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Gamma-Butyrolactone as a Lure for Traps Targeting the Asian Citrus Psyllid, *Diaphorina citri* Kuwayama (Homoptera: Psyllidae)

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ABSTRACT

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, is an invasive pest of citrus and vector of citrus greening disease. Gamma-butyrolactone (GBL), a primary component of the *D. citri* sex pheromone, failed to lure adult psyllids to sticky traps deployed in infested citrus groves in numbers greater than those captured by non-baited traps. The ineffectiveness of the single component as a trap-lure suggests that other important signals may be lacking.

Additional Index Words: Citrus greening, sticky-trap, pheromone.

The Asian citrus psyllid, *Diaphorina citri* Kuwayama, an invasive species and vector of citrus greening disease, has now infested the commercial citrus production areas of North America (Halbert and Manjunath 2004). Monitoring a pest population is a vital part of any integrated control program, and sticky traps are a standard method for censusing vagile insects in orchards (Simanton 1962, Dowell and Cherry 1981). Hall et al. (2007) reported that yellow sticky traps were the most effective method for capturing adult psyllids. However, they, and Setamou et al (2008), suggest that sampling plans for estimating pest pressure should be based on immatures rather than adults. Because immature sampling tends to be somewhat destructive, involving clipping of new growth, and because of some uncertainty with the identification of immatures, our laboratory considered ways to improve the sticky trap method.

McClain et al. (1990) found that they could increase sticky trap catches of certain aphelinid parasitoids by incorporating the pheromone of their host, the San Jose scale, *Quadraspidiotus perniciosus* (Comstock), with the trap. Wenninger et al. (2008) reported behavioral evidence for a female produced sex pheromone in *D. citri*. In November 2008, at the annual meeting of the Entomological Society of America, Onagbola et al. (2008) reported that one of the volatile ingredients in the female sex pheromone, gamma butyrolactone (hereinafter GBL), was attractive to both males of *D. citri* and to its parasitoid, *Tamarixia radiata* (Waterston) (Hymenoptera: Eulophidae). With those precedents in mind, tests were initiated using sticky traps baited with GBL.

MATERIALS AND METHODS

Commercially available laboratory-grade GBL (CAS-96-48-0) was obtained from the manufacturer (Sigma-Aldrich, St. Louis, MO). A rubber septum (Sigma-Aldrich), 6 mm diam, was filled to capacity (0.3 ml) with the clear, undiluted liquid, and inserted into the appropriate hole at the top of a yellow, 23 x 28 cm Pherocon® sticky card (Trece, Adair OK) (Fig. 1). Prior to the initiation of field testing, a preliminary test was conducted in a greenhouse containing potted citrus tree seedlings heavily infested with the psyllids. For the greenhouse tests, a trap was placed in each of the corners of the greenhouse, two with the GBL lure and two without. The traps were set on Monday and retreived on Friday. On retrieval, each individual trap was wrapped in a clear cellophane sheet to ease handling and facilitate counts of captured psyllids using a dissecting microscope. The test was repeated the subsequent week with the lure and control traps rotated from their position in the first replicate.

Field tests were conducted at two citrus groves within the city limits of Weslaco, TX, known to be

Fig. 1. A suspended sticky trap in a host tree with a lure containing septum deployed near the top edge.

infested with Asian citrus psyllid based on surveys for immatures. At each grove ten sticky traps were deployed, five with and five without the GBL loaded septa. Each trap was suspended at 1.5-2 m height within the canopy of separate, individual trees. The treatments were alternated within the row, the end row of each grove, and set in alternate trees such that each trap-tree was separated by an empty tree. Setamou et al. (2008) reported that *D. citri* tends to aggregate at the perimeter of groves. Although preliminary observations indicated that the material volatilized within two to four days depending on ambient conditions, the traps were retreived and replaced on a weekly rotation. The test was conducted from March to September 2009.

Means were compared by pairwise t-tests and the resulting probabilities calculated using the software program NCSS (NCSS Statistical Software, Kaysville, UT). Raw data was normalized by transforming to percent of monthly adult captures.

RESULTS

Greenhouse test results. In the first iteration the two traps with the GBL septa captured 302 psyllids, whereas the two control traps captured only 114 psyllids. However one of the baited traps caught a disproportionately high number, leading to the suspicion that there might have been a position effect (Table 1). The test was therefore repeated the following week with the baited and control traps rotated in position from the previous week. In terms of actual numbers, the controls captured more psyllids than baited traps in the second replication (161 vs 79 psyllids, respectively, an apparent 36% difference). However, data were highly variable and no significant differences were detected among means of the two trap types (t = 0.64, df = 6, p = 0.27) (Table 1). Apart from a likely position effect, the high density of psyllids in the artificial conditions of the greenhouse may have been a confounding factor. For example, Soroker et al. (2004) reported that male pear psyllas, *Cacopsylla bidens* (Sulc), are less responsive to female pheromones under artificial conditions. Moreover, the sex-ratio was not skewed towards males as one would expect based on the report by Onagbola et al. (2008).

Field test results. At the start of the test in March the adult psyllid population was very low. Subsequently there were peaks in trap captures during May and August. This natural waxing and waning of the population resulted in means with high standard deviations, and no significant differences were detected in comparisons of baited vs nonbaited traps (Table 2). In terms of actual numbers, the non-baited control traps actually captured more psyllids (488) than the lure baited traps (440), although the difference between means was not statistically significant (t = 0.21, df = 14, p = 0.42) even after transforming the data to mean monthly percentages (50.1% vs 49.9%, non-baited and baited traps, respectively) (t = 0.07, df = 14, p = 0.47).

Table 2. Total monthly captures of adult *D. citri* on sticky cards with or without GBL.

Month	GBL	Control
March	3	2
April	18	18
May	89	115
June	55	49
July	32	35
Aug	167	193
Sept	76	7
Totals	440	488
Mean ± SD[1]	62.9 ± 55.2 a	69.7 ± 66.1 a

[1] Means followed by the same letter are not significantly different at p = .05.

DISCUSSION

The failure of the GBL to lure citrus psyllids to the sticky traps could be attributable to a combination of factors. Most important among these is the possibility that the quantity of lure used could be suboptimal. Studies with aphids by Pettersson et al. (1998) and Quiroz et al. (1997) indicated that high levels of pheromone were actually repellent, likely because they were reflective of high population densities. Of course in this test the amount varied from high to low as the liquid material volatilized away during the course of the trap-week and would presumably reach attractive levels at some point if quantity is a factor. We found that the material was completely volatilized within about four days.

Table 1. Captures of adult *D. citri* with baited and un-baited sticky traps in a greenhouse.

	Treatment			
	Lure		Control	
Replicate	Trap 1	Trap 2	Trap 3	Trap 4
A	66	236	33	50
B	29	50	28	133
Totals	381		244	
Means ± SD[1]	95.3 ± 95.0 a		61.0 ± 48.9 a	

[1] Means followed by the same letter are not significantly different at p = .05.

Alternatively, it has recently been demonstrated that acoustical signals are used by *D. citri* to find potential mates (Wenninger et al. 2009). These acoustical signals are substrate borne. Hence, the psyllid pheromone may be a (relatively) long distance attractant luring the male to the infested tree, with the insect's entrained behavior inducing it to land on a branch for further signal acquisition; as opposed to following the chemical plume to its source. If such is the case, then a pheromone may have little efficacy in attracting the pest to a trap even if reduced amounts of GBL, or for that matter, the full pheromone blend, is more attractive than the material deployed in this test. Onagbola et al. (2009) opined that elucidation of the pheromone ecology of *D. citri* may help in the development of control strategies, such as an attractant for biological control agents, or as a mating disruption method. Even the negative results of the present study may be viewed as an incremental step in understanding the chemical ecology of this pest.

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