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Trapping for Mexican Fruit Fly (Diptera: Tephritidae) with Torula Yeast and Propylene Glycol

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ABSTRACT

In south Texas, propylene glycol is added to the liquid bait (an aqueous slurry of torula yeast) in surveillance traps for exotic fruit flies to better preserve captured specimens. In a series of tests in Texas and Mexico overall captures of Mexican fruit flies were roughly the same in traps with or without the additive. Inconsistency in the results are attributed to variables including the reproductive status of the flies (sterile vs fertile), and seasonal climatic differences during the experiments. Fertile flies were trapped in greater numbers using the additive, whereas sterile flies were not. There was also a general trend towards a female bias in captures both with and without the additive.

RESUMEN

El glicol de propilena es usado en trampas de surveo para moscas exoticas de la fruta con cebu de torula en agua para mejorar la preservación de las muestras. En una serie de pruebas en Texas y Mexico, las trampas con la preservativa ha capturado numeros de moscas Mexicanas igual, pero variable, como las trampas sin el aditivo. La inconsistencia en los resultados puede ser causado por la diferencia en la capacidad reproductiva (esteril o fertil), y diferencias en el clima temporal durante las pruebas. Tambien, estaba una preferencia hembral en ambas trampas con o sin el aditivo.

The Mexican fruit fly, *Anastrepha ludens* (Loew), is a major pest of citrus in Mexico (Aluja 1994). In Texas, quarantine restrictions are triggered when an infestation of the pest is detected either in fruit or in surveillance traps operated by the Texas Department of Agriculture (TDA) on the U.S. side of the Rio Grande (Nihlake et al. 1991). Up until October 2000 the surveillance traps, plastic versions of the traditional McPhail design, were baited with an aqueous slurry of torula yeast (Lopez et al. 1971). Beginning in October 2000 until the present, a small amount of propylene glycol has been added to the bait slurry solution to improve preservation of the captured flies. However, the impact of the additive on attractiveness of the lure had not been documented. Because southern Texas citrus is protected by inundative releases of radio-sterilized Mexican fruit flies, program managers maintain detailed records of trapping results in order to measure coverage achieved by the sterile release program. But it is not known whether catches among years are comparable given the change in lure formulation. Thomas et al. (2001) reported increased captures when propylene glycol was added to the capture liquid in surveillance traps, but not with torula yeast as the bait. Hall et al. (2005) found that propylene glycol added to the torula yeast bait reduced the capture of Caribbean fruit flies (*Anastrepha suspensa* [Loew]) in Florida citrus orchards. Therefore, we conducted a series of tests comparing the catch rate in torula yeast baited traps, with or without propylene glycol, and against both wild and sterile-released Mexican fruit fly populations.

MATERIALS AND METHODS

Studies were conducted in Texas and Mexico in citrus orchards using cylindrical plastic traps marketed as Multilure traps (Better World Manufacturing, Miami FL). The standard bait solution consisted of three 5 gm torula yeast/ borax (55:45 by weight) pellets (ERA International, Freeport, NY) dissolved in 300 ml of water. The alternative formulation was the same but with 30 ml of automotive coolant (Prestone Low-Tox™) added to 270 ml of water (10% by volume coolant). Low-Tox™ is a mixture of 40% propylene glycol in de-ionized water, with green dye and proprietary additives including lubricants and corrosion inhibitors. This formulation is preferred in fruit fly programs over industrial propylene glycol because of the lower cost and ready availability. Propylene glycol is preferred over Ethylene glycol based anti-freeze because it has significantly lower mammalian toxicity and is rated as “Generally Regarded as Safe” by the U.S. Food & Drug Administration.

The test in Mexico was conducted within the municipality of Allende, Nuevo Leon, in a Valencia orange grove where native Mexican fruit flies were known to be abundant. Ten traps were deployed in five alternate rows of citrus trees, one with and one without the anti-freeze in each row. In each row the traps with and without the additive were separated by a tree without a trap, and each week the traps were alternated among the trees to minimize position effects. The Mexican test was conducted in the spring when populations are highest, in this case, over a period of 10 weeks March to May of 2004 with all traps serviced weekly.

The Texas tests were conducted on the property of the USDA-ARS Kika de la Garza Subtropical Agriculture Research Center, Weslaco, TX, in a mixed (grapefruit, orange and tangerine) citrus grove. In 2004 the test was run for 10 weeks from August to October with all traps serviced weekly. Torula yeast baited traps

with or without anti-freeze were alternated within each of two rows with six traps of each treatment per row for a total of 24 traps in operation simultaneously. The test was repeated in the spring of 2005 (8 weeks, April-June), but with only two traps of each treatment per row for a total of eight traps in operation simultaneously. The traps were deployed as in the Mexico test and all traps were serviced weekly. Because Mexican fruit fly populations in this area are under intensive control, 4000 radio-sterilized Mexican fruit flies obtained from the USDA-APHIS facility in Mission, TX, were released weekly in the experimental plot.

USDA-APHIS maintains a surveillance grid of 1100 McPhail traps in the lower Rio Grande Valley baited with an aqueous slurry of torula yeast. These traps are serviced weekly and the trap results reported online. We compiled the results from these records over a ten yr period, 1995 to 2005, in order to compare capture rates before and after the programmatic change in formulation.

Fly captures were analyzed by two-way Analysis of Variance, separating out effects of propylene glycol and test week using super ANOVA (Abacus concepts 1989). Treatment means were compared using Fisher's Protected Least Significant Difference test . Because the effect of propylene glycol appeared to change over time in the test conducted in Mexico, a second analysis was conducted in which test week was used as a continuous variable to test for the interaction of propylene glycol with test week. Gender differences were compared by student's t-test. Probabilities were calculated with the NCSS software program PASS.

RESULTS and DISCUSSION

The results of the three experiments were not uniform (Table 1). In the 2004 Texas test about 55% more flies were caught in the traps without the propylene-glycol. The mean (± s.e.) number of flies captured weekly per trap with propylene-glycol was 7.3 ± 0.7 vs. 11.3 ± 1.0 in the control traps which was significantly different (F = 24.2; df = 1, 108; p = 0.0001).

Table 1.- Numbers of Mexflies captured weekly in traps baited with an aqueous slurry of torula yeast, with (+) or without (-) Propylene Glycol (PG) additive, in three trials.

	Texas 2004		Mexico 2004		Texas 2005	
Week	+ PG	-PG	+PG	-PG	+PG	-PG
1	14	12	41	41	49	90
2	66	117	50	51	67	75
3	13	36	31	15	180	213
4	17	22	8	6	182	121
5	34	57	25	13	54	109
6	42	38	23	4	157	112
7	37	83	3	2	151	81
8	70	109	9	3	19	61
9	72	123	30	3		
10	58	93	33	2		
Totals	423	690	253	140	859	862

In the Mexican test, however, the traps with propylene-glycol captured more flies than did the control traps. The mean number of flies captured with the propylene-glycol formulation was 5.1 ± 0.7 vs. only 2.8 ± 0.6 flies per trap-week in the traps without propylene-glycol. This difference, about 80% higher with the preservative, was also statistically significant (F = 9.47; df = 1, 89; p = 0.003).

The effect of propylene-glycol appeared to change during the course of the test in Mexico. Traps with or without propylene-glycol captured equal numbers of flies during the first two weeks, but then traps with propylene glycol captured more flies during the remainder of the test. Still, the interaction of propylene-glycol with test week was not significant at the p = .05 level (F = 3.31; df = 1, 96; p = 0.072).

An obvious factor that might account for the difference in results was that the Texas test involved factory reared, sterile flies, whereas the Mexico test involved wild, fertile flies. The torula yeast is presumptively a feeding attractant based on protein hunger (Robacker 1991). A reproductively capable fly might respond differently to a proteinaceous attractant than a sterile fly (Robacker 1998), but why this response would be influenced by the presence or absence of the propylene-glycol is unclear. To some degree the propylene-glycol may alter the rate of bacterial degradation of the yeast such as to change the quantity or composition of volatiles emanating from the trap. Even so there would seem to be confounding influences at work. In a similar test with the Caribbean fruit fly, Hall et al. (2005) also found that wild flies responded differently from radio-sterilized flies. In that study there was no significant difference in captures of sterile flies between traps with the propylene-glycol and those without. But in the case of wild flies, significantly fewer carib-flies were captured in the traps with the propylene-glycol, the opposite of our results with Mexican fruit flies.

For these reasons the Texas test was repeated in 2005. This test was conducted in the spring when fruit fly activity is expected to be higher, and indeed, much higher numbers were captured in this test, even though fewer traps were deployed. In this test no significant difference in the mean number of flies trapped weekly between the two treatments was observed: mean = 28.6 ± 3.9 flies per trap-week with the propylene glycol and mean = 28.7 ± 3.2 flies per trap-week without the propylene glycol (F = .001; df = 1, 51; p = .98). This last result would tend to support the supposition that the effect of the propylene-glycol on the attractancy of the bait liquid is negligible and that the statistically significant but opposite results of the two earlier tests have little or nothing to do with the propylene-glycol. We conclude that some unknown factor was confounding the results.

An important consideration for program managers is the ratio of male to female flies attracted to the lure formulation. Population suppression with the sterile insect technique depends primarily on the action of the males. Historically, the traps baited with the Lopez formulation are consistently female biased by a ratio of around 3:1 with sterile flies. Conversely, the same formulation produces a seasonally variable, but slightly male biased capture overall (3:2), when deployed against wild, fertile, populations (Thomas et al. 2001).

In our Mexican test there was a strong female bias (ratio 1.9:1) with the propylene-glycol formulation, and this difference was statistically significant (t = 2.66, df = 98, p = .005). By

contrast, there was no significant gender bias with the Lopez formulation, though tested simultaneously (mean = 1.44 males, vs 1.36 females, per trap week) (t = 0.17, df = 98, p = .431).

In the Texas tests, using sterile flies, there was a female bias in both years with both formulations, but the differences (range 9-35%), were not large enough to be statistically significant when the data was analyzed on a weekly basis (Table 2). This was likely because the week to week variation in total numbers tended to obscure the effect. When the numbers are compared on an individual trap basis the differences between the genders are statistically significant. For example with data from Texas in 2004 the traps with propylene-glycol captured a mean of 3.9 females per trap-week, but only 3.4 males. Those means were significantly different (t = 1.66, df = 114, p. = .049).

Table 2.- Gender of Mexflies captured in traps with (+PG) or without (-PG) propylene glycol additive. Mean ± s.d. flies trapped per week by lure treatment with Student's t-test scores for the gender bias.

	Females	Males	t	d.f.	p
Mex 04; +PG	16.5 ± 9.09	8.8 ± 6.88	2.03	18	.029
Mex 04; -PG	6.8 ± 6.79	7.2 ± 10.92	0.09	18	.463
Tex 04; +PG	22.6 ± 12.30	19.7 ± 11.52	0.52	18	.306
Tex 04; -PG	36.1 ± 20.72	32.9 ± 20.64	0.33	18	.373
Tex 05; +PG	59.0 ± 36.32	48.4 ± 32.02	0.58	14	.286
Tex 05; -PG	62.0 ± 31.53	45.8 ± 16.49	1.21	14	.124

For comparison, we examined the Texas program records for gender bias before and after deployment of the propylene-glycol additive. Table 3 shows the number and sex ratio of feral Mexican fruit flies captured in Texas over the last decade (1995-2005). As previously noted, and as shown in this table, prior to 2001 the ratio of females to males was 3.2:1. In the years where propylene-glycol was deployed in the traps, after 2000, the female bias increased to a ratio of 3.8:1.

Table 3.- Gender-ratio in non-sterile mexflies captured in 2200 surveillance traps in south Texas from 1995 to 2005. Beginning in Winter 2000-2001, propylene glycol was added to the trap liquid to improve preservation of the catch.

Season	Males	Females	Season	Males	Females
1995	161	453	2001	31	89
1996	12	38	2002	171	500
1997	48	167	2003	54	247
1998	324	1062	2004	10	75
1999	3	18	2005	9	117
2000	36	122			
Totals	584	1860		275	1028

CONCLUSION

Our results were not sufficiently consistent to draw hard conclusions regarding the effect of the propylene-glycol on attractancy of the bait solution. Taken at face value the results of our experiments suggest that under certain circumstances the additive might actually increase the sensitivity of the detection trapping to feral Mexican fruit flies, but decrease sensitivity to the sterile flies. However, given that in the largest dataset, from the experiment conducted in Texas in 2005, wherein there was no difference in captures between the formulations, it seems equally probable that the influence of the propylene-glycol is negligible, or at least, does not produce a consistent deviation from expected with the traditional Lopez formulation. There is a strong female bias in captures with the propylene-glycol additive as there was with the traditional bait solution.

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