Yeast Extract: Sucrose Ratio Effects on Egg Load, Survival, and Mortality Caused by Gf-120 in Western Cherry Fruit Fly (Diptera: Tephritidae)

Author: Yee, Wee L.

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YEAST EXTRACT: SUCROSE RATIO EFFECTS ON EGG LOAD, SURVIVAL, AND MORTALITY CAUSED BY GF-120 IN WESTERN CHERRY FRUIT FLY (DIPTERA: TEPHRITIDAE)

WEE L. YEE
United States Department of Agriculture, Agricultural Research Service, Yakima Agricultural Research Laboratory, 5230 Konnowac Pass Road, Wapato, WA 98951

ABSTRACT

It is unclear which ratios of yeast extract to sucrose result in maximum egg production and survival in many tephritid fruit flies. Objectives here were to determine yeast extract:sucrose ratios that maximize egg loads without compromising survival in western cherry fruit fly, *Rhagoletis indifferens* Curran, and their effects on mortality caused by spinosad bait (GF-120). Yeast extract:sucrose ratios of 20:80 and 30:70 maximized egg loads without reducing survival in most cases. In 1 experiment, mortality of flies with low to high egg loads exposed to fresh GF-120 for 1 or 2 h in the absence of food did not differ. In a separate experiment, egg loads were lowest in flies fed 0:100 and 1:99 diets and highest in flies fed 20:80, 30:70, and 50:50 diets. When these flies were exposed to dried GF-120 for 6 h in presence of yeast extract and sucrose, percent mortality was lower in flies fed 20:80 and 30:70 (40%) than 0:100 (69%) and 1:99 (63%) diets. In another experiment, egg loads were lowest in flies fed 0:100 and 1:99 diet and highest in flies fed 20:80 diet. When these flies were exposed to dried GF-120 for 6 h in the presence of sucrose only, percent mortality was lower in flies fed 20:80 (39%) than 0:100 (72%) and 1:99 (62%) diets. High yeast extract:sucrose ratios result in high egg loads in *R. indifferens* and may reduce the fly's feeding responses to GF-120, although not to the extent that the bait is rendered completely ineffective.

Key Words: feeding, egg production, spinosad bait, survival, diet intake

RESUMEN

No es claro cuales son las proporciones de extracto de levadura a sucrosa que resultan en la producción máxima de huevos y sobrevivencia en muchas moscas de la fruta en la familia Tephrítidae. Los objetivos fueron para determinar las proporciones de extracto de levadura a sucrosa que resultan en el máximo número de huevos sin comprometer la sobrevivencia de la mosca occidental de cereza, *Rhagoletis indifferens* Curran, y sus efectos sobre la mortalidad causada por cebo de 'spinosad' (GF-120). Proporciones de extracto de levadura: sucrosa de 20:80 y de 30:70 resultaron en la máxima cantidad (carga) de huevos sin reducir la sobrevivencia en la mayoria de los casos. En e1 experimento, la mortalidad de las moscas con cantidades bajas y altas de huevos expuestos a GF-120 fresco por 1 o 2 horas en la ausencia de comida no fue diferente. En un experimento separado, la cantidad de huevos fue la más baja en moscas alimentadas de dietas de 0:100 y 1:99 y la más alta en moscas alimentadas de dietas de 30:70, y 50:50. Cuando las moscas fueron expuestas a GF-120 seco por 6 horas en la presencia de extracto de levadura y sucrosa, el porcentaje de mortalidad fue mas bajo en moscas alimentadas de dietas de 20:80 y 30:70 (40%) que en las dietas de 0:100 (69%) y 1:99 (63%). En otro experimento, la cantidad de huevos fue más bajo en moscas alimentadas de dietas de 0:100 y 1:99 y la más alta en moscas alimentadas de una dieta de 20:80. Cuando las moscas fueron expuestas a GF-120 seco por 6 horas en la presencia de solo sucrosa, el porcentaje de mortalidad fue más bajo en moscas alimentadas de dietas de 20:80 (39%) que en dietas de 0:100 (72%) y 1:99 (62%). Proporciones altas de extracto de levadura: sucrosa resultaron en altas cantidades de huevos en *R. indifferens* y pueden reducir la respuesta de alimentación de la mosca a GF-120, aunque no al punto en que el cebo resulta completamente inefectivo.

Consumption of yeast hydrolysate or extract by many tephritid fruit flies increases egg production and benefits other physiological processes and behaviors (Webster et al. 1979; Cangussu & Zucoloto 1997; Jácome et al. 1999; Aluja et al. 2001; Meats & Leighton 2004; Prabhu et al. 2008). Particular ratios of amino acids, peptides, and protein (such as in yeast extract or yeast) to sugar may have important effects on various behavioral and physiological parameters (Prabhu et al. 2008), including egg production and survival. In nature, flies may not always have the choice of self regulating intake of protein and carbohydrates because nitrogenous and carbohydrate foods often do not occur as separate, discrete units (e.g., leachates and fruit juice are mixtures of

both) (Hendrichs et al. 1993; Yee 2003, 2008), making ratios of nutrients in foods biologically important. It remains unclear which ratios of yeast extract to sucrose result in maximum egg production and survival in many fruit fly species. Clarifying these ratios is important for producing flies with high egg loads and survival, needed for colony maintenance and experimentation, as well as for testing hypotheses about egg load effects on behaviors.

In studies to determine egg load effects on behaviors, egg loads can be manipulated by varying access to yeast hydrolysate. In *Rhagoletis* flies, increasing egg loads this way increased the propensity of flies to engage in oviposition-type behaviors, including on fruit marked by oviposition deterring pheromone (Prokopy et al. 1994; van Randen & Roitberg 1996). *Rhagoletis pomonella* (Walsh) with low egg loads were more attracted to vials with yeast hydrolysate + bird droppings than flies with high egg loads (Prokopy et al. 1995), suggesting egg loads and feeding responses to nitrogenous foods are negatively correlated. Protein-deficient *Ceratitis capitata* (Wiedemann) flies were more active and found protein bait more often than protein-fed flies and were more likely to feed on protein than protein-deprived flies (Vargas et al. 2002; Barry et al. 2003). In these cases, it can be assumed protein-fed flies had higher egg loads than protein-deficient flies.

The western cherry fruit fly, *Rhagoletis indifferens* Curran (Diptera: Tephritidae), is the major insect pest of sweet cherry, *Prunus avium* (L.) L., in the Pacific Northwest of the U.S. that benefits from feeding on yeast extract (Yee 2003). However, effects of different yeast extract:sucrose ratios on its egg production, survival, and feeding responses are not well known. A ratio of 20:80 has been used to maintain this species for experimental work (Yee 2003), but no data have been published to confirm this is the optimal ratio to use for egg production and survival.

Generating flies with low to high egg loads will help us test hypotheses about egg load effects on feeding responses in *R. indifferens*. Control of this fly is achieved mostly through use of proteinaceous-sugar bait with spinosad (GF-120® NF Naturalyte® Fruit Fly Bait, Dow AgroSciences, Indianapolis, IN) (Warner 2008) rather than broad cover sprays. GF-120 was developed to attract flies, stimulate them to feed, and then kill them (Moreno & Mangan 2003). Because *R. indifferens* begins laying eggs about 5-10 d post eclosion at 26.7°C (Frick et al. 1954), bait is applied weekly. Flies that have fed on high amounts of yeast extract should have higher egg loads than those that have fed on lower amounts and may less likely respond to GF-120 because they need less protein, resulting in lower mortality. However, the strength of any egg load-mortality relationship is unknown.

The objectives of this study were to determine yeast extract:sucrose ratios that maximize egg loads without compromising survival in *R. indifferens* and their effects on fly mortality caused by GF-120. The hypothesis that *R. indifferens* with greater egg loads (produced by feeding flies higher yeast extract diets) suffer lower mortality than flies with lower egg loads after exposure to GF-120 was tested. Intake of diets was determined.

Materials and Methods

Flies were collected as eggs or larvae in infested sweet cherries in Jun and Jul 2007 and 2008 in the cities of Richland and Yakima in central Washington state, U.S.A. Larvae emerged from cherries and pupariated in soil in tubs. Puparia were held in moist soil at 3-4°C for 4-8 months, and then removed from chilling and held at 26-28°C, 30-35% RH, and 16 h of light for adult emergence and experimentation in 2008 and 2009.

Yeast Extract:Sucrose Diets

Dried yeast extract and sucrose diets similar to dry mixes used by Prabhu et al. (2008) were tested. Yeast extract:sucrose ratios (w:w) tested were (1) 0:100, (2) 1:99, (3) 5:95, (4) 10:90, (5) 20:80, (6) 30:70, and (7) 50:50. Dry granulated yeast extract (EMD Chem., Merck KGaA, Darmstadt, Germany) was used. Based on the manufacturer's certificate of analysis, the yeast extract had a pH of 7.0 and consisted (w:w) of 10.9% N, 0.03% chloride (as NaCl), 1.3% phosphorous compounds (as P), <0.005% heavy metals (as Pb), <0.05% calcium (Ca), <0.10% magnesium (Mg), and 15.5% sulfated ash (600°C). To make the diets, yeast extract was first dissolved in water. Sucrose (Great Value Brand, Walmart, Bentonville, AR) was added and mixed until it dissolved. Each treatment when wet was a 1:1 ratio (w:w) of yeast extract + sucrose:water. A micropipette was used to place about 250 mg of wet diet onto a glass cover slip (22 × 22 mm). Diets were dried by placing them in an oven at 70-80°C for 4 h, resulting in 135-140 mg of dry diet per cover slip for exposure to flies.

Experiment 1: Egg Loads in Virgin Flies

Virgin flies were first tested because there is some evidence that in *R. pomonella* the absence of males affects fecundity (Opp & Prokopy 1986). Also, keeping sexes separate was the only way to document diet intake by males and by females. Fifteen female or 15 male flies, collected within 12 h of emergence, were placed inside a 1.9-L paper container (16.2 cm diameter × 10.5 cm high) that was white inside and covered with light organdy cloth. (The 4 other experiments below also used

this type of container.) Flies were exposed to diets (1) to (5) above. Before exposure to flies, cover slips with diet were weighed on a balance (Adventurer™, AR2140, Ohaus Corp., Pine Brook, NJ). One pre-weighed cover slip with diet was placed in a container. Water was provided on a cotton wick plugged into a 12-ml glass vial. Diets were left in containers for 5 d for 1 test and 10 d for a second test. Fly mortality was recorded daily. At the end of 5 or 10 d, females were placed in 70% ethanol and later dissected to determine numbers of fully developed, mature eggs. Flies that died before the end of 5 or 10 d were not used for egg counts because different longevities could affect egg loads (e.g., a 1-d-old fly would not have developed as many eggs as a 5-d-old fly). Cover slips with diet were reweighed after 5 or 10 d to determine diet intake, expressed as mg intake/fly/day. Flies from 5 of 11 replicates were used to determine egg loads (flies from the other 6 were used for a test not reported here), whereas all 11 replicates were used to generate survival and diet intake data, except in 10-d-old males and females fed 20:80 diet, where there were 10 replicates.

Experiment 2: Egg Loads in Mated Flies

Methods here were similar to those in Experiment 1, except 15 female and 15 male flies were held together in containers, 2 cover slips (instead of 1) with diet were provided, diets (1) to (7) were tested, and there was only a 10-d-old group. Five replicates were completed, except for (7), where 7 were completed.

Experiment 3: Mortality After 1- and 2-h-Exposures to GF-120 in Absence of Food

Diets (1) to (5) were tested with 10-d-old flies. Before exposure to GF-120, methods followed those of Experiment 2. At 10 d, diet was removed from a container, and one clear dish (9.0 cm diameter x 1.4 cm) with five 20-µL drops of fresh 20% GF-120 (v/v) (exposed to air for about 20 min at 21°C) was placed on the bottom of the white container. There was no food during exposure to GF-120. GF-120 was left in the container for 1 h in Test 1 and for 2 h in Test 2, and then removed. Three sucrose cubes were then placed in the container as food. Mortality was recorded 24 h after removal of GF-120. Flies were considered dead if they did not move or could not walk when probed. For Test 1, five replicates were completed, except for the 1:99 and 20:80 diets, where there were 4 and 6, respectively. For Test 2, five replicates were completed.

Experiment 4: Mortality After 6-h-Exposure to GF-120 in Presence of Yeast Extract Diets

As in Experiment 3, 15 females and 15 males were held together in 1.9-L containers with 2 cover slips with diet. Unlike Experiment 3, however, diets (1) to (7) were tested, flies were 7-d-old when tested, and flies were exposed to dried GF-120 on artificial silk leaves for 6 h. Eighteen h before testing, 5 drops of 20% GF-120 were placed on a 19.5-cm^2 light green silk leaf (7.0 cm long, 4.0 cm wide). Drops were dried at 21°C and were about 5 mm in diameter. Immediately before exposure to GF-120, the 2 diet slips were removed. A new pre-weighed slip with the same diet that flies had fed on was placed in the container. The leaf with GF-120 drops was then clipped onto the top edge of the container, with the leaf tip 3.5 cm above the bottom. After 6 h, the leaf and diet slip were removed, and the diet slip reweighed. One sucrose cube was then added to the container. Mortality was recorded as before. There were 5 to 9 replicates of the control and all treatments. Diets not exposed to flies inside separate containers also were weighed before and after the 6-h-exposure period to confirm weight loss was caused by feeding.

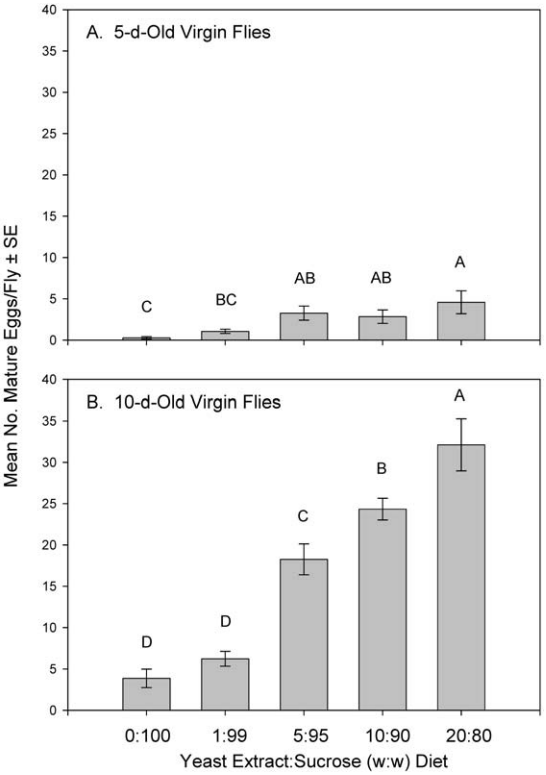

Fig. 1. Experiment 1: Mean egg loads in (A) 5-d-old and (B) 10-d-old virgin *Rhagoletis indifferens* held on various yeast extract:sucrose diets. Means with same letters are not significantly different ($P > 0.05$).

TABLE 1. EXPERIMENT 1: MEAN SURVIVAL AND DIET INTAKE (MG) ± SE IN 5- AND 10-D-OLD VIRGIN *RHAGOLETIS INDIFFERENS* USED TO DETERMINE EGG LOADS.

	Percent Alive at 5 or 10 d			
Y:S[a]	Males (5 d)	Females (5 d)	Males (10 d)	Females (10 d)
0:100	97.9 ± 0.9 Aa	97.0 ± 1.4 Aa	90.6 ± 1.5 Bb	69.1 ± 5.1 Dc
1:99	98.2 ± 1.3 Aa	95.2 ± 2.6 Aa	96.3 ± 1.1 Aa	81.8 ± 3.4 Cb
5:95	94.5 ± 2.0 Ab	99.4 ± 0.6 Aa	95.1 ± 2.0 Ab	92.7 ± 1.1 Bb
10:90	96.4 ± 1.4 Aa	98.2 ± 0.9 Aa	97.6 ± 1.4 Aa	97.0 ± 1.1 Aa
20:80	95.8 ± 2.1 Aa	95.7 ± 1.9 Aa	97.3 ± 1.1 Aa	90.7 ± 1.8 BCb
	Diet Intake/Fly/d			
Y:S[a]	Males (0-5 d)	Females (0-5 d)	Males (0-10 d)	Females (0-10 d)
0:100	0.620 ± 0.039 Ab	0.752 ± 0.024 Ca	0.567 ± 0.024 Bb	0.739 ± 0.021 Ba
1:99	0.703 ± 0.032 Ab	0.832 ± 0.024 BCa	0.590 ± 0.018 Bc	0.750 ± 0.030 Bb
5:95	0.673 ± 0.020 Ab	0.870 ± 0.042 Ba	0.587 ± 0.014 Bc	0.733 ± 0.020 Bb
10:90	0.658 ± 0.011 Ac	0.850 ± 0.024 Ba	0.598 ± 0.015 Bd	0.782 ± 0.013 Bb
20:80	0.702 ± 0.015 Ac	0.964 ± 0.029 Aa	0.673 ± 0.022 Ac	0.886 ± 0.025 Ab

11 replicates of 15 flies each (10 replicates in 10-d-old flies, 20:80 diet).
[a]Yeast Extract:Sucrose (w:w).
Means followed by same uppercase letters within columns are not significantly different ($P > 0.05$); means followed by same lowercase letters within rows are not significantly different ($P > 0.05$).

Experiment 5: Mortality After 6-h-Exposure to GF-120 in Presence of Only Sucrose

Test 1 of Experiment 5 was the same as Experiment 4, except that only sucrose was present during the 6-h-exposure to GF-120 and only diets (1), (2), (5), and (6) were tested. Five replicates were completed. In Test 2 of Experiment 5, the same methods were used, except that after exposure to GF-120, a 10:90 diet was provided in case it could reduce mortality after exposure to GF-120 compared with sucrose only. Three to 6 replicates were completed.

Statistical Analysis

In Experiment 1, three-way analysis of variance (ANOVA) was conducted, with diet, sex, and fly age as factors. One-way ANOVA was conducted among diets within sexes, age groups, and diets across sex and age groups. Fisher's protected LSD test was used for pairwise comparisons. In Experiments 2-5, three, two-, or one-way ANOVAs were conducted. In all experiments, numbers of eggs were square-root transformed for ANOVA. Fly mortality after exposure and percent survival before exposure to GF-120 were arcsine-transformed for ANOVA. Mortality data from sexes were combined when there was no sex effect and no sex × diet interaction ($P > 0.05$). In Experiments 4 and 5, Pearson correlation coefficients were calculated to describe the relationship between egg loads and female mortality irrespective of yeast extract:sucrose ratios. The Statistical Analysis System (SAS Institute Inc. 2008) was used for all analyses. Untransformed means ± SEs are presented.

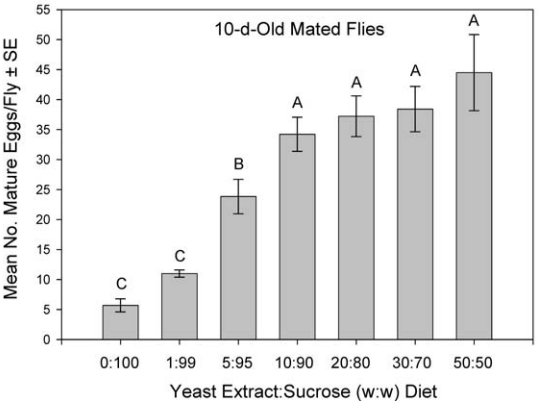

Fig. 2. Experiment 2: Mean egg loads in 10-d-old mated *Rhagoletis indifferens* held on various yeast extract:sucrose diets. Means with same letters are not significantly different ($P > 0.05$).

RESULTS

Experiment 1: Egg Loads in Virgin Flies

Egg loads were higher in 5-d-old virgin flies fed 5:95, 10:90, and 20:80 diets than in flies fed 0:100

TABLE 2. EXPERIMENT 2: MEAN SURVIVAL AND DIET INTAKE (MG) ± SE IN 10-D-OLD MATED *RHAGOLETIS INDIFFERENS* USED TO DETERMINE EGG LOADS.

Y:S[a]	Percent Alive at 10 d Males + Females	Diet Intake/Fly/d Males + Females
0:100	87.8 ± 3.1 A	0.5788 ± 0.0174 D
1:99	90.7 ± 2.9 A	0.5941 ± 0.0091 D
5:95	91.3 ± 3.9 A	0.6093 ± 0.0110 CD
10:90	97.4 ± 1.2 A	0.6589 ± 0.0142 BCD
20:80	92.5 ± 3.5 A	0.7651 ± 0.0177 BC
30:70	95.3 ± 1.4 A	0.7841 ± 0.0198 B
50:50	57.6 ± 13.9 B	1.1373 ± 0.1071 A

Five or 7 replicates of 30 flies each. [a]Yeast Extract:Sucrose (w:w).
Percent Alive: $F = 2.9$; $df = 6, 30$; $P = 0.0235$; Diet Intake: $F = 14.4$; $df = 6, 30$; $P < 0.0001$.
Means followed by same letters within columns and within parameters are not significantly different ($P > 0.05$).

diet ($F = 6.5$; $df = 4, 20$; $P = 0.0015$), but those in flies fed 0:100 and 1:99 diets did not differ (Fig. 1A). Many eggs were produced between 5 and 10 d. In 10-d-old virgins, egg load was highest in flies fed 20:80 diet ($F = 52.3$; $df = 4, 19$; $P < 0.0001$) (Fig. 1B). Inspection of containers after tests revealed no eggs, so there was no "egg dumping" caused by a lack of oviposition substrates (true in all experiments). Survival was affected by age, diet, and sex ($P < 0.05$) (no interactions). Even though the 20:80 diet resulted in the highest egg load in 10-d-old virgins, survival of virgin females on it was lower than on the 10:90 diet (Table 1). Survival of females was lower than that of males within the 0:100 diet. There were significant differences between males and females (10-d-old) in the cases of 1:99 and 20:80 diets (Table 1).

After 5 and 10 d, 70-90 and 20-60 mg of diets, respectively, remained, so diet was not limiting (also true in Experiments 2-5). Intake of the 20:80 diet was highest, except in 0-5-d old males (Table 1). Intake was affected by age, diet, and sex ($P < 0.05$) (no interactions). Based on amounts of dry sucrose in each diet and the assumption that flies ingested yeast extract and sucrose in proportion to their ratios, intake of sucrose from all diets was similar, ranging from 0.696-0.742 mg/fly/d (also true in Experiments 2-5).

Experiment 2: Egg Loads in Mated Flies

Egg loads were higher in 10-d-old mated flies fed 10:90, 20:80, 30:70, and 50:50 than 0:100, 1:99, and 5:95 diets ($F = 24.5$; $df = 6, 30$; $P < 0.0001$) (Fig. 2). Survival was lowest on the 50:50 diet (no sex effect, $P > 0.05$) (Table 2). Diet intake by 10-d-old mated flies (no sex effect, $P > 0.05$) increased numerically as yeast extract:sucrose ratios increased (Table 2).

Experiment 3: Mortality After 1- and 2-h-Exposures to GF-120 in Absence of Food

Egg loads in dead and live flies after exposure to GF-120 in Tests 1 and 2 did not differ ($P > 0.05$), so data were combined. Egg load was highest in flies fed 20:80 diet (Fig. 3A), but for both 1- and 2-h-exposures, no differences in mortality among diet treatments were detected (sex, sex × diet effect, $P > 0.05$) (Figs. 3B and 3C) (1 h: $F = 0.8$; $df = 4, 20$; $P = 0.5508$; 2 h: $F = 1.3$; $df = 4, 20$; $P = 0.2890$). Survival of females on 0:100 was lower than on other diets (Table 3). The diet intake pattern before GF-120 exposure (Table 3) was similar to that in Experiment 2.

Experiment 4: Mortality After 6-h-Exposure to GF-120 in Presence of Yeast Extract Diets

Egg load data from dead and live flies after exposure to GF-120 were combined ($P > 0.05$). Egg loads were lowest in flies fed 0:100 and 1:99 diets and highest in flies fed 20:80, 30:70, and 50:50 diets (Fig. 4A). Mortality caused by GF-120 was lower in flies fed 20:80 and 30:70 than 0:100 and 1:99 diets (sex, sex × diet effect, $P > 0.05$) (Fig. 4B) ($F = 3.6$; $df = 6, 35$; $P = 0.0067$). Although not significant, there was an unexpected increase in mortality of flies fed 50:50 compared with 20:80 and 30:70 diets. There was a significant negative correlation between egg loads and mortality caused by GF-120 ($r = -0.4684$; $P = 0.0018$). Flies consumed 0.092-0.416 mg yeast extract diet/fly during the 6-h-exposure to GF-120. Greater amounts of higher than lower yeast extract diets were consumed. Survival before exposure to GF-120 (Table 4) was lowest in flies fed 50:50 diet, even though egg load in these flies was similar to that in flies fed 20:80 diet (Fig. 4A). Survival of flies fed 0:100 diet was as high as that of flies fed 1:99 to 30:70 diets before exposure to GF-120 (Table 4), but flies fed 0:100 diet suffered higher mortality than flies fed 5:95 to 30:70 diets after GF-120 exposure (Fig. 4B), suggesting the absence of yeast extract in diet caused a greater response to GF-120. Food intake increased as yeast extract in diets increased (Table 4).

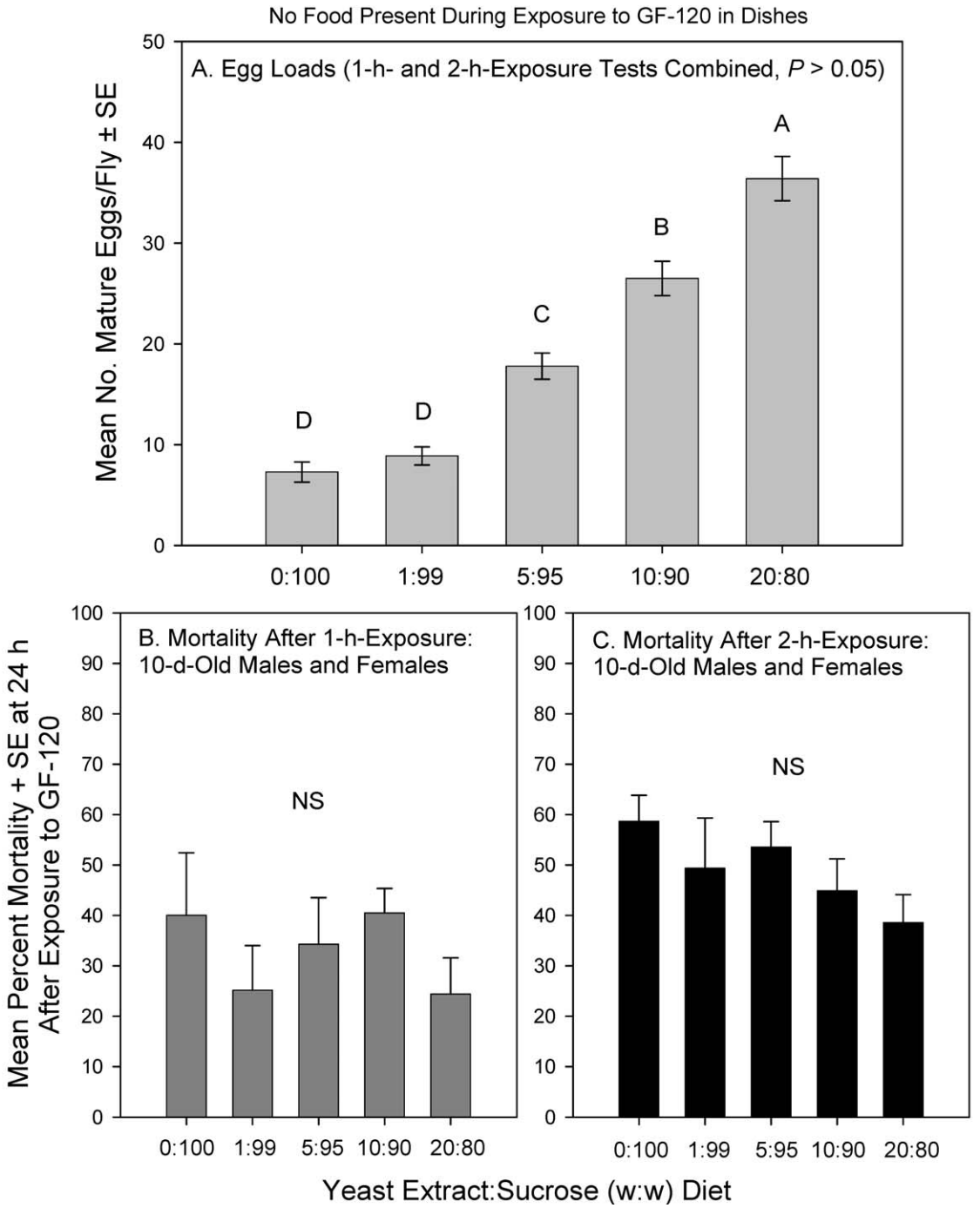

Fig. 3. Experiment 3: (A) Mean egg loads and (B) percent mortality of 10-d-old mated male and female *Rhagoletis indifferens* held on various yeast extract:sucrose diets at 24 h after 1-h- and (C) 2-h-exposures to fresh GF-120 in dishes. Means with same letter or no letters (NS) are not significantly different ($P > 0.05$).

Experiment 5: Mortality After 6-h-Exposure to GF-120 in Presence of Only Sucrose

There were no differences between Tests 1 and 2 for any parameters ($P > 0.05$), so data were combined. Egg loads in dead and live flies after exposure to GF-120 did not differ ($P > 0.05$), and were lowest in flies fed 0:100 and 1:99 diets and highest in flies fed 20:80 diet (Fig. 5A). Mortality caused by GF-120 was higher in flies fed 0:100 and 1:99

TABLE 3. EXPERIMENT 3: MEAN SURVIVAL AND DIET INTAKE (MG) ± SE IN 10-D-OLD MATED *RHAGOLETIS INDIFFERENS* TESTED FOR MORTALITY CAUSED BY 1- AND 2-H-EXPOSURES TO GF-120.

	Percent Alive at 10 d		Diet Intake/Fly/d
Y:S[a]	Males	Females	Males + Females
0:100	88.8 ± 2.8 A	59.8 ± 5.1 C	0.634 ± 0.022 C
1:99	92.6 ± 2.1 A	83.0 ± 3.7 B	0.630 ± 0.012 C
5:95	92.7 ± 1.9 A	95.2 ± 1.8 A	0.649 ± 0.010 BC
10:90	95.3 ± 2.2 A	90.7 ± 2.2 AB	0.684 ± 0.015 B
20:80	93.9 ± 3.0 A	89.0 ± 4.5 AB	0.792 ± 0.016 A

1-h- and 2-h-exposure tests combined: 9 to 11 replicates of 15 (percent alive) or 30 flies (diet intake) each. [a]Yeast Extract:Sucrose (w:w). Percent Alive, Males: $F = 1.6$; $df = 4, 45$; $P = 0.1940$; Percent Alive, Females: $F = 11.2$; $df = 4, 45$; $P < 0.0001$; Diet Intake: $F = 21.7$; $df = 4, 43$; $P < 0.0001$.

Means followed by same letters within columns are not significantly different ($P > 0.05$).

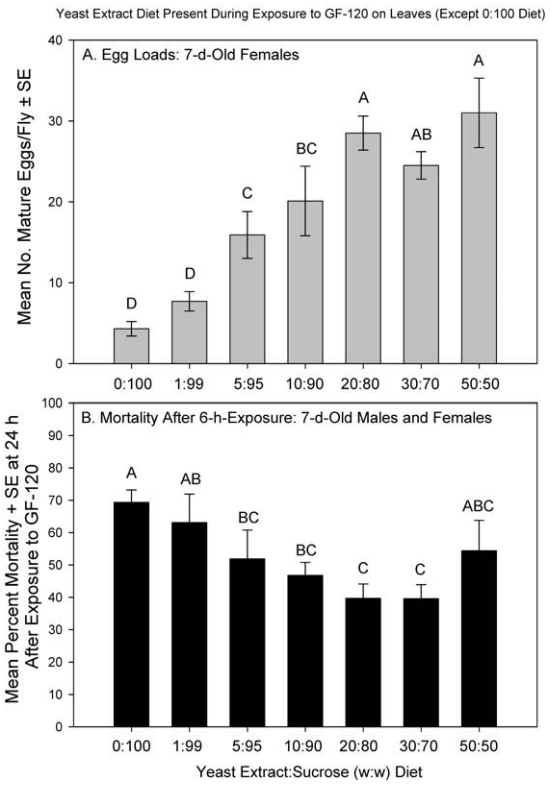

Fig. 4. Experiment 4: (A) Mean egg loads and (B) percent mortality in 7-d-old mated male and female *Rhagoletis indifferens* held on various yeast extract:sucrose diets at 24 h after a 6-h-exposure to dried GF-120 on artificial silk leaves, with yeast extract:sucrose diet present during exposure to GF-120. Means with same letters are not significantly different ($P > 0.05$).

than 20:80 diets (sex, sex × diet effect, $P > 0.05$) (Fig. 5B) ($F = 11.1$; $df = 3, 32$; $P < 0.0001$). Mortality of flies fed 20:80 diet was lower than of flies fed 0:100 and 1:99 diets. There was a significant correlation between egg loads and mortality (r = -0.5893, $P = 0.0002$). Flies in all diet treatments consumed 0.198-0.292 mg sucrose/fly during the 6-h-exposure to GF-120 ($P > 0.05$). Survival of females fed 0:100 diet before exposure to GF-120 was lower than that of females fed other diets (Table 5), and flies fed 0:100 diet suffered higher mortality than flies fed 20:80 and 30:70 diets after GF-120 exposure (Fig. 5B). The diet intake pattern before GF-120 exposure (Table 5) followed that in Experiment 4.

TABLE 4. EXPERIMENT 4: MEAN SURVIVAL AND DIET INTAKE (MG) ± SE IN 7-D-OLD MATED *RHAGOLETIS INDIFFERENS* TESTED FOR MORTALITY CAUSED BY GF-120.

Y:S[a]	Percent Alive at 7 d Males + Females	Diet Intake/Fly/d Males + Females
0:100	91.6 ± 1.4 B	0.609 ± 0.016 C
1:99	93.8 ± 1.7 AB	0.599 ± 0.013 C
5:95	99.3 ± 0.7 A	0.607 ± 0.013 C
10:90	95.2 ± 2.6 AB	0.628 ± 0.023 C
20:80	95.1 ± 1.0 AB	0.764 ± 0.025 B
30:70	97.3 ± 1.9 AB	0.833 ± 0.031 B
50:50	67.2 ± 14.3 C	1.210 ± 0.127 A

Five to 9 replicates of 30 flies each.

[a]Yeast Extract:Sucrose (w:w). Percent Alive: $F = 3.0$; $df = 6, 35$; $P = 0.0178$; Diet Intake: $F = 21.1$; $df = 6, 43$; $P < 0.0001$. Means followed by same letters within columns are not significantly different ($P > 0.05$).

DISCUSSION

Results from 5 experiments with virgin and mated *R. indifferens* showed that overall the yeast extract:sucrose ratios of 20:80 and 30:70, which equated to 2.2 and 3.3% nitrogen in diets, maximized egg loads without reducing survival up to 7 or 10 d. However, there were exceptions. In 1 of 3 experiments, the 30:70 diet resulted in lower egg loads than the 20:80 diet, suggesting that yeast extract above that found in a 20:80 diet is unnec-

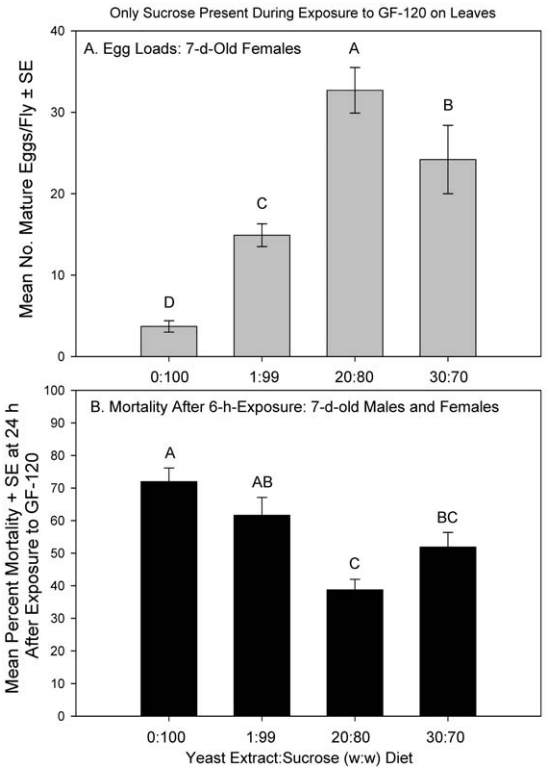

Fig. 5. Experiment 5: (A) Mean egg loads and (B) percent mortality in 7-d-old mated male and female *Rhagoletis indifferens* held on various yeast extract:sucrose diets at 24 h after a 6-h-exposure to dried GF-120 on artificial silk leaves, with only sucrose present during exposure to GF-120. Means with same letters are not significantly different ($P > 0.05$).

essary to maximize egg production. This result supports the 20:80 ratio currently used for maintenance of *R. indifferens*. The 50:50 (5.4% nitrogen) diet also maximized egg loads, but it compromised survival. Similarly, diets with 50-91% yeast hydrolysate reduced longevity in *Bactrocera tryoni* Froggatt (Prabhu et al. 2008). Even though the 20:80 diet lowered survival in one instance, ingesting a diet with 2.2% nitrogen may have an advantage for an insect such as *R. indifferens*, which in nature probably lives only about 1 month (Frick et al. 1954). In *Drosophila melanogaster* Meigen, egg laying was maximized at a protein:carbohydrate ratio of 1:2, but longevity was highest at 1:16, and when offered a choice, flies regulated food intake to maximize lifetime egg production rather than longevity (Lee et al. 2008).

The hypothesis that *R. indifferens* with greater egg loads suffer lower mortality than flies with lower egg loads after exposure to GF-120 was not supported in Experiment 3 with 1- and 2-h-exposures to GF-120, but it was in Experiments 4 and 5, so results remain somewhat inconclusive. Possibly the fresh drops, which were brown in color, stood out against the white background of the containers in Experiment 3, making them more easily seen and found by flies with high loads than the dry drops presented on green leaves in Experiments 4 and 5. Also, fresh drops may have had more attractive odor than dry drops. GF-120 began losing attractiveness to *Bactrocera cucurbitae* Coquillett after aging for 1-5 h and lost all attractiveness after 24 h (Prokopy et al. 2003; Revis et al. 2004). Mortality in *R. indifferens* presumably resulted from feeding on GF-120 by flies (Yee 2006a). Interestingly, within diet treatments, mortality of males and females caused by GF-120 did not differ in any test, suggesting yeast extract:sucrose ratios affected sexes similarly.

High yeast extract:sucrose ratios result in high egg loads and may reduce feeding responses by *R. indifferens* to GF-120, although not to the extent that the bait is rendered completely ineffective. In Experiments 4 and 5, about 60% of flies fed 20:80 diet apparently did not respond to dried GF-120 within 6 h. Based on this finding, it may be important to apply bait in a way that results in a very high percentage of flies with high egg loads finding it quickly, perhaps a way that results in a

TABLE 5. EXPERIMENT 5: MEAN SURVIVAL AND DIET INTAKE (MG) ± SE IN 7-D- OLD MATED *RHAGOLETIS INDIFFERENS* TESTED FOR MORTALITY CAUSED BY GF-120.

	Percent Alive at 7 d		Diet Intake/Fly/d
Y:S[a]	Males	Females	Males + Females
0:100	96.9 ± 1.1 A	82.4 ± 3.1 B	0.648 ± 0.023 B
1:99	97.6 ± 1.7 A	95.0 ± 2.7 A	0.677 ± 0.014 B
20:80	94.8 ± 1.5 A	100.0 ± 0.0 A	0.838 ± 0.015 A
30:70	96.7 ± 1.7 A	92.2 ± 0.8 A	0.838 ± 0.024 A

Tests 1 and 2 combined: 8 to 11 replicates of 15 (percent alive) or 30 flies (diet intake) each. [a]Yeast Extract:Sucrose (w:w).

Percent Alive, Males: $F = 0.8$; $df = 3, 32$; $P = 0.4821$; Percent Alive, Females: $F = 17.2$; $df = 3, 32$; $P < 0.0001$; Diet Intake: $F = 25.8$; $df = 3, 32$; $P < 0.0001$.

Means followed by same letters within columns are not significantly different ($P > 0.05$).

uniform rather than a spotty bait distribution. Flies that do not find the bait quickly could oviposit before feeding and dying. This could contribute to the variable control (42-91%) using GF-120 in heavily infested residential trees after 1 season of spraying (Yee & Chapman 2005; Yee 2006b).

Rhagoletis indifferens consumed greater amounts of diets with higher than lower yeast extract: sucrose ratios. Flies apparently extracted similar amounts of sucrose from all diets irrespective of yeast extract levels, and coincidentally ingested the yeast extract that was mixed in them. This would suggest intake of mixed diets is regulated by sucrose. In general, this explanation for the observed intake pattern is consistent with work on *C. capitata* (Nestel et al. 1985; Plácido-Silva et al. 2006) and *Anastrepha obliqua* (Macquart) (Fontellas & Zucoloto 1999; Cresoni-Pereira & Zucoloto 2001; Medeiros & Zucoloto 2006), and now has been extended to *R. indifferens*.

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