent fecundity during the first 4 d of evaluation of diets containing only 15, 30, 50% sucrose, and a 30% sucrose diet supplemented with 0.5% yeast extract.

Diets	Mean number born during the first 4 d[a]	Percentage of fecundity during the first 4 d
Sucrose 30%	28.9 ± 10.3 a	86.2%
Sucrose 50%	23.7 ± 7.3 ab	88.0%
Sucrose 15%	19.1 ± 9.4 bc	95.9%
Sucrose 30%/yeast 0.5%	14.7 ± 6.5 c	89.2%
Tap water (control)	2.2 ± 1.81*	Does not apply

[a]Means with a different letter are significantly different, Holm Sidak multiple comparison test (P = 0.05). *Average of nymphs born during of the first 2 d.

complete aphid mortality on the third day (Table 1). Overall, the 30% and 50% sucrose-alone diets produced the best survival results at the end of the study. Although survival rates for 30% and 50% sucrose were not significantly different, the 30% diet exhibited higher mean daily survival values with lower standard deviations (Fig. 2). Approximately 20% of newborn nymphs remained alive at 48 h after transfer from the original feeding chamber.

Fecundity was greatest for all diets during the first 4 d of evaluation and represented > 80% of the total, then steadily decreased after this period (Table 2, Fig. 3). Daily abundance of newborn aphids differed according to diet (Table 2). For the whitefly diet with 30% sucrose supplemented with 0.5% yeast extract, a total of 66 aphids were born during the evaluation period. A total of 80 aphids were born from adults that fed on the 15% sucrose diet, 134 aphids from the 30% sucrose diet, and 108 aphids from the 50% sucrose diet.

Discussion

The use of artificial diets has played a very important role in facilitating the development of novel bioassay screening procedures to identify bioactive substances for possible development as insecticides. In the family Aphididae, several diets have already been developed and used in toxicological, physiological, and behavioral research, as well as in studies of endosymbiosis, virus transmission, mortality, and nutrition (Hunter & Hsu 1999). However, no dietary studies for *M. sacchari* have been reported in this regard. Generally, in vitro feeding assays usually require a minimum evaluation time that varies between 2 and 7 d (Liu et al. 2002). Moreover, a 10 d evaluation period has been considered sufficient time in which to screen natural toxins, entomopathogens, or synthetic chemicals for insecticidal properties for aphids (Sabtharishi & Naveen 2017; Torres-Quintero et al. 2016; Han et al. 2014). We found that the 30% sucrose diet (herein designated as the "SA" [sugarcane aphid] diet) yielded an 87.4% survival rate of aphids up to 10 d. We expect that this artificial diet will provide researchers a simple in vitro method for screening chemicals or toxins for biocidal activity for *M. sacchari*.

Acknowledgments

The authors gratefully acknowledge CONACYT (National Council of Science and Technology) for the scholarship granted to Erubiel Toledo-Hernández for his doctorate degree (CVU 419143). This work is part of the Ph.D. thesis; appreciation is extended to Lucrecia Barboza for her critical reading of the manuscript and 2 anonymous reviewers who improved previous drafts.

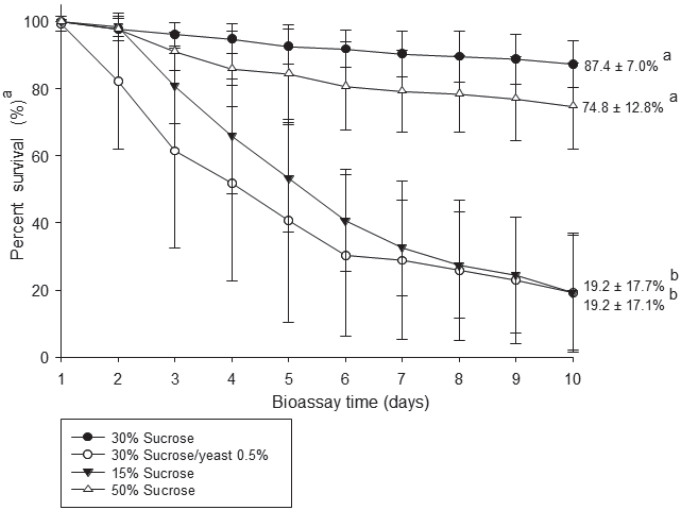

Fig. 2. Daily cumulative mean (± SE) survival of *Melanaphis sacchari* during a 10 d feeding evaluation of diets containing only 15, 30, 50% sucrose, and a 30% sucrose diet supplemented with 0.5% yeast extract. [a]Means with a different letter are significantly different, Holm Sidak multiple comparison test (P = 0.05).

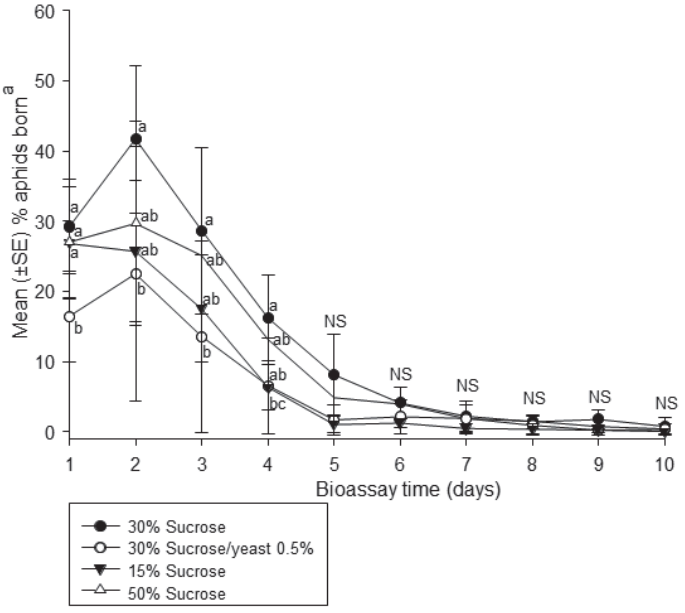

Fig. 3. Mean (± SE) number of aphids born daily during a 10 d evaluation of diets containing only 15, 30, 50% sucrose, and a 30% sucrose diet supplemented with 0.5% yeast extract. [a]Means with a different letter are significantly different, Holm Sidak multiple comparison test (P = 0.05).

References Cited

Bowling RD, Brewer MJ, Kerns DL, Gordy J, Seiter N, Elliott NE, Buntin GD, Way MO, Royer TA, Biles S, Maxson E. 2016. Sugarcane aphid (Hemiptera: Aphididae): a new pest on sorghum in North America. Journal of Integrated Pest Management 7: 1–13.

De Geyter E, Smagghe G, Rahbé Y, Geelen D. 2012. Triterpene saponins of *Quillaja saponaria* show strong aphicidal and deterrent activity against the pea aphid *Acyrthosiphon pisum*. Pest Management Science 68: 164–169.

Denmark HA. 1988. Sugarcane aphids in Florida (Homoptera: Aphididae). Florida Department of Agriculture and Consumer Services. Division of Plant Industry, Entomology circular No. 302.

Han JH, Jin BR, Kim JJ, Lee SY. 2014. Virulence of entomopathogenic fungi *Metarhizium anisopliae* and *Paecilomyces fumosoroseus* for the microbial control of *Spodoptera exigua*. Mycobiology 42: 385–390.

Hunter WB, Hsu HT. 1999. Synthetic diets and piercing-sucking insect research. International Journal of Ecology and Environmental Science 25: 259–266.

Jancovich JK, Davidson EW, Lavine M, Hendrix DL. 1997. Feeding chamber and diet for culture of nymphal *Bemisia argentifolii* (Homoptera: Aleyrodidae). Journal of Economic Entomology 90: 628–633.

Liu TX, Sparks Jr AN, Chen W, Liang GM, Brister C. 2002. Toxicity, persistence, and efficacy of indoxacarb on cabbage looper (Lepidoptera: Noctuidae) on cabbage. Journal of Economic Entomology 95: 360–367.

Manikandan S. 2010. Data transformation. Journal of Pharmacology and Pharmacotherapeutics 1: 126–127.

Mead FW. 1978. Sugarcane Aphid, *Melanaphis sacchari* (Zehntner) – Florida – New Continental United States Record. Cooperative Plant Pest Report 3.

Mittler TE, Koski P. 1976. Development of meridic and oligidic diets for rearing the aphid *Myzus persicae*. Journal of Insect Physiology 22: 1135–1141.

Raga A, Sato ME. 2011. Toxicity of neonicotinoids to *Ceratitis capitata* and *Anastrepha fraterculus* (Diptera: Tephritidae). Journal of Plant Protection Research 51: 413–419.

Reyes H, Arzuffi R, Robledo N. 2015. Effects of male age and mating status on response to the female sex pheromone of *Copitarsia decolora* (Lepidoptera: Noctuidae). Florida Entomologist 98: 47–51.

Rodríguez-del-Bosque L, Terán A. 2015. *Melanaphis sacchari* (Hemiptera: Aphididae): a new sorghum insect pest in Mexico. Southwestern Entomologist 40: 433–434.

Sabtharishi S, Naveen NC. 2017. Bioassay for monitoring insecticide toxicity in *Bemisia tabaci* populations. Protocol Exchange. Nature Publishing Group. http://dx.doi.org/10.1038/protex.2017.015 (last accessed 12 May 2018).

Sarwar M. 2015. The killer chemicals as controller of agriculture insect pests: the conventional insecticides. International Journal of Chemical and Biomolecular Science 1: 141–147.

Singh BU, Padmaja PG, Seetharama N. 2004. Biology and management of the sugarcane aphid, *Melanaphis sacchari* (Zehntner) (Homoptera: Aphididae), in sorghum: a review. Crop Protection 23: 739–755.

Torres-Quintero MC, Arenas-Sosa I, Hernández-Velázquez VM, Suárez-Rodríguez R, Peña-Chora G. 2016. Characterization of *Bacillus thuringiensis* (Bacillaceae) strains pathogenic to *Myzus persicae* (Hemiptera: Aphididae). Florida Entomologist 99: 639–643.

Torres-Quintero MC, Arenas-Sosa I, Peña-Chora G, Hernández-Velázquez VM. 2013. Feeding chamber for *Myzus persicae* culture (Hemiptera: Aphididae). Florida Entomologist 96: 1600–1602.

Villanueva RT, Brewer M, Way MO, Biles S, Sekula D, Bynum E, Swart J, Crumley C, Knutson A, Porter P, Parker R, Odvody G, Allen C, Ragsdale D, Rooney W, Peterson G, Kerns D, Royer T, Armstrong S. 2014. Sugarcane aphid: a new pest of sorghum. Texas A&M Agrilife Extension. Ento-035. Weslaco, Texas, USA.

White W, Reagan T, Hall D. 2001. *Melanaphis sacchari* (Homoptera: Aphididae), a sugarcane pest new to Louisiana. Florida Entomologist 84: 435–436.